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The molecular basis of programmed cell death and neuroinflammation in neurodegenerative diseases

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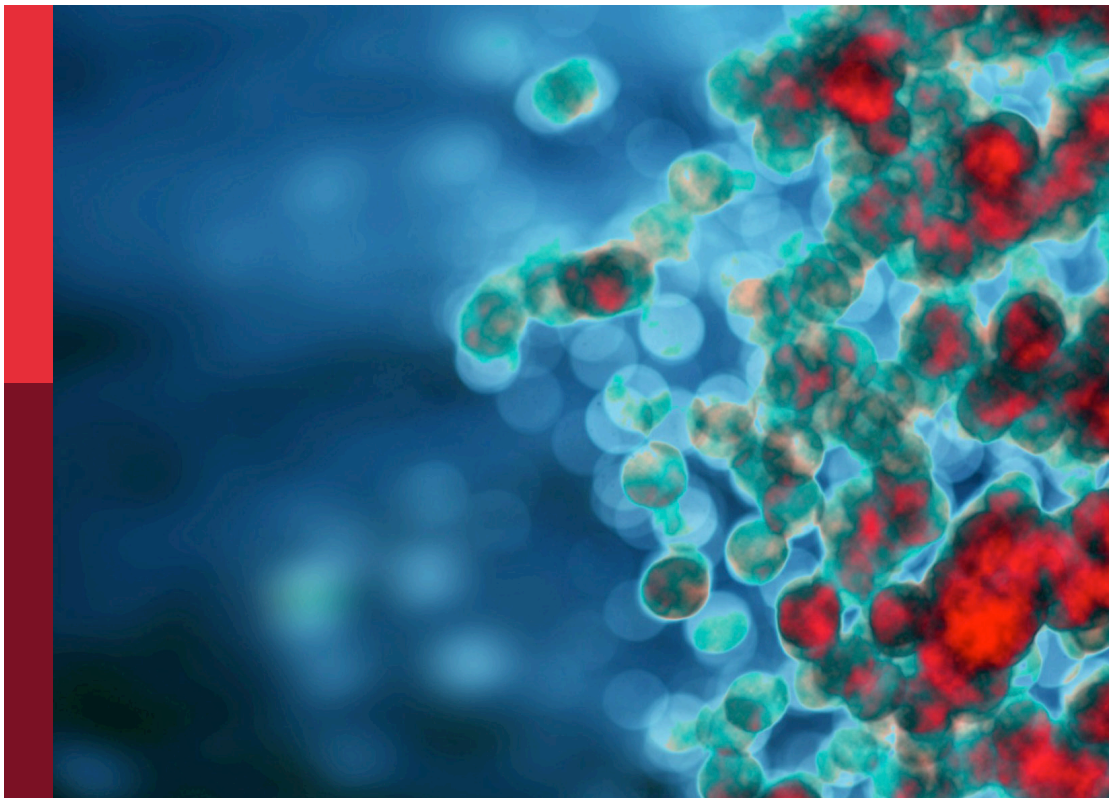

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The molecular basis of programmed cell death and neuroinflammation in neurodegenerative diseases

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Editorial: The molecular basis of programmed cell death and neuroinflammation in neurodegenerative diseases

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Editorial on the Research Topic
The molecular basis of programmed cell death and neuroinflammation in neurodegenerative diseases

The pathogenesis of neurodegenerative diseases is closely linked to the occurrence of programmed cell death, also known as apoptosis and neuroinflammation. Despite the presence of a distinct mode of cell death exhibiting unique morphological characteristics, the identification and implications of this phenomenon in neurological diseases remain unknown. Current scientific investigations are diligently striving to identify and understand distinct cellular and molecular targets that can be selectively engaged in therapeutic intervention for Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). This special edition is dedicated to a diverse array of original research, comprehensive literature reviews, and expert opinions that its aim is to provide a deeper understanding of the intricate molecular mechanisms underlying programmed cell death in the context of neurodegenerative disorders.

The perspective article from Zhang et al. presents a comprehensive analysis of the landscape of necroptosis research spanning the period from 2012 to 2021. The investigation of necroptosis has exhibited a sustained and consistent expansion throughout the previous decade. The current focus of research revolves around the phenomenon of synergistic interactions with ferroptosis, with an increasing emphasis on the exploration of RIPK1/RIPK3/MLKL pathways. This line of investigation aims to elucidate the intricate connections between ferroptosis, inflammation, and oxidative stress while also exploring the translational applications and therapeutic prospects for combating cancer and neurological disorders.

The seminal study conducted by Lee et al. introduced a chronic constriction injury (CCI) model as an effective animal model for investigating neuropathic pain. Their research

further demonstrated that the administration of Mesenchymal stem cells (MSCs) in spheroid form exhibited enhanced efficacy in promoting pain resolution and cell viability when compared to monolayer cells. In this study, the researchers observed that MSC spheroids consisting of 10,000 cells displayed enhanced resistance to apoptosis, a programmed cell death process, and exhibited decreased secretion of immune response factors after CCI. This study presents compelling evidence supporting the notion that spheroids possess the capacity to enhance the efficacy of MSC-based therapeutic interventions targeting induced neuropathic pain.

The review article authored by Li et al. provides an analysis of the intricate metabolic and regulatory pathways associated with tryptophan (TRP). A substantial body of preclinical research has been conducted to investigate the modulation of TRP metabolism as a potential therapeutic approach for neurological and psychiatric disorders. However, the majority of these studies have primarily concentrated on examining the involvement of 5-HT, TRP, KYN metabolites, and IDO expression, while paying limited attention to the intricate interplay of complex enzymes. The investigation of enzymes involved in TRP metabolism necessitates the utilization of molecular, cellular, and tissue methodologies, as they serve as crucial regulators within the TRP pathway.

The current knowledge regarding the involvement of the central nervous system (CNS) and peripheral inflammatory changes in Huntington's disease (HD) remains limited. In the comprehensive review, Jia et al. provide a concise synthesis of current scientific literature about immune and inflammatory changes observed in individuals with HD. This study encompasses investigations conducted on animal models of HD as well as clinical observations of patients diagnosed with HD. The investigation into the specific impact of inflammation on the development of Huntington's disease has promising implications for the development of innovative treatment approaches.

Genomic instability is a key driver of various neurodegenerative disorders and central nervous system malignancies. The comprehensive review by Suptela et al. presents the occurrence of cytosolic DNA sensors in resident central nervous system (CNS) cells and their capacity to actively modulate their reactions to self-DNA. In this study, the author discusses how glial DNA sensors can prevent cancer and neuroinflammation, which are known to cause neurodegenerative diseases. The processes by which glia detect cytosolic DNA and the relative involvement of each pathway at different stages of CNS diseases will help us understand their pathophysiology while developing new treatments.

The growing study has shown chlorogenic acid's anti-inflammatory and immunomodulatory effects. In this seminal research article, Xiong et al. elucidate the molecular mechanisms and specific targets associated with the potential therapeutic effects of chlorogenic acid on neuroinflammation. The present study demonstrates the notable inhibitory impact of Chlorogenic acid on the polarization of microglia towards the M1 phenotype. Furthermore, it amplifies the cognitive impairment induced by neuroinflammation in a murine model.

The Lipopolysaccharide (LPS)-induced depression-like model in mice is a widely employed approach for investigating the underlying mechanisms of depression associated with inflammation as well as evaluating the potential therapeutic benefits of various drugs. Yin et al. conducted a thorough systematic review aimed at identifying appropriate animal models for future investigations on inflammation-related depression and highlighted the significant influence of mouse strains and lipopolysaccharide (LPS) administration on the assessment of behavioral outcomes in relevant experimental models.

Levodopa (L-DOPA) is frequently administered to patients with Parkinson's disease (PD) by healthcare providers in clinical settings to improve their symptoms. Despite long-term administration of L-DOPA, a significant proportion of patients experience various complications, including the "on-off phenomenon," decreased therapeutic efficacy, and levodopa-induced dyskinesia (LID). The review by Zhang et al. examines the effects of widely used anti-inflammatory medicines and NMDA receptor antagonists on central nervous system neuroinflammation and LID progression. This study presents a novel theoretical framework for the identification of potential therapeutic targets for LID.

Finally, in a recent study, the therapeutic potential of Olfactory ensheathing cells (OECs), a specific type of glial cell, has been demonstrated in the treatment of various neurological disorders. OECs have been shown to possess therapeutic potential for the treatment of neurological disorders. In their comprehensive review, Zhang et al. highlight the significance of OECs as a promising therapeutic approach for various nervous system diseases.

Overall, the Research Topics focus on novel and impactful investigations into the mechanisms of neuronal cell death and the activation of microglia. These processes are known to play a crucial role in the development of neuroinflammation and neurodegenerative disorders. The insights gained from this Research Topic will certainly improve our understanding of the consequences of programmed cell death and the molecular mechanisms involved in mitigating neurodegenerative diseases.

Author contributions

CS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Writing – review & editing. AT: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – review & editing. XW: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. RM: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Knowledge Mapping of Necroptosis From 2012 to 2021: A Bibliometric Analysis

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Background: Necroptosis, a recently discovered programmed cell death, has been pathologically linked to various diseases and is thus a promising target for treating diseases. However, a comprehensive and objective report on the current status of entire necroptosis research is lacking. Therefore, this study aims to conduct a bibliometric analysis to quantify and identify the status quo and trending issues of necroptosis research in the last decade.

Methods: Articles were acquired from the Web of Science Core Collection database. We used two bibliometric tools (CiteSpace and VOSviewer) to quantify and identify the individual impact and cooperation information by analyzing annual publications, journals, co-cited journals, countries/regions, institutions, authors, and co-cited authors. Afterwards, we identified the trending research areas of necroptosis by analyzing the co-occurrence and burst of keywords and co-cited references.

Results: From 2012 to 2021, a total of 3,111 research articles on necroptosis were published in 786 academic journals by 19,687 authors in 885 institutions from 82 countries/regions. The majority of publications were from China and the United States, of which the United States maintained the dominant position in necroptosis research; meanwhile, the Chinese Academy of Sciences and Ghent University were the most active institutions. Peter Vandenabeele published the most papers, while Alexei Degterev had the most co-citations. *Cell Death & Disease* published the most papers on necroptosis, while *Cell* was the top 1 co-cited journal, and the major area of these publications was molecular, biology, and immunology. High-frequency keywords mainly included those that are molecularly related (MLKL, TNF-alpha, NF-κB, RIPK3, RIPK1), pathological process related (cell-death, apoptosis, necroptosis, necrosis, inflammation), and disease related (cancer, ischemia/reperfusion injury, infection, carcinoma, Alzheimer's disease).

Conclusion: Necroptosis research had a stable stepwise growth in the past decade. Current necroptosis studies focused on its cross-talk with other types of cell death, potential applications in disease treatment, and further mechanisms. Among them, the

synergy with ferroptosis, further RIPK1/RIPK3/MLKL studies, its association with inflammation and oxidative stress and translational applications, and the therapeutic potential to treat cancer and neurodegenerative diseases are the trending research area. These might provide ideas for further research in the necroptosis field.

Keywords: necroptosis, programmed cell death, CiteSpace, VOSviewer, bibliometric

1 INTRODUCTION

Necroptosis is a form of programmed cell death (PCD) mediated by receptor interacting protein kinase-3 (RIPK3) and its substrate mixed lineage kinase domain-like protein (MLKL) (1). It was first observed in 1988 as a tumor necrosis factor (TNF)–induced new necrotic cell death, with the "classical" features of apoptosis but a "balloon-like" morphology without unclear disintegration (2). In 2005, Junying Yuan and colleagues proposed the term "necroptosis" and identified a specific small-molecule inhibitor of necroptosis, necrostatin-1, which blocks a critical step in necroptosis (3). Necroptosis is morphologically similar to necrosis but rigorously regulated by intracellular signaling cascades. Apoptosis and necroptosis are both PCD; compared to the former, necroptosis is an inflammatory process characterized by swollen organelles, membrane pores, and the eventual rupture of the plasma membrane and organelles (4). The initiation of necroptosis is usually triggered by the activation of death receptors (e.g., TNF receptor), which subsequently recruit receptor interacting protein kinase-1 (RIPK1). The elimination or inhibition of caspase-8 enables the activation of RIPK1 and recruitment of RIPK3. RIPK3 then recruits and phosphorylates MLKL, and phosphorylated MLKL oligomerizes and moves to the plasma membrane and forms a pore, leading to the membrane rupture (1, 2). Necroptosis has been pathologically related to various human diseases, such as cancers (5–7), Alzheimer's disease (AD) (8), Parkinson's disease (PD) (9), multiple sclerosis (10), stroke (11), infection (12), inflammatory bowel disease (13), pancreatitis (14), and atherosclerosis (15). Consequently, necroptosis is considered to be a promising target for many diseases.

According to its great potential, necroptosis has gained researchers' keen interest with a rapidly increasing number of publications. Many reviews have summarized necroptosis studies from various aspects (7, 16–20), including a bibliometric analysis (21); however, this bibliometric study only focused on neuroscience and included both research articles and reviews. Therefore, to the best of our knowledge, there is no report on the whole picture of necroptosis research.

Although a quantitative overview could be conducted through many approaches, such as traditional review, systematic review, main path analysis, evidence map, and bibliometrics (22), only bibliometrics could qualitatively and quantitatively analyze the contribution and cooperation of authors, institutions, countries, and journals and evaluate the knowledge base and trending research topics at the same time (23, 24).

Therefore, the present study aims to use two bibliometric software, CiteSpace and VOSviewer, to quantify the whole picture of necroptosis research and identify trending research questions in the last decade, which may help to generate hypotheses for future studies in the necroptosis field.

2 MATERIALS AND METHODS

2.1 Data Collection

The Web of Science Core Collection (WoSCC) database is widely used in bibliometrics, which contains Science Citation Index Expanded (SCIE), Social Science Citation Index (SSCI), and Emerging Sources Citation Index (ESCI) (21, 24). Data were obtained from the WoSCC database on March 24, 2022. The search formula was [TS = ("necroptosis" OR "necroptotic")] AND [Publication type = (Article)] AND [Language = (English)], and the publication year was limited to (2012-2021). Search results were downloaded as "Full Record and Cited References" and "Plain Text". For further analysis, we subsequently renamed the files as "download_*.txt", which CiteSpace software could read.

2.2 Data Analysis

We used CiteSpace 5.8.R3 (Chaomei Chen, 2006), VOSviewer 1.6.16 (Nees Jan van Eck and Ludo Waltman, 2010), and Microsoft Excel 2019 to perform bibliometric analysis and visualization. Data cleaning was the first step, for instance, "tumor cell" and "tumor-cells" were merged as "tumor cells", "reactive oxygen" and "ros" were unified as "ros", and meaningless terms such as "age" and "assay" were deleted (25).

CiteSpace is a bibliometric and visual analysis tool that excels at detecting cooperation, key points, internal structure, potential trends, and dynamics in a scientist field (26). Therefore, we used CiteSpace to analyze the co-occurrence of countries/regions and institutions, the dual-map of journals, reference timeline, citation bursts, keyword timeline, and keyword bursts. The settings were as follows: timespan (2012–2021), years per slice (1), pruning (none), selection criteria (Top N=100), minimum duration of burstness (2 years), cluster labels were extracted by light semantic indexing (LSI) and the log-likelihood ratio (LLR) algorithm, and others followed the default. In CiteSpace visualization, the size of node reflects the co-occurrence frequencies, and the links indicate the co-occurrence relationships. The colors of the node and line represent different years; the colors vary from purple to red as time goes from 2012 to 2021. Nodes with purple round mean a high betweenness centrality (≥0.10), which acts as a bridge between different networks (26–28).

VOSviewer is another bibliometric software that is good at creating and visualizing knowledge maps, showing the types of

clusters, overlays, or density colors (29, 30). It was used to perform the co-occurrence of authors and co-cited authors, journals and co-cited journals, co-cited references, and keywords. We set the counting method as full counting; other thresholds were shown in the corresponding chapter. In the cluster map, the size of node reflects the co-occurrence frequencies, and the same color represents the same cluster; furthermore, the link indicates the co-occurrence relationship, and the thickness of the link depends on a calculated strength value, which is proportional to the number of publications two researchers co-authored or the number of publications in which two keywords occur together (30). In density maps, the size of word and round and the opacity of yellow are positively related to the co-cited frequency. In the overlay map, the color indicates the average published year.

We used Excel software to analyze the annual publications. Furthermore, the impact factor (IF) and Journal citation reports (JCR) division of journals and the H-index of scholars were obtained from the Web of Science on April 5, 2022.

3 RESULTS

3.1 Annual Growth Trend

We obtained 3,194 papers from the WoSCC database and finally included 3,111 eligible articles (**Figure 1**; **Supplementary Table 1**). As shown in **Figure 2**, the number of necroptosis-related articles has steadily increased over the past decade.

3.2 Distribution of Countries/Regions and Institutions

A total of 3,111 papers were from 82 different countries/regions and 885 institutions (**Table 1**). China published the most articles (n=1,213), followed by the United States (n=955) and Germany (n=318). However, the centrality of China was less than 0.10, which means China might not be a "bridge" node in necroptosis studies. By contrast, Germany (centrality=0.41), the United States (centrality=0.23), and France (centrality=0.14) had high centrality, which is presented with a purple circle in **Figure 3A**. The density of country/region co-occurrence (**Figure 3B**) was 0.14, indicating active cooperation among them. Chinese Academy of Sciences is the most productive institution, but its centrality is relatively low (n=86, centrality=0.07). By contrast, Harvard Medical School (n=70, centrality=0.14), Ghent University (n=77, centrality=0.12), and St. Jude Children's Research Hospital (n=55, centrality=0.10) had a high centrality.

3.3 Authors and Co-Cited Authors

A total of 19,687 authors were involved in necroptosis research; among them, 91 authors published at least ten papers (**Figure 4A**; **Supplementary Table 2**). Peter Vandenabeele from the University of Ghent published the highest number of necroptosis-related

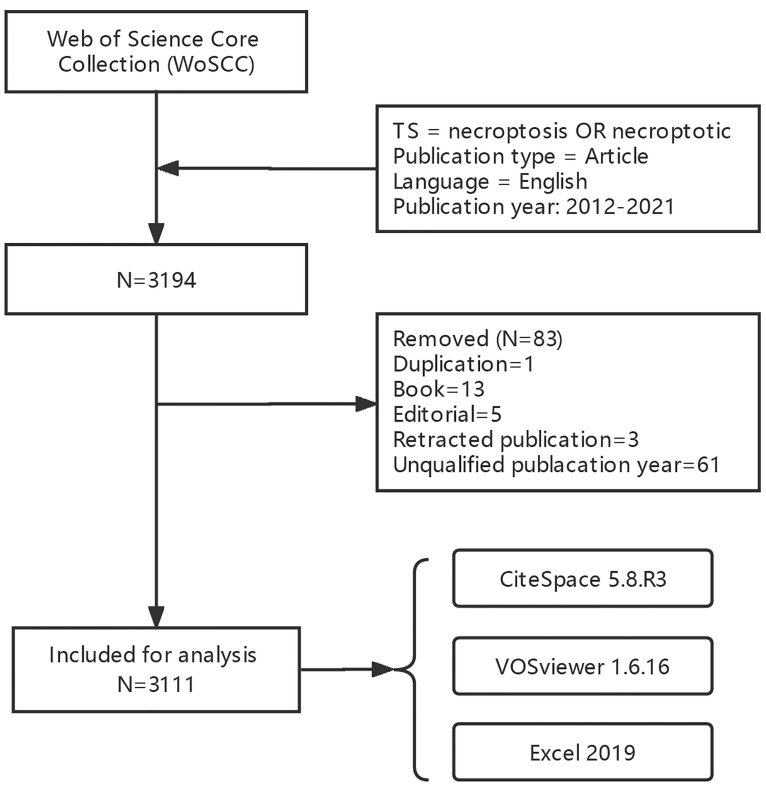

FIGURE 1 | Flow chart of data collection.

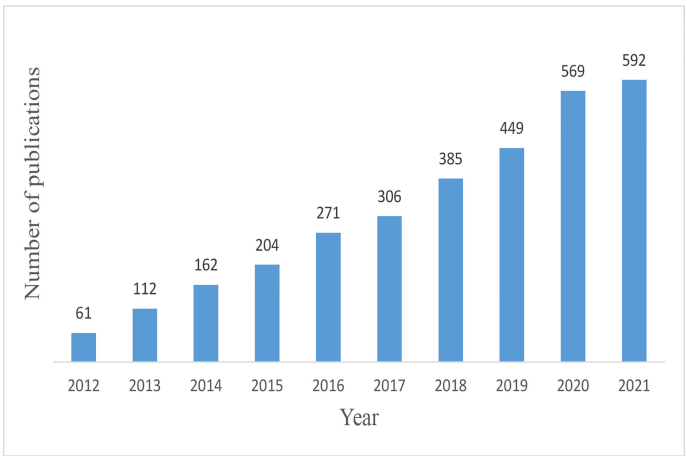

FIGURE 2 | Trends of necroptosis publications over the past decade.

articles (n=45), followed by John Bertin and James M. Murphy (**Table 2**). There were fifteen colors in **Figure 4A**, representing 15 clusters among authors. Active collaborations usually exist in the same cluster, such as Bertin John and Gough Peter J. There were also collaborations among linked two nodes in different clusters, such as John Bertin and Peter Vandenabeele.

Co-cited authors are the authors who were cited in one article (31). Among 61,882 co-cited authors, 99 had over 100 co-citations (**Figure 4B**; **Supplementary Table 2**). **Figure 4B** presented them as a density map, which could clearly show the high-frequency co-cited authors. The more citations, the warmer the color. As shown in **Table 2** and **Figure 4B**, Alexei Degterev, Sudan He, and Kim Newton had the most co-cited.

Given the inherent limitations of the CiteSpace and VOSviewer visualization, the pictures cannot show all the information. Therefore, we placed the complete data in **Supplementary Table 2**, as well as the figures below.

3.4 Journals and Co-Cited Academic Journals

A total of 786 academic journals published articles on necroptosis research. The top 15 journals published 821 papers, accounting for 26.39% of all publications (**Table 3**). *Cell Death & Disease* had the greatest number of publications (n=164), followed by *Cell Death and Differentiation* (n=87) and *Scientific Reports* (n=84).

Among 6,555 co-cited sources, 49 journals had >500 citations; among which, *Cell* (n=6,414), *Nature* (n=5,083), and *Proceedings of the National Academy of Sciences of the United States of America* (*PNAS*) (n=4,210) had the greatest number of citations. Furthermore, the top 15 co-cited journals accounted for 28.20% citation of all cited sources (**Table 3**).

The dual-map overlay of journals stands for the topic distribution of academic journals (32) (**Figure 5**). Citing journals are on the left, cited journals are on the right, and colored paths indicate citation relationships. **Figure 5** showed there was only one primary citation path from Molecular/Biology/Genetics journals to Molecular/Biology/Immunology journals.

3.5 Co-Cited References and Reference Burst

Of the 89,431 cited references, 71 were cited at least 100 times (**Supplementary Table 3**). **Table 4** showed that the top 10 co-cited references were co-cited at least 348 times. Among them, the most co-cited reference is an article published in *Cell* by Liming Sun et al. in 2011 (n=714). Furthermore, seven of the top 10 were research articles, two were reviews, and one was a report.

TABLE 1 | Top 10 countries/regions and institutions related to necroptosis.

Rank	Countries/Regions	Centrality	Count	Institution	Centrality	Count
1	China	0.06	1213	Chinese Acad Sci (China)	0.07	86
2	United States	0.23	955	Univ Ghent (Belgium)	0.12	77
3	Germany	0.41	318	Univ Melbourne (Australia)	0.07	74
4	Japan	0.07	195	Harvard Med Sch (United States)	0.14	70
5	South Korea	0.09	172	Fudan Univ (China)	0.07	67
6	England	0.09	130	Shanghai Jiao Tong Univ (China)	0.06	65
7	Australia	0.08	116	Zhejiang Univ (China)	0.08	62
8	Canada	0.05	116	Walter & Eliza Hall Inst Med Res (Australia)	0.04	60
9	Belgium	0.08	97	Sun Yat Sen Univ (China)	0.06	58
10	France	0.14	94	St Jude Childrens Res Hosp (USA)	0.10	55

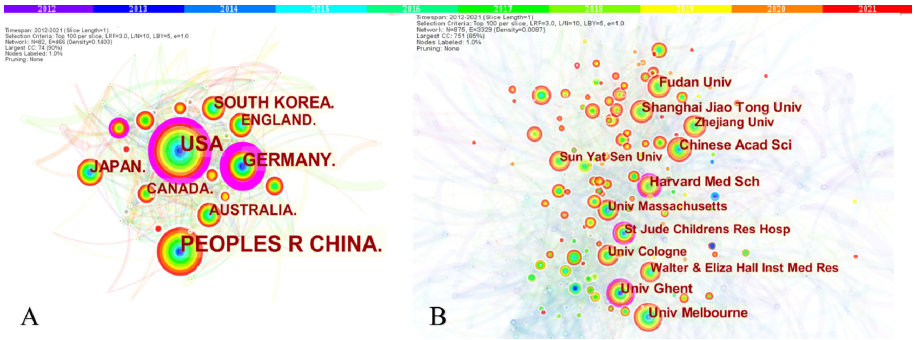

FIGURE 3 | The co-occurrence map of countries/regions **(A)** and institutions **(B)** in necroptosis research. **(A)** Country/regions (n≥100); **(B)** Institution (n≥50). The node size reflects the co-occurrence frequencies, and the links indicate the co-occurrence relationships. The color of node and line represent different years; colors vary from purple to red as time goes from 2012 to 2021, and nodes with purple round mean high betweenness centrality (≥0.1).

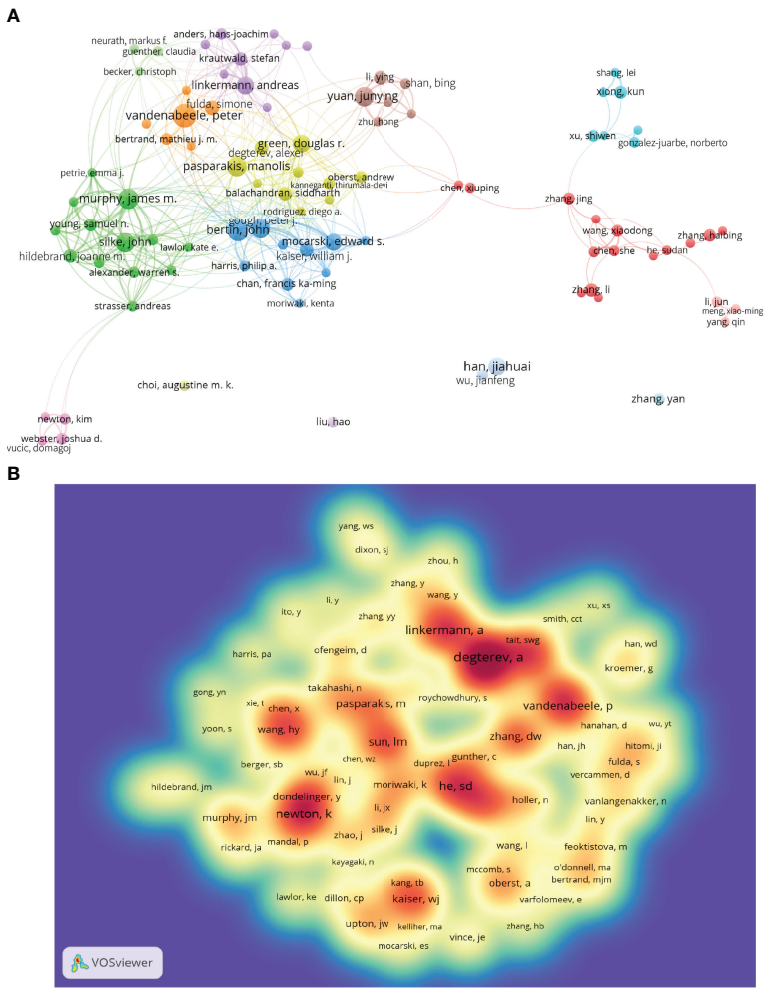

FIGURE 4 | The co-occurrence authors' **(A)** and co-cited authors' **(B)** map of necroptosis research. **(A)** Authors with documents ≥10 (cluster map). The size of node reflects the authors' co-occurrence frequencies, the link indicates the co-occurrence relationship between authors, the thickness of the link is proportional to the number of publications two researchers have co-authored, and the same color of node represents the same cluster. **(B)** Co-cited authors with citations ≥100 (density map). The size of word, the size of round, and the opacity of yellow are positively related to the co-cited frequency.

TABLE 2 | Top 10 authors and co-cited authors related to necroptosis.

Rank	Author	Count	H-index	Co-cited author	Count	H-index
1	Peter Vandenabeele	45	118	Alexei Degterev	1363	40
2	John Bertin	37	66	Sudan He	866	20
3	James M. Murphy	36	18	Kim Newton	865	39
4	Manolis Pasparakis	34	76	Andreas Linkermann	843	47
5	Junying Yuan	33	63	Galluzzi L	765	107
6	Peter J. Gough	32	38	Liming Sun	752	14
7	John Silke	32	72	Peter Vandenabeele	742	118
8	Douglas R. Green	29	23	Young-Sik Cho	705	19
9	Andreas Linkermann	29	47	William J. Kaiser	616	51
10	Edward S. Mocarski	27	82	Duanwu Zhang	597	11

TABLE 3 | Top 15 journals and co-cited journals related to necroptosis.

Rank	Journal	Count	JCR (2020)	IF (2020)	Cited journal	Cited count	JCR (2020)	IF (2020)
1	Cell Death & Disease	164	Q1	8.469	Cell	6414	Q1	41.584
2	Cell death and Differentiation	87	Q1	15.828	Nature	5083	Q1	49.962
3	Scientific Reports	84	Q1	4.380	Proceedings of the national academy of sciences of the United States of America	4210	Q1	11.205
4	Proceedings of the National Academy of Sciences of the United States of America	55	Q1	11.205	Cell Death and Differentiation	4183	Q1	15.828
5	Plos One	51	Q2	3.240	Journal of Biological Chemistry	3941	Q2	5.157
6	Nature Communications	47	Q1	14.919	Science	2791	Q1	47.728
7	Cell Reports	46	Q1	9.423	Immunity	2472	Q1	31.745
8	International Journal of Molecular Sciences	45	Q1/Q2	5.924	Cell Death & Disease	2410	Q1	8.469
9	Biochemical and biophysical research communications	40	Q2/Q3	3.575	Molecular Cell	2299	Q1	17.970
10	Journal of Immunology	40	Q2	5.422	Plos One	2205	Q2	3.240
11	Oncotarget	40	/	/	Journal of Immunology	2148	Q2	5.422
12	Cell death Discovery	33	Q2	5.241	Nature Reviews Molecular Cell Biology	1812	Q1	94.444
13	Journal of Biological Chemistry	33	Q2	5.157	Nature Communications	1496	Q1	14.919
14	Frontiers in Immunology	30	Q1	7.561	Nature Chemical Biology	1396	Q1	15.040
15	Molecular Medicine Reports	26	Q3/Q4	2.952	Cell Reports	1335	Q1	9.423

The references timeline view could visualize the evolution of research hotspots over time. The terms with the highest frequency in each cluster were tagged as cluster labels, and the rest were listed in **Supplementary Table 2**. As shown in **Figure 6**, cluster #0 (necroptosis/MLKL), #1 (apoptosis/TNF-α), #3 (RIPK3/necrostatin-1), #5 (inducing factor/astrocytes), and #6 (RIP1/AIF) started earlier; while cluster #2 (RIPK1/ZBP1) and #4 (lymphocytes/ferroptosis) are still ongoing, which could be considered as the frontier.

References with citation bursts are those that have been cited significantly more frequently over a period (28). A total of 243 references were detected as citation bursts, and we listed the top 20 in **Figure 7**. The strongest burstness (strength=79.48) occurred in a paper entitled "Molecular mechanisms of

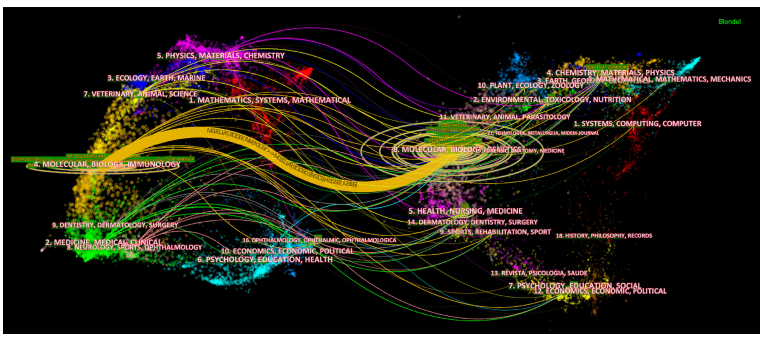

FIGURE 5 | The dual-map overlay of journals on necroptosis. The citing journals are on the left, the cited journals are on the right, and the colored path represents the citation relationship.

TABLE 4 | Top 10 co-cited references related to necroptosis.

	Title	First Author	Journals	Citations	Type	Year
1	Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase (33)	Liming Sun	Cell	714	Article	2011
2	Phosphorylation-driven assembly of the RIP1–RIP3 complex regulates programmed necrosis and virus-induced inflammation (34)	Young Sik Cho	Cell	657	Article	2009
3	Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury (3)	Alexei Degterev	Nat Chem Biol	652	Article	2005
4	Receptor Interacting Protein Kinase-3 Determines Cellular Necrotic Response to TNF-a (35)	Sudan He	Cell	647	Article	2009
5	Molecular mechanisms of necroptosis: an ordered cellular explosion (36)	Peter Vandenabeele	Nat Rev Mol Cell Biol	542	Review	2010
6	RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis (37)	Duan-Wu Zhang	Science	539	Report	2009
7	Identification of RIP1 kinase as a specific cellular target of necrostatins (38)	Alexei Degterev	Nat Chem Biol	520	Article	2008
8	Necroptosis and its role in inflammation (17)	Manolis Pasparakis	Nature	463	Review	2015
9	Mixed lineage kinase domain–like protein MLKL causes necrotic membrane disruption upon phosphorylation by RIP3 (39)	Huayi Wang	Mol Cell	438	Article	2014
10	Plasma membrane translocation of trimerized MLKL protein is required for TNF-induced necroptosis (40)	Zhenyu Cai	Nat Cell Biol	348	Article	2014

necroptosis: an ordered cellular explosion" (36), published in *Nat Rev Mol Cell Biol* by Vandenabeele et al. in 2010, with citation burstness from 2012 to 2015. Notably, four references (16–18, 41) were still in burstness. Respectively, Pasparakis et al. (17) reviewed necroptosis and its role in inflammation; Weinlich et al. reviewed necroptosis in development, inflammation, and disease (18); the Nomenclature Committee on Cell Death prompted a recommendation on the molecular mechanisms of cell death (41); Galluzzi et al. (16) reviewed the mechanisms of necroptosis and its relevance to disease.

3.6 Keyword Analysis of Trending Research Topic

A total of 5,639 keywords were extracted, among which 297 keywords appeared at least ten times and 62 keywords appeared at least 50 times. As we can see from **Table 5**, cell death was the most frequent keyword (n=1,211), followed by apoptosis (n=1,104) and necroptosis (n=885). **Table 6** showed the top 10 keywords of molecules, pathological processes, and diseases related to necroptosis. It could be seen that MLKL (n=521), TNF-alpha (n=332), NF-κB (n=315), RIPK3 (n=233), and RIPK1 (n=155) were the most studied molecules; cell-death (n=1211), apoptosis (n=1,104), necroptosis (n=885), necrosis (n=557), inflammation (n=443), and oxidative stress (n=273) were the most mentioned pathological process; and cancer (n=170), ischemia/reperfusion injury (n=110), infection (n=50), carcinoma (n=32), and Alzheimer's disease (n=31) were the most studied diseases in necroptosis studies.

Figure 8A showed the high-frequency keywords (n≥50) as an overlay map, where the color indicated the average published year. As we can see, inflammation, oxidative stress, phosphorylation, and protection are emerging fields that were colored yellow. The timeline view (**Figure 8B**) presented the top 3 (if any) high-frequency keywords in each cluster over time. We could see that seven of the eight clusters (except #6) are still ongoing. Among them, #0 (reperfusion injury/rat model) is the biggest cluster, followed by #1 (cell death/anticancer effect), #2 (necroptotic cell death/molecular switch), and #3 (oxidative stress/smoke-induced necroptosis). More information was listed in **Supplementary Table 2**.

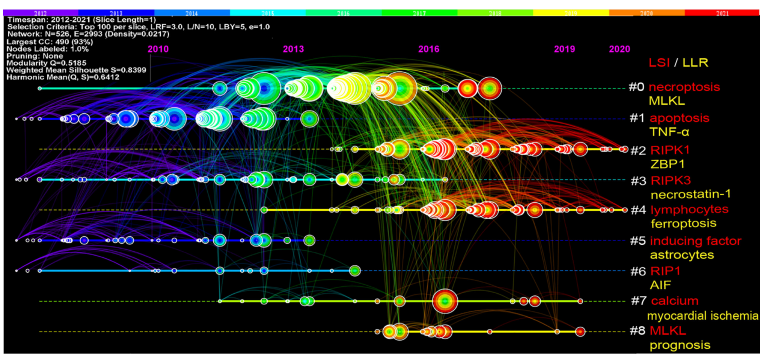

FIGURE 6 | Timeline view of co-cited references related to necroptosis. Each horizontal line represents a cluster; the smaller the number, the larger the cluster, and #0 is the largest cluster. The node size reflects the co-cited frequencies, and the links indicate the co-cited relationships; the color of node and line represent different years; nodes are at their first co-cited year. Cluster labels were extracted from title by LSI (red) and LLR (yellow).

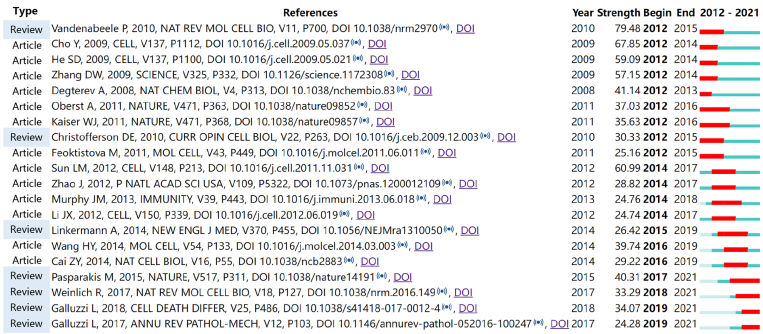

FIGURE 7 | Top 20 references with the strongest citation bursts involved in necroptosis (sorted by the starting year). The Blue bars mean the reference had been published; the red bars represent citation burstness.

Keyword bursts are those that were cited significantly frequently over a period (28). As shown in **Figure 9**, receptor interacting protein had the strongest bursts (strength=9.96), followed by TNF (strength=9.42) and l929 cell (strength=8.06). Notably, rat, ferroptosis, and protect were in burstness until 2021.

4 DISCUSSION

4.1 General Information

Based on the data from the WoSCC database from 2012 to 2021, a total of 3,111 necroptosis articles were published in 786

TABLE 5 | Top 20 keywords related to necroptosis.

Rank	Keywords	Counts	Rank	Keywords	Counts
1	cell-death	1,211	11	TNF-alpha	358
2	apoptosis	1,104	12	NF-κB	315
3	necroptosis	885	13	oxidative stress	273
4	activation	711	14	kinase	259
5	necrosis	557	15	phosphorylation	254
6	MLKL	521	16	inhibition	238
7	programmed necrosis	467	17	RIPK3	233
8	inflammation	443	18	autophagy	204
9	mechanism	393	19	pathway	194
10	expression	391	20	cancer	170

MLKL, mixed lineage kinase domain-like protein; TNF-alpha, tumor necrosis factor-alpha; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells; RIPK3, receptor-interacting protein kinase-3.

TABLE 6 | Top 15 molecules, pathological process and disease related to necroptosis.

Rank	Molecules	Counts	Pathological process	Counts	Diseases	Counts
1	MLKL	521	Cell-death	1,211	Cancer	170
2	TNF-alpha	358	Apoptosis	1,104	Ischemia/reperfusion injury	110
3	NF-κB	315	Necroptosis	885	Infection	50
4	RIPK3	233	Necrosis	557	Carcinoma	32
5	RIPK1	155	Inflammation	443	Alzheimer's disease	31
6	Complex	89	Oxidative stress	273	Brain injury	30
7	Caspase-8	82	Phosphorylation	254	Fibrosis	26
8	NLRP3	65	Autophagy	204	Stroke	26
9	ROS	50	Proliferation	70	Acute kidney injury	25
10	FADD	38	ER stress	65	Breast cancer	25
11	Necrostatin-1	37	Ubiquitination	45	Tumor	23
12	Inflammasome	34	DNA damage	40	Drug resistance	22
13	p53	33	Pyroptosis	26	Hepatocellular carcinoma	22
14	bcl-2	32	Ferroptosis	25	Inflammatory bowel disease	20
15	Toll-like receptors	19	Mitophagy	19	Parkinson's disease	19

MLKL, mixed lineage kinase domain-like protein; TNF-alpha, tumor necrosis factor-alpha; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells; RIPK3, receptor-interacting protein kinase-3; RIPK1, receptor-interacting protein kinase-1; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; ROS, reactive oxygen species; FADD, Fas-associated via death domain; er stress, endoplasmic reticulum stress; bcl-2, B-cell lymphoma-2.

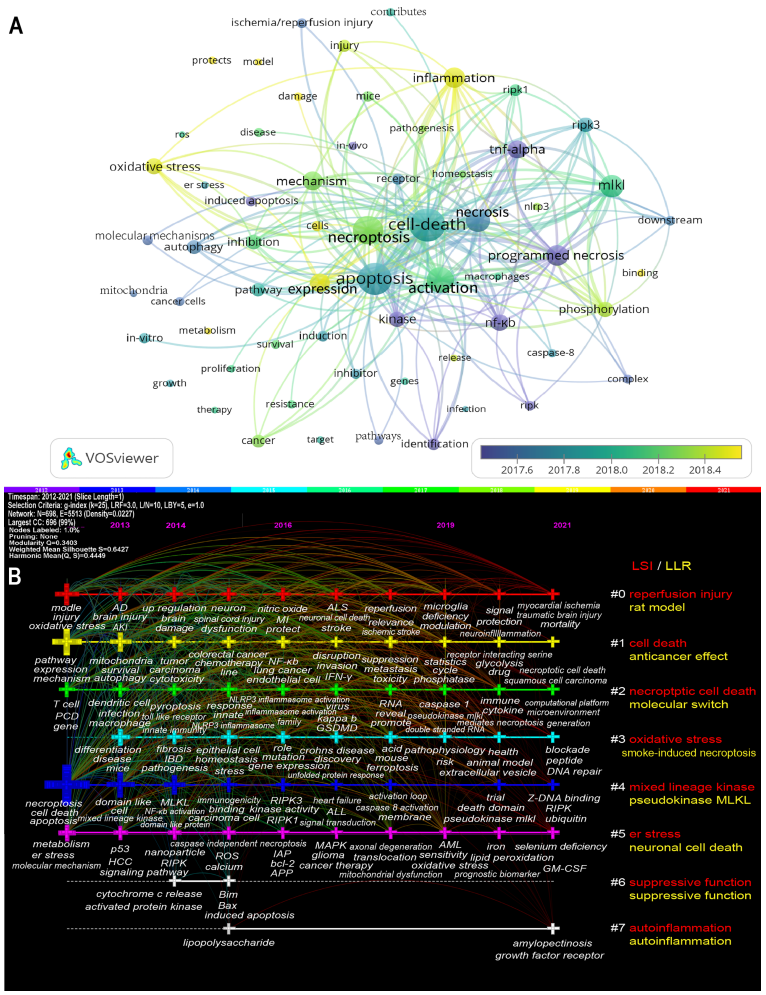

FIGURE 8 | The overlay map **(A)** and timeline view **(B)** of keywords related to necroptosis. **(A)** Keywords appeared ≥50, max lines = 200. The node size reflects the co-occurrence frequencies, the link indicates the co-occurrence relationship, the thickness of the link is proportional to the number of times two keywords co-occur, and the color indicates the average published year. **(B)** Each horizontal line represents a cluster; the smaller the number, the larger the cluster, and #0 is the largest. The time is at the top, and keywords are located at their first co-occurrence time in the cluster. Cluster labels were extracted from title and abstract information by LSI (red) and LLR (yellow). LSI, light semantic indexing; LLR, log-likelihood ratio; AD, Alzheimer's disease; AKI, acute kidney injury; ALL, acute lymphoblastic leukemia; ALS, amyotrophic lateral sclerosis; AML, Acute Myelocytic leukemia; APP, amyloid precursor protein; bcl-2, B-cell lymphoma-2; er stress, endoplasmic reticulum stress; GM-CSF, granulocyte/macrophage colony-stimulating factor; GSDMD, gasdermin-D; HCC, hepatocellular carcinoma; IAP, inhibitors of apoptosis; IBD, inflammatory bowel disease; INF-γ, interferon-γ; MI, myocardial infarction; MLKL, mixed lineage kinase domain-like protein; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; PCD, programmed cell death; RIPK, receptor-interacting protein kinase; RIPK1, receptor-interacting protein kinase-1; RIPK3, receptor-interacting protein kinase-3; ROS, reactive oxygen species.

academic journals by 19,687 authors in 885 institutions from 82 countries/regions.

The increasing trend of publications indicated that necroptosis is attracting mounting attention and interest. The necroptosis research officially started in 2005, when Junying Yuan proposed the term "necroptosis" (3). Since then, necroptosis research has grown rapidly. In the past decade, necroptosis research had stable stepwise growth, and the published article in 2021 is almost ten times that of 2012.

In country/region analysis, the number of publications and betweenness centrality are two vital indicators, in which high centrality (≥0.10) nodes imply the "bridge" effects of those countries/regions in the global cooperation network (26, 27, 42, 43). According to **Table 1** and **Figure 3**, China and the United States contributed the most to publications on necroptosis. Of the top 10 institutions that published the most research items, five were in China, two in the United States, two in Australia, and one in Belgium (1/10). However, the centrality of China and Chinese institutions was less than 0.1 while that of the United States was 0.23, indicating that the United States might maintain the dominant position in necroptosis research. Moreover, Germany, the United States, and France had high centrality, which means they played key roles in the global cooperation of necroptosis research. Furthermore, in terms of

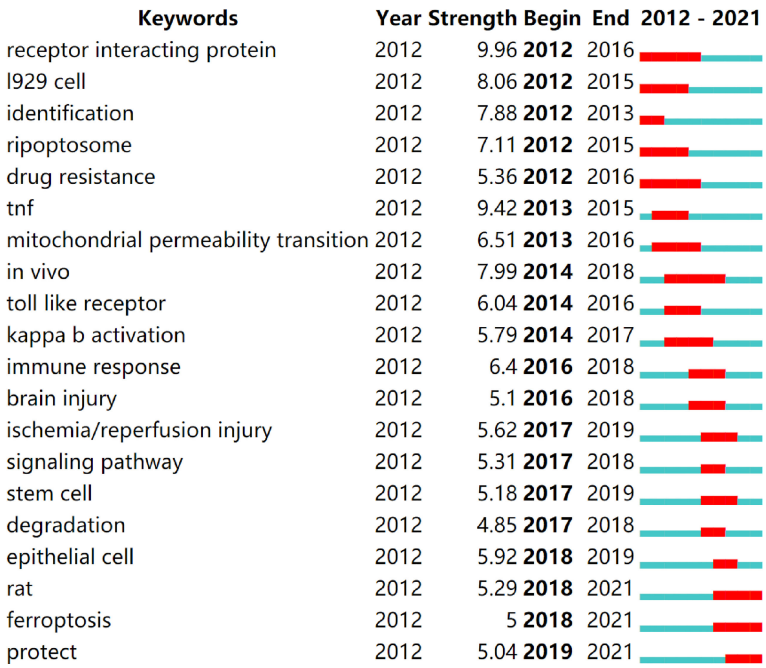

FIGURE 9 | Top 20 keywords with the strongest citation bursts (sorted by the starting year). The red bars mean citation burstness.

network density, there was active cooperation among countries and institutions.

Among the top 10 authors and co-cited authors (**Table 2**), Peter Vandenabeele not only published the greatest number of necroptosis-related papers but is also among the top seven co-cited authors, indicating his outstanding contribution to necroptosis research. Vandenabeele is a professor at Ghent University, focusing on cell death, immunology, and inflammation. In 2010, his group published a review (36) that described the molecular mechanisms of necroptosis in detail and discussed its immunological outcomes and pathophysiological implications, which was co-cited up to 542 times and had the strongest citation bursts in this study. Among both top 10 authors and co-cited authors, Andreas Linkermann is also a professor working in Technische Universitat Dresden; his laboratory is interested in multiple regulated cell deaths in kidney transplantation, acute kidney injury, antibody-mediated rejection, and ischemia-reperfusion injury. In 2014, the *New England Journal of Medicine* published a Linkermann and Green's review entitled Necroptosis (1). They summed the regulatory mechanism and pathophysiology of necroptosis and pointed out the therapeutic strategies for preventing necroptotic diseases; this reference had a strong burst from 2015 to 2019. Notably, the most co-cited author, Alexei Degterev, an associate professor at Tufts University, recently centered on analyzing the roles of RIPK1/RIPK3 in necroptosis and inflammation. He published two top 10 co-cited articles that proposed the concept "necroptosis" (3) and identified the RIP1 kinase as a specific cellular target of necrostatins distinct from necrostatin-1 (38), respectively.

In a journal analysis (**Table 3**), *Cell Death & Disease* published the most necroptosis studies, and it was also the eighth most cited journal. *Cell death and differentiation* and *PNAS* were both the top 5 publication journals and top 5 co-cited journals, indicating their essential role in disseminating necroptosis research. *Cell* received the most co-citations, partly because three of the top four highly cited papers (33–35) were published in *Cell* (**Table 4**), followed by *Nature* and *PNAS*. The journals were mainly in the cell biology, immunology, and comprehensive fields. This is consistent with the dual-map analysis (**Figure 5**), which showed that the main citation path in necroptosis research is related to molecular, biology, and immunology.

The collection of co-cited references cited by the corresponding research community could partly represent the knowledge base (28, 44–46). Among the top 10 co-cited references, six mainly elaborated RIPK1 or RIPK3 (33–35, 37–39), three are about the key role of MLKL (33, 39, 40), two are related to the necroptosis in inflammation (17, 34), one prompted the term "necroptosis" and regarded it as a therapeutic potential for ischemic brain injury (3), and one reviewed the molecular mechanisms of necroptosis (36). As for reference burst analysis, four references are still in burst and worth our attention: three are about necroptosis-related diseases and inflammation (16–18), and one is a recommendation on the molecular mechanisms of cell death (41).

4.2 The Hotspots and Trending

In such an era of information explosion, keeping abreast of the trending research area in the research field is critical to researchers.

Bibliometrics provides a method in which keyword co-occurrence can reflect the hotspots of an academic area (47), the overlay and timeline view can present the evolution of new hotspots (21, 48), and reference clusters and citation bursts can characterize the emerging topics in the discipline (23, 28, 42). In this study, we tried to objectively evaluate the hotspots and frontiers of necroptosis research through the analysis of keyword co-occurrence (**Table 5** and **Table 6**), keyword overlay and timeline (**Figure 8**), keyword burst (**Figure 9**), reference timeline (**Figure 6**), and reference burst (**Figure 7**). We summarized three aspects as follows.

4.2.1 The Cross-Talk of Necroptosis With Other Types of Cell Death

PCD is a form of cell death that results from the activation of signal transduction modules, including apoptosis, necroptosis, pyroptosis, and ferroptosis, and hence can be pharmacologically or genetically modulated (41). Over the last two decades, numerous studies have highlighted the cross-talk between multiple types of PCD rather than linear pathways with defined immunological outputs (49, 50).

The pathways of necroptosis and apoptosis are tightly linked through caspase-8 (51, 52), which not only is a typical activator of extrinsic apoptosis but also inhibits necroptosis through the cleavage of RIPK1 and possibly RIPK3 (53, 54). That is, the cell commits three fates when TNF engages its receptor and forms Complex I, which consists of TNFR1-associated death domain protein (TRADD), TNFR-associated factors (TRAF), RIPK1, the cellular inhibitor of apoptosis protein 1 (cIAP1), and the cellular inhibitor of apoptosis protein 2 (cIAP2) (55). If RIPK1 is ubiquitinated, cell death is aborted; if caspase-8 activity is inhibited, necroptosis occurs; otherwise, the cells undergo apoptosis (56).

Necroptosis and pyroptosis are both regulated necrosis, among which pyroptosis can be initiated by the NLRP3 inflammasome when it suffers changes in cellular ion homeostasis, while this property also allows its activation in response to membrane disruption caused by MLKL, the terminal effector of necroptosis (57, 58).

Ferroptosis is an iron-dependent PCD caused by unrestricted lipid peroxidation and subsequent membrane damage (59). In this study, the keyword ferroptosis is not only in keyword co-occurrence; especially, it is also among the top 20 keywords with the strongest citation bursts and still on bursts and is the cluster label (#4) of the reference timeline (**Figure 6**) in which the cluster remains ongoing. That means ferroptosis still occurs frequently in recent necroptosis studies, indicating that the cross-talk between necroptosis and ferroptosis might be a new rising research area. Multiple structural, functional, and mechanistic evidence proved their cross-talk (60). One of the mechanisms of ferroptosis is that iron overload leads to a mitochondrial permeability transition pore (MPTP) opening, which exacerbates RIPK1 phosphorylation and contributes to necroptosis (61, 62). Moreover, heat shock protein 90 (HSP90) facilitates necroptosis and ferroptosis by promoting RIPK1 phosphorylation and inhibiting glutathione peroxidase 4 (GPX4) activation (63, 64). The cross-talk between necroptosis and ferroptosis has drawn attention in the areas of ischemic stroke (60), neuronal death after hemorrhagic stroke (65), kidney disease (66), pulmonary disease (67), and so on, which might have huge prospects.

Autophagy is also frequently mentioned in necroptosis research. Necroptosis and autophagy could cross-talk through RIPK1. When RIPK1 activates ERK, ERK negatively regulates transcription factor EB (TFEB) and, subsequently, basal autophagy through phosphorylation at the serine 142 site; if RIPK1 forms DISC and caspase-8 is activated, it leads to necroptosis (68).

Recently, a review discussed the emerging connectivity of PCD pathways and its physiological implications (50). The authors pointed out that the various types of PCD might be a single, coordinated cell death system in which the individual pathways are highly interconnected and can flexibly compensate for one another. Similar to our findings, the cross-talk between necroptosis and other cell death modalities and the synergy in disease might be the trending research area in the field of necroptosis.

4.2.2 Potential Applications of Necroptosis in Various Diseases

According to our results (**Table 6**, **Figures 6**–**9**), necroptosis-related disease is a hot area in necroptosis research, including cancers (cancer, tumor, carcinoma, breast cancer, hepatocellular carcinoma, leukemia, drug resistance), neurological diseases (AD, brain injury, stroke, PD, ischemia/reperfusion injury), acute kidney injury, other ischemia/reperfusion injuries, infection, autoinflammatory disease, and myocardial ischemia. We discussed the first two fields below: cancers and neurological diseases.

The role of necroptosis in cancer is still controversial (6, 69). On the one hand, the dysregulation of necroptosis regulatory molecules such as MLKL and RIPK3 is associated with cancer development (70–72), and the activation of RIPK1/RIPK3 may potentiate antitumor immunity (73) and reverses drug resistance (70). On the other hand, there is also evidence that necroptosis may promote carcinogenesis by inducing adaptive immunosuppression; for instance, RIPK1 is overexpressed in glioblastoma, lung cancer, and pancreatic ductal adenocarcinoma (PDAC) (74, 75), and RIPK3 and MLKL are highly expressed in PDAC (75, 76). Among the clinically approved drugs, 5-FU (77, 78) and shikonin (79, 80) might enhance antitumor immunity through necroptosis induction. Overall, although the molecular mechanism of necroptosis has been studied well, its application and regulation in cancer only began to emerge and need further investigation (20).

Similar to our findings, accumulating evidence indicates that age-related neurodegenerative diseases, such as AD and PD, and acute neuronal injury, such as stroke, traumatic brain injury, and ischemia/reperfusion injury of brain, are strongly associated with necroptosis (8, 81, 82). Notably, degradation is among the top 20 keywords with the strongest citation bursts (**Figure 9**), indicating that it is a hot research field from 2017 to 2018; moreover, neuronal cell death is extracted as the label of cluster #5 (**Figure 8B**) that is still ongoing, meaning it remains as the

research foci for now. AD is characterized by severe neuronal loss in which necroptosis is observed and correlated positively with the Braak stage and negatively with brain mass and cognition (8, 83). RIPK1, a key regulator of necroptosis, has emerged as a promising therapeutic target for neurodegenerative disease (81, 84, 85). It might be involved in regulating transcriptional responses in AD, and the inhibition of RIPK1 might promote the ability of microglia to degrade amyloid-β, reduce inflammatory microglia, and restore the phagocytic capacity of microglia (84). Recently, Park et al. demonstrated that O-GlcNAcylation (O-linked β-N-acetylglucosaminylation) plays a protective role in AD by inhibiting necroptosis through ameliorated AD pathology, including Aβ burden, neuronal loss, neuroinflammation, and damaged mitochondria and recovered the M2 phenotype and phagocytic activity of microglia (86). The increased levels of RIPK1, RIPK3, and MLKL were also observed in the PD model (81); furthermore, the necrostatin-1, an inhibitor of necroptosis, improves the survival of optic atrophy type 1 mutant human iPSC–derived neurons *in vitro* and attenuate MPTP-induced dopaminergic neuron loss (9). Necroptosis was also observed in traumatic brain injury, stroke, and ischemia/reperfusion injury of brain (87–89). Moreover, the inhibitor of necroptosis, necrostatin-1, has been regarded as a promising treatment target for neurodegenerative diseases (88, 90, 91). Necroptosis has shown a great therapeutic promise in neurodegenerative diseases and acute neuronal injury, which has attracted the researchers' interests and is becoming a trending topic.

4.2.3 Mechanism of Necroptosis

As shown in **Tables 5**, **6**, in the necroptosis research field, the hotspots of key players include RIPK1, RIPK3, and MLKL; the hotspots of triggering factors include TNF-alpha, NF-κB, toll-like receptors, and ZBP1, as supported by the timeline analysis (**Figures 6**, **8**). We will not repeat them because previous reviews (1, 36, 92) have thoroughly explained these. Notably, our results show that inflammation and oxidative stress are at the forefront of the current necroptosis research.

It is known that necroptosis is an inflammatory form of PCD; when cells die and the membrane ruptures, damage-associated molecular patterns (DAMPs) that can cause inflammatory responses are released (18, 93). In addition, previous studies suggested that RIPKs facilitate the activation of the NLRP3 inflammasome (94, 95). Indeed, necroptosis is associated with some inflammatory diseases, such as neuroinflammatory disease (96), infection (12), autoinflammation (97), inflammatory bowel disease (13), and idiopathic inflammatory myopathy (98). Therapeutically, the inhibition of RIPK3 or RIPK1 exhibited anti-inflammatory effects in animal disease models, suggesting that the inhibitors of these kinases may have a therapeutic potential to treat inflammatory injuries (99).

Oxidative stress is labeled as the fourth cluster that is still ongoing (**Figure 8**, #3); it is an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage (100). Oxidative stress is caused by an imbalance between the production of ROS and the antioxidant capacity. Oxidative damage is not only the cause of necroptosis but also its consequence (101, 102). Excess ROS leads to lipid peroxidation and damage to proteins and DNA, and the latter is an important cause of genomic instability in age-related diseases (102). For instance, oxidative stress could promote Aβ deposition, tau hyperphosphorylation, and synaptic and neuronal loss and subsequently contributes to the development of AD (103).

4.3 Strength and Limitations

Overall, this is the first bibliometric study to systematically analyze the necroptosis-related publications in the past decade. Compared to traditional reviews, the bibliometric analysis provides a novel and objective insight into the evolving research foci and trends (24). Meanwhile, we used various bibliometric software to perform an analysis, which could provide richer results in multiple dimensions (24, 104). This study will inform the public of the importance of necroptosis, provide scholars a whole picture of necroptosis research, and further serve as a comprehensive and objective guide for the future development of the necroptosis research field.

Inevitably, this study has some limitations. Firstly, we exclusively retrieved the articles published in English from the WoSCC database, thus omitting articles that are not in WoSCC or not English. Nevertheless, English articles in WoSCC are the most commonly used data source in bibliometrics, which could represent most of the information to a degree (24, 105). Secondly, bibliometric methods are based on natural language processing, which may be biased, as reported by other bibliometric studies (21, 24). However, our results are consistent with recent traditional reviews (92, 106, 107) while providing more comprehensive and objective information.

CONCLUSION

In conclusion, research on necroptosis had a stable stepwise growth with active cooperation worldwide in the past decade, of which the United States might maintain the dominant position in necroptosis research. Peter Vandenabeele contributed to most of the publications, and Alexei Degterev was the most co-cited in necroptosis field. Current necroptosis studies are focused on its cross-talk with other types of cell death, potential applications in disease, and further mechanisms. Among them, the synergy with ferroptosis, further RIPK1/RIPK3/MLKL studies, the mechanism and translational applications with inflammation and oxidative stress, and the therapeutic potential to treat cancer and neurodegenerative diseases might be the rising and promising research areas. These might provide guidance and new insight for further research in the necroptosis field.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

HX, JQJ, JL, and JZ designed this study. JDJ and WT collected and cleaned the data. JZ and LS performed the analysis. RL normalized the pictures. ZZ and JDJ re-checked data. JZ and LS wrote the original draft. All authors reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.917155/full#supplementary-material

Supplementary Table 1 | Included studies (.xlsx).

Supplementary Table 2 | Additional information of figures (.xlsx).

Supplementary Table 3 | High-frequency co-cited references (.xlsx).

REFERENCES

1. Linkermann A, Green DR. Necroptosis. *N Engl J Med* (2014) 370(5):455–65. doi: 10.1056/NEJMra1310050
2. Laster SM, Wood JG, Gooding LR. Tumor Necrosis Factor Can Induce Both Apoptic and Necrotic Forms of Cell Lysis. *J Immunol* (1988) 141(8):2629–34.
3. Degterev A, Huang Z, Boyce M, Li Y, Jagtap P, Mizushima N, et al. Chemical Inhibitor of Nonapoptotic Cell Death With Therapeutic Potential for Ischemic Brain Injury. *Nat Chem Biol* (2005) 1(2):112–9. doi: 10.1038/nchembio711
4. Zhang Y, Chen X, Gueydan C, Han J. Plasma Membrane Changes During Programmed Cell Deaths. *Cell Res* (2018) 28(1):9–21. doi: 10.1038/cr.2017.133
5. Gong Y, Fan Z, Luo G, Yang C, Huang Q, Fan K, et al. The Role of Necroptosis in Cancer Biology and Therapy. *Mol Cancer* (2019) 18(1):100. doi: 10.1186/s12943-019-1029-8
6. Tang R, Xu J, Zhang B, Liu J, Liang C, Hua J, et al. Ferroptosis, Necroptosis, and Pyroptosis in Anticancer Immunity. *J Hematol Oncol* (2020) 13(1):110. doi: 10.1186/s13045-020-00946-7
7. Chen X, Zeh HJ, Kang R, Kroemer G, Tang D. Cell Death in Pancreatic Cancer: From Pathogenesis to Therapy. *Nat Rev Gastroenterol Hepatol* (2021) 18(11):804–23. doi: 10.1038/s41575-021-00486-6
8. Jayaraman A, Htike TT, James R, Picon C, Reynolds R. Tnf-Mediated Neuroinflammation Is Linked to Neuronal Necroptosis in Alzheimer's Disease Hippocampus. *Acta Neuropathol Commun* (2021) 9(1):159. doi: 10.1186/s40478-021-01264-w
9. Iannielli A, Bido S, Folladori L, Segnali A, Cancellieri C, Maresca A, et al. Pharmacological Inhibition of Necroptosis Protects From Dopaminergic Neuronal Cell Death in Parkinson's Disease Models. *Cell Rep* (2018) 22(8):2066–79. doi: 10.1016/j.celrep.2018.01.089
10. Zelic M, Pontarelli F, Woodworth L, Zhu C, Mahan A, Ren Y, et al. Ripk1 Activation Mediates Neuroinflammation and Disease Progression in Multiple Sclerosis. *Cell Rep* (2021) 35(6):109112. doi: 10.1016/j.celrep.2021.109112
11. Naito MG, Xu D, Amin P, Lee J, Wang H, Li W, et al. Sequential Activation of Necroptosis and Apoptosis Cooperates to Mediate Vascular and Neural Pathology in Stroke. *Proc Natl Acad Sci USA* (2020) 117(9):4959–70. doi: 10.1073/pnas.1916427117
12. Jorgensen I, Rayamajhi M, Miao EA. Programmed Cell Death as a Defence Against Infection. *Nat Rev Immunol* (2017) 17(3):151–64. doi: 10.1038/nri.2016.147
13. Patankar JV, Becker C. Cell Death in the Gut Epithelium and Implications for Chronic Inflammation. *Nat Rev Gastroenterol Hepatol* (2020) 17(9):543–56. doi: 10.1038/s41575-020-0326-4
14. Boonchan M, Arimochi H, Otsuka K, Kobayashi T, Uehara H, Jaroonwitchawan T, et al. Necroptosis Protects Against Exacerbation of Acute Pancreatitis. *Cell Death Dis* (2021) 12(6):601. doi: 10.1038/s41419-021-03847-w
15. Leeper NJ. The Role of Necroptosis in Atherosclerotic Disease. *JACC Basic Transl Sci* (2016) 1(6):548–50. doi: 10.1016/j.jacbts.2016.08.002
16. Galluzzi L, Kepp O, Chan FK, Kroemer G. Necroptosis: Mechanisms and Relevance to Disease. *Annu Rev Pathol* (2017) 12:103–30. doi: 10.1146/annurev-pathol-052016-100247
17. Pasparakis M, Vandenabeele P. Necroptosis and Its Role in Inflammation. *Nature* (2015) 517(7534):311–20. doi: 10.1038/nature14191
18. Weinlich R, Oberst A, Beere HM, Green DR. Necroptosis in Development, Inflammation and Disease. *Nat Rev Mol Cell Biol* (2017) 18(2):127–36. doi: 10.1038/nrm.2016.149
19. Tonnus W, Belavgeni A, Beuschlein F, Eisenhofer G, Fassnacht M, Kroiss M, et al. The Role of Regulated Necrosis in Endocrine Diseases. *Nat Rev Endocrinol* (2021) 17(8):497–510. doi: 10.1038/s41574-021-00499-w
20. Yan J, Wan P, Choksi S, Liu ZG. Necroptosis and Tumor Progression. *Trends Cancer* (2022) 8(1):21–7. doi: 10.1016/j.trecan.2021.09.003
21. Yan WT, Lu S, Yang YD, Ning WY, Cai Y, Hu XM, et al. Research Trends, Hot Spots and Prospects for Necroptosis in the Field of Neuroscience. *Neural Regener Res* (2021) 16(8):1628–37. doi: 10.4103/1673-5374.303032
22. Dong Q, Liang Q, Chen Y, Li J, Lu L, Huang X, et al. Bibliometric and Visual Analysis of Vascular Calcification Research. *Front Pharmacol* (2021) 12:690392. doi: 10.3389/fphar.2021.690392
23. Chen C, Song M. Visualizing a Field of Research: A Methodology of Systematic Scientometric Reviews. *PloS One* (2019) 14(10):e0223994. doi: 10.1371/journal.pone.0223994
24. Zhang J, Song L, Xu L, Fan Y, Wang T, Tian W, et al. Knowledge Domain and Emerging Trends in Ferroptosis Research: A Bibliometric and Knowledge-Map Analysis. *Front Oncol* (2021) 11:686726. doi: 10.3389/fonc.2021.686726
25. Paunkov A, Chartoumpekis DV, Ziros PG, Sykiotis GP. A Bibliometric Review of the Keap1/Nrf2 Pathway and Its Related Antioxidant Compounds. *Antioxid (Basel)* (2019) 8(9):353. doi: 10.3390/antiox8090353
26. Chen C. Searching for Intellectual Turning Points: Progressive Knowledge Domain Visualization. *Proc Natl Acad Sci USA* (2004) 101 Suppl 1(Suppl 1):5303–10. doi: 10.1073/pnas.0307513100
27. Chen C, Hu Z, Liu S, Tseng H. Emerging Trends in Regenerative Medicine: A Scientometric Analysis in Citespace. *Expert Opin Biol Ther* (2012) 12(5):593–608. doi: 10.1517/14712598.2012.674507
28. Chen C. Science Mapping:A Systematic Review of the Literature. *J Data Inf Sci* (2017) 2(02):1–40. doi: 10.1515/jdis-2017-0006

29. van Eck NJ, Waltman L. Software Survey: Vosviewer, a Computer Program for Bibliometric Mapping. *Scientometrics* (2010) 84(2):523–38. doi: 10.1007/s11192-009-0146-3
30. Eck N, Waltman L. Vosviewer Manual. Leiden Leiden Universiteit. (2020).
31. Li C, Wu K, Wu J. A Bibliometric Analysis of Research on Haze During 2000-2016. *Environ Sci Pollut Res Int* (2017) 24(32):24733–42. doi: 10.1007/s11356-017-0440-1
32. Chen C, Leydesdorff L. Patterns of Connections and Movements in Dual-Map Overlays: A New Method of Publication Portfolio Analysis. *J Assoc Inf Sci Technol* (2014) 65(2):334–51. doi: 10.1002/asi.22968
33. Sun L, Wang H, Wang Z, He S, Chen S, Liao D, et al. Mixed Lineage Kinase Domain-Like Protein Mediates Necrosis Signaling Downstream of Rip3 Kinase. *Cell* (2012) 148(1-2):213–27. doi: 10.1016/j.cell.2011.11.031
34. Cho YS, Challa S, Moquin D, Genga R, Ray TD, Guildford M, et al. Phosphorylation-Driven Assembly of the Rip1-Rip3 Complex Regulates Programmed Necrosis and Virus-Induced Inflammation. *Cell* (2009) 137(6):1112–23. doi: 10.1016/j.cell.2009.05.037
35. He S, Wang L, Miao L, Wang T, Du F, Zhao L, et al. Receptor Interacting Protein Kinase-3 Determines Cellular Necrotic Response to Tnf-Alpha. *Cell* (2009) 137(6):1100–11. doi: 10.1016/j.cell.2009.05.021
36. Vandenabeele P, Galluzzi L, Vanden Berghe T, Kroemer G. Molecular Mechanisms of Necroptosis: An Ordered Cellular Explosion. *Nat Rev Mol Cell Biol* (2010) 11(10):700–14. doi: 10.1038/nrm2970
37. Zhang DW, Shao J, Lin J, Zhang N, Lu BJ, Lin SC, et al. Rip3, an Energy Metabolism Regulator That Switches Tnf-Induced Cell Death From Apoptosis to Necrosis. *Science* (2009) 325(5938):332–6. doi: 10.1126/science.1172308
38. Degterev A, Hitomi J, Germscheid M, Ch'en IL, Korkina O, Teng X, et al. Identification of Rip1 Kinase as a Specific Cellular Target of Necrostatins. *Nat Chem Biol* (2008) 4(5):313–21. doi: 10.1038/nchembio.83
39. Wang H, Sun L, Su L, Rizo J, Liu L, Wang LF, et al. Mixed Lineage Kinase Domain-Like Protein Mlkl Causes Necrotic Membrane Disruption Upon Phosphorylation by Rip3. *Mol Cell* (2014) 54(1):133–46. doi: 10.1016/j.molcel.2014.03.003
40. Cai Z, Jitkaew S, Zhao J, Chiang HC, Choksi S, Liu J, et al. Plasma Membrane Translocation of Trimerized Mlkl Protein Is Required for Tnf-Induced Necroptosis. *Nat Cell Biol* (2014) 16(1):55–65. doi: 10.1038/ncb2883
41. Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P, et al. Molecular Mechanisms of Cell Death: Recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ* (2018) 25(3):486–541. doi: 10.1038/s41418-017-0012-4
42. Ma L, Ma J, Teng M, Li Y. Visual Analysis of Colorectal Cancer Immunotherapy: A Bibliometric Analysis From 2012 to 2021. *Front Immunol* (2022) 13:843106. doi: 10.3389/fimmu.2022.843106
43. Liu S, Xia K, Liu X, Duan Y, Hu M, Xia H, et al. Bibliometric Analysis of Birt-Hogg-Dubé Syndrome From 2001 to 2021. *Front Med (Lausanne)* (2022) 9:857127. doi: 10.3389/fmed.2022.857127
44. Chaomei C. Citespace Ii: Detecting and Visualizing Emerging Trends and Transient Patterns in Scientific Literature. *J Am Soc Inf Sci Technol* (2005) 57(3):1–4. doi: 10.1002/asi.20317
45. Lu C, Liu M, Shang W, Yuan Y, Li M, Deng X, et al. Knowledge Mapping of Angelica Sinensis (Oliv.) Diels (Danggui) Research: A Scientometric Study. *Front Pharmacol* (2020) 11:294. doi: 10.3389/fphar.2020.00294
46. Chen C, Song M. Science Mapping Tools and Applications, In Representing Scientific Knowledge: The Role of Uncertainty. Springer (2017). p. 57–137.
47. Xiao F, Li C, Sun J, Zhang L. Knowledge Domain and Emerging Trends in Organic Photovoltaic Technology: A Scientometric Review Based on Citespace Analysis. *Front Chem* (2017) 5:67. doi: 10.3389/fchem.2017.00067
48. Wang Y, Jia Y, Li M, Jiao S, Zhao H. Hotspot and Frontier Analysis of Exercise Training Therapy for Heart Failure Complicated With Depression Based on Web of Science Database and Big Data Analysis. *Front Cardiovasc Med* (2021) 8:665993. doi: 10.3389/fcvm.2021.665993
49. Snyder AG, Oberst A. The Antisocial Network: Cross Talk Between Cell Death Programs in Host Defense. *Annu Rev Immunol* (2021) 39:77–101. doi: 10.1146/annurev-immunol-112019-072301
50. Bedoui S, Herold MJ, Strasser A. Emerging Connectivity of Programmed Cell Death Pathways and Its Physiological Implications. *Nat Rev Mol Cell Biol* (2020) 21(11):678–95. doi: 10.1038/s41580-020-0270-8
51. Oberst A, Dillon CP, Weinlich R, McCormick LL, Fitzgerald P, Pop C, et al. Catalytic Activity of the Caspase-8-Flip(L) Complex Inhibits Ripk3-Dependent Necrosis. *Nature* (2011) 471(7338):363–7. doi: 10.1038/nature09852
52. Kaiser WJ, Upton JW, Long AB, Livingston-Rosanoff D, Daley-Bauer LP, Hakem R, et al. Rip3 Mediates the Embryonic Lethality of Caspase-8-Deficient Mice. *Nature* (2011) 471(7338):368–72. doi: 10.1038/nature09857
53. Newton K, Wickliffe KE, Dugger DL, Maltzman A, Roose-Girma M, Dohse M, et al. Cleavage of Ripk1 by Caspase-8 Is Crucial for Limiting Apoptosis and Necroptosis. *Nature* (2019) 574(7778):428–31. doi: 10.1038/s41586-019-1548-x
54. Feng S, Yang Y, Mei Y, Ma L, Zhu DE, Hoti N, et al. Cleavage of Rip3 Inactivates Its Caspase-Independent Apoptosis Pathway by Removal of Kinase Domain. *Cell Signal* (2007) 19(10):2056–67. doi: 10.1016/j.cellsig.2007.05.016
55. Haas TL, Emmerich CH, Gerlach B, Schmukle AC, Cordier SM, Rieser E, et al. Recruitment of the Linear Ubiquitin Chain Assembly Complex Stabilizes the Tnf-R1 Signaling Complex and Is Required for Tnf-Mediated Gene Induction. *Mol Cell* (2009) 36(5):831–44. doi: 10.1016/j.molcel.2009.10.013
56. Ketelut-Carneiro N, Fitzgerald KA. Apoptosis, Pyroptosis, and Necroptosis-Oh My! The Many Ways a Cell Can Die. *J Mol Biol* (2022) 434(4):167378. doi: 10.1016/j.jmb.2021.167378
57. Conos SA, Chen KW, De Nardo D, Hara H, Whitehead L, Núñez G, et al. Active Mlkl Triggers the Nlrp3 Inflammasome in a Cell-Intrinsic Manner. *Proc Natl Acad Sci USA* (2017) 114(6):E961–e9. doi: 10.1073/pnas.1613305114
58. Gutierrez KD, Davis MA, Daniels BP, Olsen TM, Ralli-Jain P, Tait SW, et al. Mlkl Activation Triggers Nlrp3-Mediated Processing and Release of Il-1β Independently of Gasdermin-D. *J Immunol* (2017) 198(5):2156–64. doi: 10.4049/jimmunol.1601757
59. Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, et al. Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. *Cell* (2017) 171(2):273–85. doi: 10.1016/j.cell.2017.09.021
60. Zhou Y, Liao J, Mei Z, Liu X, Ge J. Insight Into Crosstalk Between Ferroptosis and Necroptosis: Novel Therapeutics in Ischemic Stroke. *Oxid Med Cell Longev* (2021) 2021:9991001. doi: 10.1155/2021/9991001
61. Zhu P, Hu S, Jin Q, Li D, Tian F, Toan S, et al. Ripk3 Promotes Er Stress-Induced Necroptosis in Cardiac Ir Injury: A Mechanism Involving Calcium Overload/Xo/Ros/Mptp Pathway. *Redox Biol* (2018) 16:157–68. doi: 10.1016/j.redox.2018.02.019
62. Basit F, van Oppen LM, Schöckel L, Bossenbroek HM, van Emst-de Vries SE, Hermeling JC, et al. Mitochondrial Complex I Inhibition Triggers a Mitophagy-Dependent Ros Increase Leading to Necroptosis and Ferroptosis in Melanoma Cells. *Cell Death Dis* (2017) 8(3):e2716. doi: 10.1038/cddis.2017.133
63. Wang Z, Guo LM, Wang Y, Zhou HK, Wang SC, Chen D, et al. Inhibition of Hsp90α Protects Cultured Neurons From Oxygen-Glucose Deprivation Induced Necroptosis by Decreasing Rip3 Expression. *J Cell Physiol* (2018) 233(6):4864–84. doi: 10.1002/jcp.26294
64. Chen C, Wang D, Yu Y, Zhao T, Min N, Wu Y, et al. Legumain Promotes Tubular Ferroptosis by Facilitating Chaperone-Mediated Autophagy of Gpx4 in Aki. *Cell Death Dis* (2021) 12(1):65. doi: 10.1038/s41419-020-03362-4
65. Zille M, Karuppagounder SS, Chen Y, Gough PJ, Bertin J, Finger J, et al. Neuronal Death After Hemorrhagic Stroke *in Vitro* and *in Vivo* Shares Features of Ferroptosis and Necroptosis. *Stroke* (2017) 48(4):1033–43. doi: 10.1161/strokeaha.116.015609
66. Belavgeni A, Meyer C, Stumpf J, Hugo C, Linkermann A. Ferroptosis and Necroptosis in the Kidney. *Cell Chem Biol* (2020) 27(4):448–62. doi: 10.1016/j.chembiol.2020.03.016
67. Minagawa S, Yoshida M, Araya J, Hara H, Imai H, Kuwano K. Regulated Necrosis in Pulmonary Disease. A Focus on Necroptosis and Ferroptosis. *Am J Respir Cell Mol Biol* (2020) 62(5):554–62. doi: 10.1165/rcmb.2019-0337TR
68. Yonekawa T, Gamez G, Kim J, Tan AC, Thorburn J, Gump J, et al. Rip1 Negatively Regulates Basal Autophagic Flux Through Tfeb to Control

Sensitivity to Apoptosis. *EMBO Rep* (2015) 16(6):700–8. doi: 10.15252/embr.201439496
69. Koren E, Fuchs Y. Modes of Regulated Cell Death in Cancer. *Cancer Discov* (2021) 11(2):245–65. doi: 10.1158/2159-8290.Cd-20-0789
70. Koo G-B, Morgan MJ, Lee D-G, Kim W-J, Yoon J-H, Koo JS, et al. Methylation-Dependent Loss of Rip3 Expression in Cancer Represses Programmed Necrosis in Response to Chemotherapeutics. *Cell Res* (2015) 25(6):707–25. doi: 10.1038/cr.2015.56
71. Li X, Guo J, Ding A-P, Qi W-W, Zhang P-H, Lv J, et al. Association of Mixed Lineage Kinase Domain-Like Protein Expression With Prognosis in Patients With Colon Cancer. *Technol Cancer Res Treat* (2017) 16(4):428–34. doi: 10.1177/1533034616655909
72. He L, Peng K, Liu Y, Xiong J, Zhu F-f. Low Expression of Mixed Lineage Kinase Domain-Like Protein Is Associated With Poor Prognosis in Ovarian Cancer Patients. *OncoTargets Ther* (2013) 6:1539. doi: 10.2147/OTT.S52805
73. Snyder AG, Hubbard NW, Messmer MN, Kofman SB, Hagan CE, Orozco SL, et al. Intratumoral Activation of the Necroptotic Pathway Components Ripk1 and Ripk3 Potentiates Antitumor Immunity. *Sci Immunol* (2019) 4(36):eaaw2004. doi: 10.1126/sciimmunol.aaw2004
74. Park S, Hatanpaa KJ, Xie Y, Mickey BE, Madden CJ, Raisanen JM, et al. The Receptor Interacting Protein 1 Inhibits P53 Induction Through Nf-Kb Activation and Confers a Worse Prognosis in Glioblastoma. *Cancer Res* (2009) 69(7):2809–16. doi: 10.1158/0008-5472.CAN-08-4079
75. Seifert L, Werba G, Tiwari S, Giao Ly NN, Alothman S, Alqunaibit D, et al. The Necrosome Promotes Pancreatic Oncogenesis *Via* Cxcl1 and Mincle-Induced Immune Suppression. *Nature* (2016) 532(7598):245–9. doi: 10.1038/nature17403
76. Colbert LE, Fisher SB, Hardy CW, Hall WA, Saka B, Shelton JW, et al. Pronecrotic Mixed Lineage Kinase Domain-Like Protein Expression Is a Prognostic Biomarker in Patients With Early-Stage Resected Pancreatic Adenocarcinoma. *Cancer* (2013) 119(17):3148–55. doi: 10.1002/cncr.28144
77. Oliver Metzig M, Fuchs D, Tagscherer K, Gröne H, Schirmacher P, Roth W. Inhibition of Caspases Primes Colon Cancer Cells for 5-Fluorouracil-Induced Tnf-A-Dependent Necroptosis Driven by Rip1 Kinase and Nf-Kb. *Oncogene* (2016) 35(26):3399–409. doi: 10.1038/onc.2015.398
78. Zhao D, Zhang H, Tao W, Wei W, Sun J, He Z. A Rapid Albumin-Binding 5-Fluorouracil Prodrug With a Prolonged Circulation Time and Enhanced Antitumor Activity. *Biomater Sci* (2017) 5(3):502–10. doi: 10.1039/C6BM00884D
79. Fu Z, Deng B, Liao Y, Shan L, Yin F, Wang Z, et al. The Anti-Tumor Effect of Shikonin on Osteosarcoma by Inducing Rip1 and Rip3 Dependent Necroptosis. *BMC Cancer* (2013) 13(1):1–10. doi: 10.1186/1471-2407-13-580
80. Wang H, Tang Y, Fang Y, Zhang M, Wang H, He Z, et al. Reprogramming Tumor Immune Microenvironment (Time) and Metabolism *Via* Biomimetic Targeting Codelivery of Shikonin/Jq1. *Nano Lett* (2019) 19(5):2935–44. doi: 10.1021/acs.nanolett.9b00021
81. Degterev A, Ofengeim D, Yuan J. Targeting Ripk1 for the Treatment of Human Diseases. *Proc Natl Acad Sci USA* (2019) 116(20):9714–22. doi: 10.1073/pnas.1901179116
82. Moujalled D, Strasser A, Liddell JR. Molecular Mechanisms of Cell Death in Neurological Diseases. *Cell Death Differ* (2021) 28(7):2029–44. doi: 10.1038/s41418-021-00814-y
83. Caccamo A, Branca C, Piras IS, Ferreira E, Huentelman MJ, Liang WS, et al. Necroptosis Activation in Alzheimer's Disease. *Nat Neurosci* (2017) 20(9):1236–46. doi: 10.1038/nn.4608
84. Ofengeim D, Mazzitelli S, Ito Y, DeWitt JP, Mifflin L, Zou C, et al. Ripk1 Mediates a Disease-Associated Microglial Response in Alzheimer's Disease. *Proc Natl Acad Sci USA* (2017) 114(41):E8788–e97. doi: 10.1073/pnas.1714175114
85. Li S, Qu L, Wang X, Kong L. Novel Insights Into Ripk1 as a Promising Target for Future Alzheimer's Disease Treatment. *Pharmacol Ther* (2022) 231:107979. doi: 10.1016/j.pharmthera.2021.107979
86. Park J, Ha HJ, Chung ES, Baek SH, Cho Y, Kim HK, et al. O-Glcnacylation Ameliorates the Pathological Manifestations of Alzheimer's Disease by Inhibiting Necroptosis. *Sci Adv* (2021) 7(3):eabd3207. doi: 10.1126/sciadv.abd3207
87. Wang CF, Zhao CC, Weng WJ, Lei J, Lin Y, Mao Q, et al. Alteration in Long Non-Coding Rna Expression After Traumatic Brain Injury in Rats. *J Neurotrauma* (2017) 34(13):2100–8. doi: 10.1089/neu.2016.4642
88. Jun-Long H, Yi L, Bao-Lian Z, Jia-Si L, Ning Z, Zhou-Heng Y, et al. Necroptosis Signaling Pathways in Stroke: From Mechanisms to Therapies. *Curr Neuropharmacol* (2018) 16(9):1327–39. doi: 10.2174/1570159X16666180416152243
89. Tuo QZ, Zhang ST, Lei P. Mechanisms of Neuronal Cell Death in Ischemic Stroke and Their Therapeutic Implications. *Med Res Rev* (2022) 42(1):259–305. doi: 10.1002/med.21817
90. Wang YQ, Wang L, Zhang MY, Wang T, Bao HJ, Liu WL, et al. Necrostatin-1 Suppresses Autophagy and Apoptosis in Mice Traumatic Brain Injury Model. *Neurochem Res* (2012) 37(9):1849–58. doi: 10.1007/s11064-012-0791-4
91. Deng XX, Li SS, Sun FY. Necrostatin-1 Prevents Necroptosis in Brains After Ischemic Stroke *Via* Inhibition of Ripk1-Mediated Ripk3/Mlkl Signaling. *Aging Dis* (2019) 10(4):807–17. doi: 10.14336/ad.2018.0728
92. Khan I, Yousif A, Chesnokov M, Hong L, Chefetz I. A Decade of Cell Death Studies: Breathing New Life Into Necroptosis. *Pharmacol Ther* (2021) 220:107717. doi: 10.1016/j.pharmthera.2020.107717
93. Bibo-Verdugo B, Salvesen GS. Caspase Mechanisms in the Regulation of Inflammation. *Mol Aspects Med* (2022) 88:101085. doi: 10.1016/j.mam.2022.101085
94. Kang S, Fernandes-Alnemri T, Rogers C, Mayes L, Wang Y, Dillon C, et al. Caspase-8 Scaffolding Function and Mlkl Regulate Nlrp3 Inflammasome Activation Downstream of Tlr3. *Nat Commun* (2015) 6:7515. doi: 10.1038/ncomms8515
95. Moriwaki K, Bertin J, Gough PJ, Chan FK. A Ripk3-Caspase 8 Complex Mediates Atypical Pro-Il-1β Processing. *J Immunol* (2015) 194(4):1938–44. doi: 10.4049/jimmunol.1402167
96. Yu Z, Jiang N, Su W, Zhuo Y. Necroptosis: A Novel Pathway in Neuroinflammation. *Front Pharmacol* (2021) 12:701564. doi: 10.3389/fphar.2021.701564
97. Taft J, Markson M, Legarda D, Patel R, Chan M, Malle L, et al. Human Tbk1 Deficiency Leads to Autoinflammation Driven by Tnf-Induced Cell Death. *Cell* (2021) 184(17):4447–63.e20. doi: 10.1016/j.cell.2021.07.026
98. Shi J, Tang M, Zhou S, Xu D, Zhao J, Wu C, et al. Programmed Cell Death Pathways in the Pathogenesis of Idiopathic Inflammatory Myopathies. *Front Immunol* (2021) 12:783616. doi: 10.3389/fimmu.2021.783616
99. Newton K, Manning G. Necroptosis and Inflammation. *Annu Rev Biochem* (2016) 85:743–63. doi: 10.1146/annurev-biochem-060815-014830
100. Sies H, Jones D. *Oxidative Stress. 2nd*. Amsterdam: Elsevier (2007).
101. Canli Ö, Alankuş YB, Grootjans S, Vegi N, Hültner L, Hoppe PS, et al. Glutathione Peroxidase 4 Prevents Necroptosis in Mouse Erythroid Precursors. *Blood* (2016) 127(1):139–48. doi: 10.1182/blood-2015-06-654194
102. Tang D, Kang R, Berghe TV, Vandenabeele P, Kroemer G. The Molecular Machinery of Regulated Cell Death. *Cell Res* (2019) 29(5):347–64. doi: 10.1038/s41422-019-0164-5
103. Chen Z, Zhong C. Oxidative Stress in Alzheimer's Disease. *Neurosci Bull* (2014) 30(2):271–81. doi: 10.1007/s12264-013-1423-y
104. Shen J, Shen H, Ke L, Chen J, Dang X, Liu B, et al. Knowledge Mapping of Immunotherapy for Hepatocellular Carcinoma: A Bibliometric Study. *Front Immunol* (2022) 13:815575. doi: 10.3389/fimmu.2022.815575
105. Ke L, Lu C, Shen R, Lu T, Ma B, Hua Y. Knowledge Mapping of Drug-Induced Liver Injury: A Scientometric Investigation (2010-2019). *Front Pharmacol* (2020) 11:842. doi: 10.3389/fphar.2020.00842
106. Newton K, Dixit VM, Kayagaki N. Dying Cells Fan the Flames of Inflammation. *Science* (2021) 374(6571):1076–80. doi: 10.1126/science.abi5934
107. Choi ME, Price DR, Ryter SW, Choi AMK. Necroptosis: A Crucial Pathogenic Mediator of Human Disease. *JCI Insight* (2019) 4(15):e128834. doi: 10.1172/jci.insight.128834

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Mesenchymal stem cell spheroids alleviate neuropathic pain by modulating chronic inflammatory response genes

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Chronic neuropathic pain is caused by dysfunction of the peripheral nerves associated with the somatosensory system. Mesenchymal stem cells (MSCs) have attracted attention as promising cell therapeutics for chronic pain; however, their clinical application has been hampered by the poor *in vivo* survival and low therapeutic efficacy of transplanted cells. Increasing evidence suggests enhanced therapeutic efficacy of spheroids formed by three-dimensional culture of MSCs. In the present study, we established a neuropathic pain murine model by inducing a chronic constriction injury through ligation of the right sciatic nerve and measured the therapeutic effects and survival efficacy of spheroids. Monolayer-cultured and spheroids were transplanted into the gastrocnemius muscle close to the damaged sciatic nerve. Transplantation of spheroids alleviated chronic pain more potently and exhibited prolonged *in vivo* survival compared to monolayer-cultured cells. Moreover, spheroids significantly reduced macrophage infiltration into the injured tissues. Interestingly, the expression of mouse-origin genes associated with inflammatory responses, Ccl11/Eotaxin, interleukin 1A, tumor necrosis factor B, and tumor necrosis factor, was significantly attenuated by the administration of spheroids compared to that of monolayer. These results suggest that MSC spheroids exhibit enhanced *in vivo* survival after cell transplantation and reduced the host inflammatory response through the regulation of main chronic inflammatory response-related genes.

KEYWORDS

mesenchymal stem cells, 3D spheroids, chronic inflammatory response genes, neuropathic pain, chronic constriction injury

Introduction

Peripheral neuropathic pain is a complex, chronic, and debilitating condition that severely worsens a patient's quality of life (1). Chronic peripheral neuropathic pain is caused by pathological changes or diseases of the peripheral somatosensory nervous system (2), and is characterized by neuropathic pain such as hyperalgesia (painful stimuli) or allodynia (painless stimuli) (3, 4). Although neuronal processes most likely trigger the onset of neuropathic pain, accumulating evidence suggests the involvement of immune cells and chronic inflammation in the induction and maintenance of neuropathic pain (5). Several pharmacological treatment approaches have been introduced; however, despite these effects of pain relief, chronic peripheral neuropathic pain does not respond to conventional drug treatments (6).

Stem cells have drawn considerable attention in the therapy of chronic peripheral neuropathic pain (7). Mesenchymal stem cells (MSCs) represent a good source for therapy of various diseases and regeneration of injured tissues through direct differentiation into tissue-type cells or paracrine functions, including pro-angiogenesis, anti-apoptosis, and immunomodulatory effects (8–10). However, the clinical application of MSCs has been hampered by the poor survival and therapeutic efficacy of transplanted cells. MSC spheroids or aggregates, which are formed by three-dimensional (3D) suspension culture, not only enhance the survival of administered MSCs but also better mimic the physiological microenvironment compared to two-dimensional (2D) monolayer-cultured MSCs (11–15). Accordingly, conditions with cell-to-cell interaction and cell-extracellular matrix can be used to preserve viable cells throughout cell transplantation and repair the pain response. However, it has not been explored whether MSC spheroids can affect the survival and therapeutic efficacy of MSCs in chronic peripheral neuropathic pain.

The chronic constriction injury (CCI) model is one of the most frequently used models for studying neuropathic pain. The CCI model is based on the unilateral loose ligation of the sciatic nerve, which corresponds to the pathophysiological properties of chronic neuropathic pain in humans (16). Ligation knots are placed on the sciatic nerve to induce chronic nerve injury (17–19). However, some studies reported the alleviation of symptoms after 2-4 weeks (20), and Urban et al. reported that they rarely observed signs of pain (21). Therefore, it is necessary to perform compression tests on the ligations before applying the CCI model to avoid inconsistencies in the results.

In this study, we aim to demonstrate the MSC implantation in 3D spheroid form can effectively relieve injured pain in the CCI model, and to explore the main mechanisms that effectively respond to inflammatory signaling by improving the survival of the engrafted MSCs. We believe that this strategy may benefit the post-engrafted survival of MSCs and their paracrine potential to improve the therapeutic efficiency of MSC transplantation in neuropathic pain.

Materials and methods

Animals and surgical procedure of the CCI model

All animal experiments were performed in accordance with the guidelines of the Pusan National University Institutional Animal Care and Use Committee (IACUC: PNU-2021-0063). Adult Balb/C male and female mice weighing 20–25g were housed under a standard 12-h light/dark cycle with a regulated ambient temperature of 22–24°C. Mice were anesthetized with a single intraperitoneal injection of 2% Avertin (200 mg/kg) in accordance with the methods described in IACUC policy (The preparation, storage, and use of Tribromoethanol (Avertin) in mice). The CCI to sciatic nerve was used to induce the neuropathic pain as previously reported (16). Under aseptic conditions, the sciatic nerve was exposed by a skin incision along the femur, followed by the separation of biceps femoris muscles and superficial gluteal muscles without damaging the muscle bundles. The CCI was then induced by placing one, two, four ligatures (4/0 silk suture, 1 mm apart) around the nerve. The animals were returned to their cages following wound closure to recover, and the von Frey test was performed every 3–4 days for all groups.

Isolation of tonsil-derived mesenchymal stem cells (TMSCs)

TMSCs were isolated as previously described (Doi: 10.3390/cells9010089). Briefly, tonsils were obtained from patients with chronic tonsillitis who had undergone tonsillectomy. To isolate stem cells, tonsil tissues were chopped using an autoclaved blade and scissors. Next, the tissues were digested in Type1 collagenase (Sigma-Aldrich, C9891) and hyaluronidase (Worthington Biochemical Corporation, LS002592) at 37°C for 1 h. The enzyme was neutralized with a 10% fetal bovine serum (FBS)-containing medium, and the samples were centrifuged at 3000 rpm for 10 min. After centrifugation, the samples were filtered through a 40-μm nylon mesh to remove debris and diluted in culture media for overnight incubation at 37°C under 5% CO_2 conditions.

Differentiation of TMSCs to adipocytes, osteoblasts, and chondrocytes

To induce the differentiation of MSCs to osteoblasts and adipocytes, TMSCs were seeded on 0.1% gelatin-coated 6-well

plate at a density with 1×10^5 cells. The osteogenic and adipogenic differentiation of TMSCs were induced by using StemPro osteogenesis differentiation kit (Thermo Fisher, A1007201) and StemPro adipogenesis differentiation kit (Thermo Fisher, A1007101), respectively. To induce the chondrogenic differentiation, pellets containing 5×10^4 TMSCs were formed in 96-well polypropylene plates *via* centrifugation for 5 min at 300 × *g*. Cells were maintained in chondrogenesis media (StemPro chondrogenesis differentiation kit, A10071-01) at 37°C with 5% CO_2. The differentiation induction media were changed every other day. Osteogenic differentiation of TMSCs was visualized by staining with Alizarin Red (Sigma Aldrich, TMS008) after 7 days of induction. The differentiation of TMSCs to adipocytes was demonstrated by staining the cytoplasmic inclusion of lipid droplet with Oil Red O (Sigma Aldrich, MAK194) at day 7. Toluidine Blue stain (Sigma Aldrich, 89640) was used to examine the formation of chondrogenic structure after 14 days under induction medium. The stained cells were observed on the microscope.

MSC monolayer (2D) and spheroid (3D) cell cultures

TMSCs were cultured in gelatin-coated dishes containing α-MEM (Invitrogen, 12000022), 10% FBS (Invitrogen, 16000044), and 1% penicillin/streptomycin (Invitrogen, 15140122) for maintenance and 2D cell culture. To establish MSC spheroids, cells were harvested and cultured by hanging-drop methods at 1000, 2500, 5000, 10000, and 25000 cells/spheroid in a 150 mm *petri* dish and incubated for 3 days at 37°C under 5% CO_2. After 3 days, the spheroids were collected from the dishes.

Live and dead cell counting

MSC spheroids were collected from the plate and fragmented to single-cell conditions using Accutase. The enzyme-treated cells were neutralized with an FBS-containing medium. Neutralized cells were centrifugated at 1000 rpm for 4 min, and the pellets were diluted in 1 ml media. 10 µl of cell-contained media was mixed with 10 µl of trypan blue for staining of dead cells, and cell viability was assessed using a LUNA cell counter (LUNA-II Automated Cell Counter, Logos Biosystems, L40001).

Cell transplantation

For cell transplantation, 1×10^6 cells were suspended in the solution of Matrigel (1:20 dilution in the α-MEM, Corning, BC-354230) to obtain 1×10^6 cells/100 µL for the 2D condition and 100 spheroids/100 µL for the 3D condition. For MSC transplantation, either single cells or spheroids were vertically injected into the thigh muscle region of CCI-induced mice using a 0.5 ml Hamilton syringe with 26-gauge needle. The injection was maintained at a slow flow rate (about 100 µL/min) and the needle was kept in place for at least 2 min post-injection to avoid leakage of the injecting solution. To prevent cell death by host defense, mice were treated with cyclosporine A (Sigma-Aldrich, 30024), an immunosuppressive drug, at a dose of 10 mg/kg every other day.

Immunohistochemistry

The gastrocnemius muscle with sciatic nerves were fixed in acetone and incubated overnight at -20°C. Fixed tissues were washed and incubated in sucrose solution at 10%, 20%, and 30% dosage over 4 h. Tissues were embedded in O.C.T compounds and stored at -80°C to prepare cryomolds. Molds were sectioned to 20-µm and stored at -80°C. Sectioned samples were deparaffinized by cooling with acetone and blocked with M.O.M. IgG blocking reagents (Vector Laboratories, BM000-2202). For macrophage and inflammation staining of normal, 2 and 4 ligations, anti-CD68 (1:200, Bio-Rad, MCA 1957GA) and anti-Iba1 (1:200, Cell signaling, 17198S) were stained with DAPI. To measure neuroinflammation by CCI, anti-CD68 (1:200, Bio-Rad, MCA 1957GA) was co-stained with anti-human nuclei (1:200, Merck, MP-MAB1281) antibody. Primary antibodies were incubated overnight at 4°C. The sections were washed with PBS three times, and stained with goat anti-rat Alexa Fluor 488 dye (1:200, Invitrogen, A11006) for human nuclei antibody and goat anti-mouse Alexa Fluor 568 dye (1:200, Invitrogen, A11004) for CD68 or Iba1 antibody at room temperature for 2 h. The stained tissues were mounted using an anti-fade mounting solution containing DAPI for counter-staining of nuclei and visualized using the EVOS M5000 imaging system.

Enzyme-linked immunosorbent assay (ELISA)

On day 9 after cell transplantation, all mice were anesthetized with 2% Avertin and 0.5~0.8 ml of blood was obtained directly from mouse hearts using a heparin-coated 1 mL syringe with 26-gauge needle. After collection, blood samples were centrifugated at 1000 g for 30 min to isolate sera. The levels of TNF-α and INF-γ in serum were measured by using ELISA assay kits (TNF-α: Biolegend, 430904 and IFN-γ: Biolegend, 43804). Briefly, TNF-α and IFN-γ antibodies (diluted 1:200) were coated on 96-well non-coated plate overnight at 4°C. The plate was then washed four times and blocked for 1 h at room temperature. After washing four times, sera were added to a plate and incubated for 2 h. Sera-incubated plates were washed four times to remove

overexpression and reacted with detection antibodies (diluted 1:200) at room temperature. After 1 h, the plate was washed four times, treated with horseradish peroxidase (HRP) (diluted to 1:1000) for 30 min at room temperature, and washed five times to remove residual HRP. The washed plates were incubated with HRP substrate solution for 15 min before stopping the reaction with the stop solution. The plate was analyzed using a microplate reader (TECAN Sunrise, TECAN Life Science).

Staining of 2D and 3D MSCs using *in vivo* tracker

MSCs were stained using an *in vivo* tracker (IVISense 680 Fluorescent Cell Labeling Dye; PerkinElmer, NEV12000). Briefly, cells were harvested from the culture dishes and stained with an *in vivo* tracker at a dosage of 2.5×10^8/ml, for 15 min of 2D MSCs and 30 min of 3D MSCs, at room temperature, protected from light. The stained cells were washed three times in PBS and diluted in Matrigel for cell transplantation. The cells were vertically transplanted close to the sciatic nerve using a 26-gauge syringe needle. To confirm the bioluminescence levels of the transplanted cells, mice were visualized using an *in vivo* imaging system (CRI Maestro) to measure cell engraftment and viability 24 h, 1 week, and 2 weeks after cell transplantation.

Von Frey test (PWT)

To confirm mechanical allodynia, the PWT was measured using the von Frey test. CCI-induced mice were placed on a stainless mesh and adapted for 10 min. After adaptation, von Frey filaments (JEUNG DO BIO & PLANT, JD-SI-11F) were stimulated in the hind paws. Von Frey filaments were selected from 0.16 g, and the filament force was gradually increased until the paw was raised. To select mice for CCI modeling, the PWT was measured three times at 2-day intervals, and the 1.0 g value mice were selected before surgery. After surgery, the PWTs were recorded every 3 days and quantified at the filament force levels of the paw reaction.

Target gene qPCR screening

To extract total RNA, the injured sciatic nerve was isolated from all groups, and RNA was separated using TRIzol reagent (Sigma-Aldrich, T9424) according to the manufacturer's protocols. The RNA concentration was measured using a NanoDrop spectrophotometer. RNA was reverse transcribed with oligo dT primers using Superscript II. For target gene screening, we performed real-time quantitative RT-PCR using SYBR Green PCR Master Mix (Applied Biosystems, ABS-4309155). PCR amplification was performed using the inflammatory response and autoimmunity gene-specific primers (Accutarget ™ qPCR Screening kit, Bioneer, SH-000-10 for human origin; SM-000-10 for mouse origin) as follows: 40 cycles of denaturation at 95°C for 5 s, annealing at 58°C for 25 s, and elongation at 72°C for 30 s. Target gene expression was determined by normalization to endogenous GAPDH or actin using the comparative cycle time method.

Statistical analysis

Statistical differences between groups were analyzed using the t-test and one-way analysis of variance (ANOVA) for multiple comparisons. T-test was performed two-tailed Student's t-test and one-way ANOVA was performed using Tukey's posthoc test. All values are presented as mean ± standard error of the mean, and the p-values are indicated as follows: * $P < 0.05$, ** $P < 0.01$, *** $p< 0.005$ and **** $p< 0.001$. All statistical analyses were performed using GraphPad Prism 9 (GraphPad Software, Inc.).

Results

Induction of the optimal CCI pain model by two ligation knots

The CCI animal model is a well-established neuropathic pain model reported previously (17–19) and is particularly useful for quantitative assessment of the therapeutic efficacy of cell transplantation on chronic neuropathic pain. To apply a suitable CCI animal model for cell therapy, we first validated a ligation-knot-based constriction injury generated by one, two, or four ligations on the partial sciatic nerve of the right hindlimb (Figure 1A). To measure the nerve-induced pain response, we performed the von Frey test in each mouse every 2 days. The von Frey test assesses mechanical pain response-like behavior and measures the paw withdrawal threshold (PWT) by flicking its paw away from the filament force. As shown in Figure 1B, before the injury, the baseline (D0) threshold, the PWT, was not significantly different among the groups before modeling. However, after surgery applying the ligation, the PWT decreased significantly in the groups with either two or four ligations compared to the control group, and the group with one ligation spontaneously recovered at 7 days compared to day 3 after surgery (one knot: 0.85 ± 0.44 g; two knots: 0.32 ± 0.13 g; four knots: 0.22 ± 0.12 g). In addition to the paw response to ligation, we examined the involvement of inflammatory markers. In all groups, the suture knots were carefully removed under a microscope (Figures 1C–E), followed by staining with anti-CD68 and anti-Iba1 antibodies. The group with one ligation exhibited mild inflammation in the middle part

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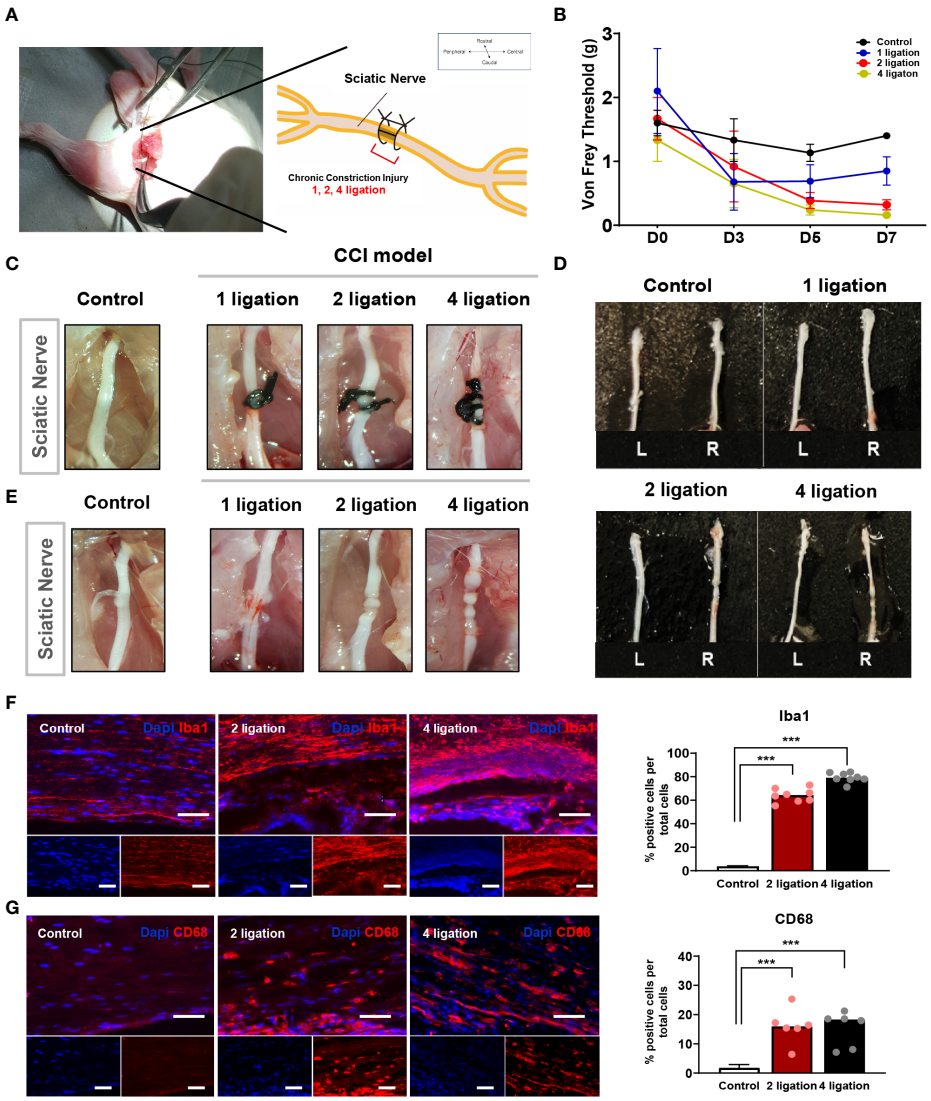

FIGURE 1
Induction of chronic constriction injury mouse model and inflammatory factors. **(A)** The location of ligation knots on the right hindlimb sciatic nerve. **(B)** Von Frey test of 1, 2, 4 ligations CCI mouse model. Day 0 is baseline threshold before the injury (n = 5 mice for each group). Morphological features of **(D)** sciatic nerve **(C)** before and **(E)** after ligations. **(F)** Immunofluorescence staining of Iba-1 positive cells (Red) with DAPI (Blue). Percentage of Iba-1 positive cells in 2 and 4 ligations CCI model. Scale bar: 150 μm. **(G)** Immunofluorescence staining of CD68-positive cells (Red) and DAPI (Blue). Percentage of CD68-positive cells in 2 and 4 ligations compared to control. Scale bar: 150 μm. Data are presented as mean ± SEM. Statistical differences were analyzed using one-way ANOVA, followed by Tukey's *post hoc* test, ***$p < 0.005$.

of the nerve but no strong CD68 expression. Consistently, both the two and four ligation groups exhibited a strong increase in the number of Iba1-positive cells and moderate increase of CD68-positive macrophages in tissues (Figures 1F, G; Supplementary Figure 1). This finding suggests that either two or four ligations can induce hyperalgesia and produces the CCI neuropathic pain phenotype.

Subsequently, the weight of the gastrocnemius muscle was validated in the CCI model. The muscle weight (g) of all groups was estimated as a ratio to compare the left hindlimb muscle to the right contralateral site. Partial gastrocnemius muscle atrophy was detected in both the two and four ligation groups, as they exhibited a significant loss (approximately 30–40%) of muscle weight compared to the control and one ligation groups (Figure 2B). Taken together, these results indicate that either two or four ligations induced significant muscle atrophy and moderate nerve damage in mice compared with one ligation and that four ligations group mice had increased severe nerve damage, such as sensory loss (Figure 2A and Supplementary Figure 1). Therefore, according to the results of the ligation test,

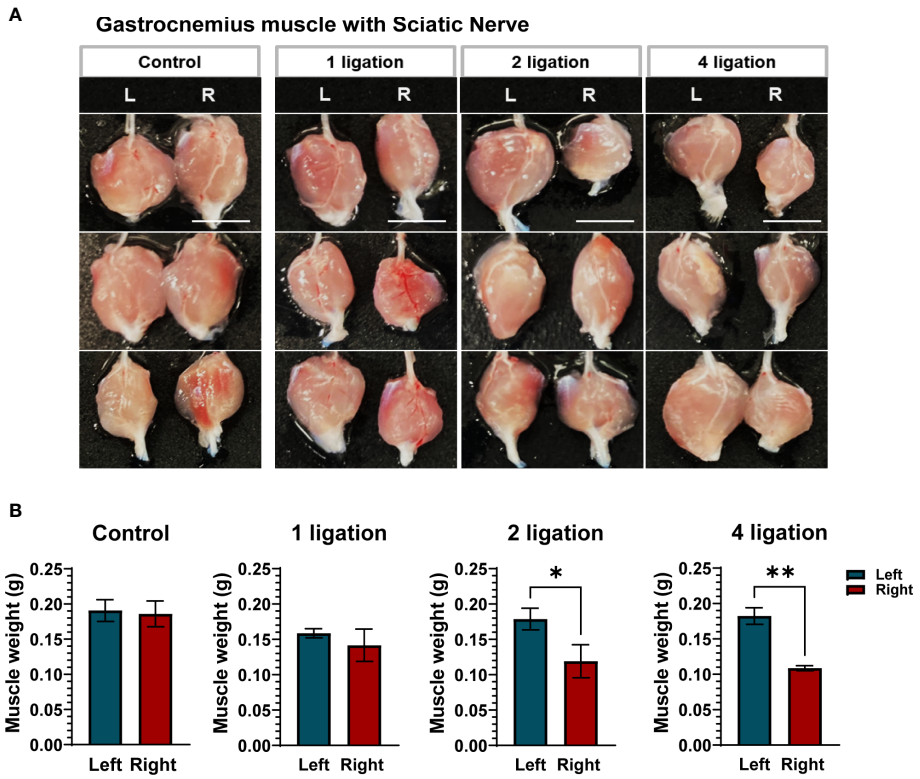

FIGURE 2
Gastrocnemius muscle weight according to the ligations. **(A)** Gastrocnemius muscles on control, 1, 2 and 4 ligations. Scale bar: 1 cm. **(B)** The muscle weight (g) was estimated as a ratio to compare the left hindlimb muscle (Blue bar) to the right contralateral site (Red bar) (n = 5 per group). Data are presented as mean ± SEM. Statistical significance was analyzed using two-tailed Student's t-test, *$p < 0.05$, ** $p < 0.01$.

we selected two ligations to generate the CCI pain mouse model for this study.

MSC spheroids with a density of 10000 cells are suitable for cell transplantation

Human tonsil-derived mesenchymal stem cells (TMSCs) were isolated from patient tonsil tissues and digested into small clumps. These clumps appeared heterogeneous at passage 0 (P0) and expanded through progressive subculture. By passages 3 and 4, the cells developed a typical MSC-like morphology with elongated and heterogeneous single cells. The surface phenotypes of MSCs were analyzed by flow cytometry analysis. TMSCs were positive for several MSC-specific cell surface markers, including CD105, CD90, and CD44 (Supplementary Figure 2A), and they were negative for hematopoietic cell markers, such as CD34 and CD45 (data not shown). TMSCs also had the capacity to differentiate into tri-lineage cell types including adipocytes, osteoblasts, and chondrocytes (Supplementary Figure 2B). Not only 2D MSCs but also 3D MSC spheroids expressed the three MSC markers (Supplementary Figure 2C). Therefore, these results suggest that TMSCs exhibit MSC phenotype and multipotent differentiation potential.

Numerous laboratories have investigated the applicability of spheroid MSCs for cell therapy (22, 23). Based on the findings that spheroids offer various advantages and can significantly enhance the viability of grafted cells, spheroids have recently been used more frequently than monolayer cultured cells for the purpose of cell transplantation. As the size of spontaneously formed spheroids varies between experiments, we sought to establish spheroids with a more suitable cell density and size for stem cell therapy. For spheroid aggregation, TMSCs were cultured in suspension using the hanging drop method (24–26) (Figures 3A, B), and spheroids were produced at five cell densities (1000, 2500, 5000, 10000, and 25000 cells). As shown in Figures 3C, D, all spheroids were visible to the naked eye after 1 day of culture and grew in size by day 3. The diameters of the formed spheroids ranged from 60 to 600 μm (1K: 68.9 ± 5.03 μm; 2.5K: 113 ± 8.91 μm; 5K: 176 ± 9.23 μm; 10K: 326 ± 35.2 μm; 25K: 510 ± 89.52 μm) depending on the cell densities, demonstrating that spheroid sizes can be precisely manipulated by the cell-seeding densities. Moreover, we counted the live/dead

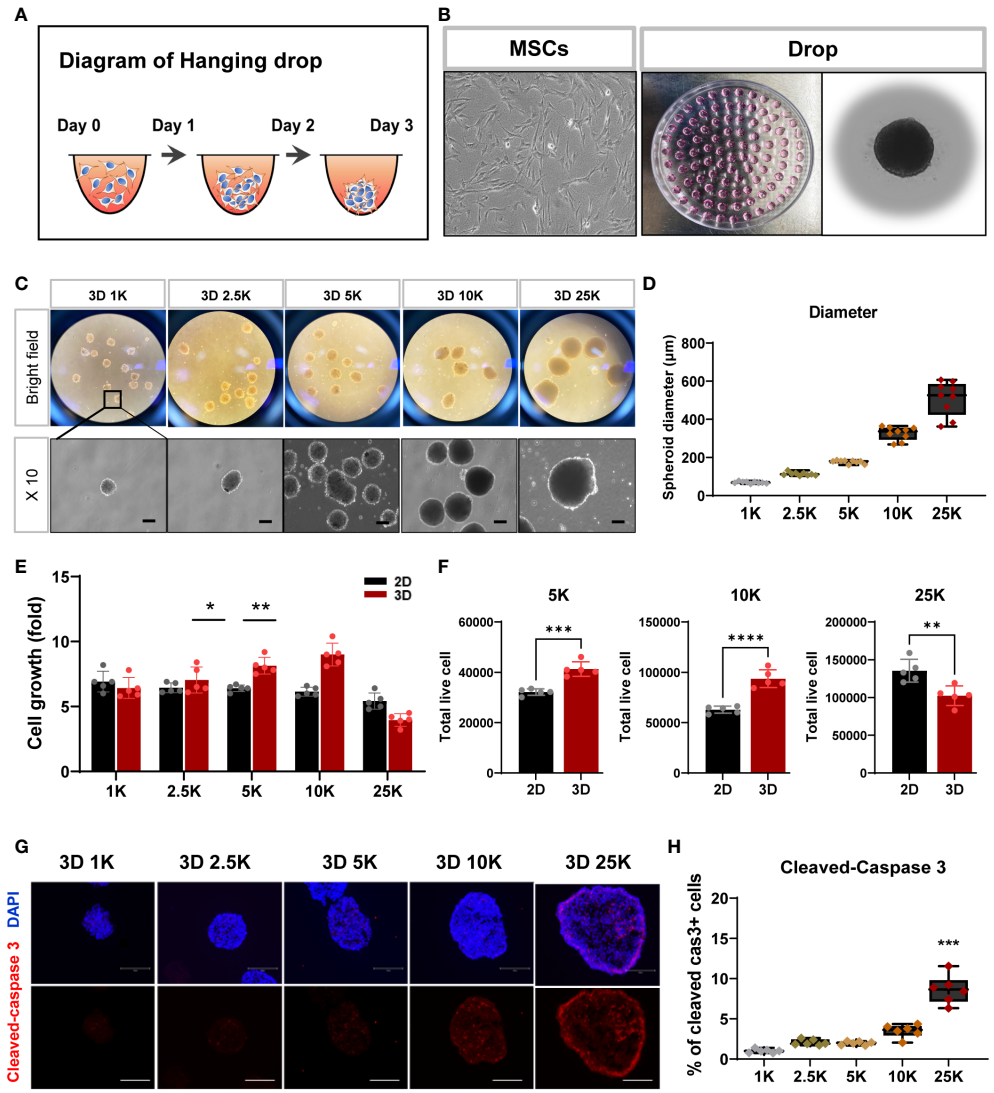

FIGURE 3
Structural and cell viability of tonsil-derived MSC spheroids (3D) depending on cell densities. **(A, B)** Suspension culture of MSC spheroids using hanging drop method. **(A)** Schematic diagram of hanging drop. **(B)** Bright-field images for tonsil-derived MSCs (TMSCs) and drops in a *petri* dish. **(C)** Morphological features of the spheroids formed at five different cell densities. Spheroids incubated for 3 days and then collected them from the *petti* dish. Scale bar: 100 μm. **(D)** The diameter of spheroids (μm). **(E)** Fold change of spheroid cell growth. **(F)** The number of live cells in a density of 5000, 10000 and 250000 cell spheroids. Spheroids were dissociated into single cells and counted the total viable cells by trypan blue assay. **(G)** Immunofluorescence staining of cleaved-caspase 3 positive cell (Red) with DAPI (Blue) according to spheroids. Scale bar: 100 μm. **(H)** Quantification of the expression of cleaved-caspase 3. The high-density spheroid was increased the cleaved-caspase positive cells compare to smaller spheroids. Data are presented as mean ± SEM. Statistical significance for spheroid diameters, spheroid cell growth and % of cleaved caspase3 cells were analyzed using one-way ANOVA, followed by Tukey's *post hoc* test, $*p < 0.05$, $**p < 0.01$, $***p < 0.005$. Statistical significance for total live cells was analyzed using two-tailed Student's t-test, $**p < 0.01$, $***p < 0.005$, $****p < 0.001$. Cell densities are 1K, 1000 cells; 2.5K, 2500 cells; 5K, 5000 cell; 10K, 10000 and 25K, 25000.

cells in all spheroids to determine the number of living cells in the final spheroid formed on day 3. To calculate cell viability and growth, we dissociated the spheroids into single cells using Accutase and performed trypan blue staining. The total number of live cells increased linearly with increasing cell numbers; however, with 25000 cells, the number of live cells was dramatically reduced (Figures 3E, F). Furthermore, to investigate apoptosis in spheroids of different sizes, the assembled spheroids were harvested and analyzed for apoptosis markers by immunostaining. Cleaved caspase 3-positive cells showed significantly increased in the high-density spheroids (up to 25000 cells) compared to that observed in smaller spheroids and was mostly expressed on the outer surface of the spheroids (Figures 3G, H).

As an extension of the previous experiment, the inflammatory environment in the injured region may affect survival after transplantation, and spheroid cell damage may occur first during the application of the injection needle. To investigate whether spheroids of various sizes exhibited alterations in their surface shape after syringe application, all spheroids were passed through a 26-gauge syringe with a needle. The surface of small spheroids (< 10000 cells) appeared to have a grainy texture and exhibited a light-silver sheen compared to that of large-sized spheroids. Moreover, larger spheroids (> 10000 cells) exhibited wall damage during flow through a syringe needle (Supplementary Figure 3). We did not check for surface markers, but variations in the formation of spheroids with a characteristic spherical shape were observed, as shown in Supplementary Figure 3. Thus, we suggest that 10000 cells for spheroids are suitable for cell transplantation in this study and highlight the importance of determining spheroid size/cell density to obtain consistent and reliable results of cell transplantation in animal models of disease.

MSC spheroids exhibit improved efficiency of cell survival at the target site and afford pain relief and downregulation of inflammatory factors

Based on the optimal spheroid size and cell density established in this study, we examined the *in vivo* effects of optimized spheroid MSCs (10000 cells) against neuropathic pain. Mice were randomized into the following four groups: normal, vehicle, 2D MSCs, and 3D MSCs. CCI neuropathic pain was induced in all mice, except for the normal group, *via* two ligations of the sciatic nerve. Before CCI surgery, we performed von Frey tests and observed that the baseline threshold in all groups was 1.2–1.6 g. After surgery, the von Frey thresholds of the CCI-induced groups significantly decreased in between day 0 and day 6 relative to the baseline normal group, consistent with the result of Figure 1B. On day 6, we prepared vehicle and cell groups (1×10^6 cells/100 μL for the 2D condition and 100 spheroids/100 μL for the 3D condition) and transplanted them into the sciatic muscle area of the injured hindlimb. The von Frey test was performed every 3–4 days (Figure 4A). Compared to baseline, the vehicle group showed significant mechanical allodynia and gradually decreased von Frey thresholds. Conversely, cell injection, especially in the 3D condition, significantly improved the pain response from 0.16 to 0.8 g on day 15 and gradually increased the von Frey threshold from 9 days compared to the 2D condition (Figure 4B). This suggests that MSC spheroids are sufficient to ameliorate the impaired paw response because of their good graft survival.

To support the previous data, we evaluated the distribution of the transplanted 2D and 3D MSCs. Monolayer and spheroid MSCs labeled with an *in vivo* tracker (27) were directly injected into the gastrocnemius muscle close to the sciatic nerve, and measurements were performed using optical fluorescence imaging at 680 nm after cell transplantation. Interestingly, on day15, the results showed that 3D MSCs persisted well in the target region and yielded a signal intensity in grafted cells that was significantly greater than that detected in 2D MSCs (Figure 4C). The stereological estimates of surviving human nuclei cells show an average of 83 ± 27 cells and 125 ± 34 cells in 2D and 3D graft site close to the sciatic nerve (Figures 4D, E). As it is possible to establish correlations with inflammation signaling through macrophages, we harvested the gastrocnemius muscle and performed staining for the pan-macrophage marker CD68, which triggers the release of cytokines *via* macrophage infiltration. As shown in Figures 5C, D, the groups with CCI injury exhibited high expression levels of CD68 in the mid-nerve region, while the 2D and 3D MSC injection groups showed significantly reduced expression of CD68. Therefore, our results demonstrated that spheroids have better survival at the engrafting site compared to monolayer cultured MSCs and alleviates hyperalgesia induced by nerve damage by reducing macrophage infiltration. Next, we screened for inflammation-response-related genes in the cell transplantation groups, including the control group, for further analysis of a key mechanism that reduces inflammation.

Good graft survival of MSC spheroids modulates the host inflammatory response induced by sciatic nerve injury pain

After nerve injury, proinflammatory cytokines such as tumor necrosis factor- α (TNF-α), interferon- γ (IFN-γ), and interleukin-6 are upregulated in CCI animal models and are important effectors in modulating immune responses (12, 28–30). In this context, we collected blood from six mice in each group on day 9 after cell injection and assessed TNF-α and IFN-γ levels in the isolated serum. As shown in Figures 5A, B, we observed a significant difference between CCI and spheroid groups as follows: the TNF-α levels in the CCI, 2D MSC, and 3D MSC groups were 130.1 ± 18.1, 126.6 ± 25.3, and 61.8 ± 3.6 pg/ml, whereas IFN-γ levels were 386 ± 67.8, 366.5 ± 89.9, and 213.3 ± 53.5 pg/ml, respectively. As a result, the plasma TNF-α levels in the spheroid group decreased by approximately 47% and the IFN-γ levels by approximately 55% compared to the CCI group. Hence, MSC spheroids exerted a good survival effect, which dramatically reduced the levels of proinflammatory cytokines.

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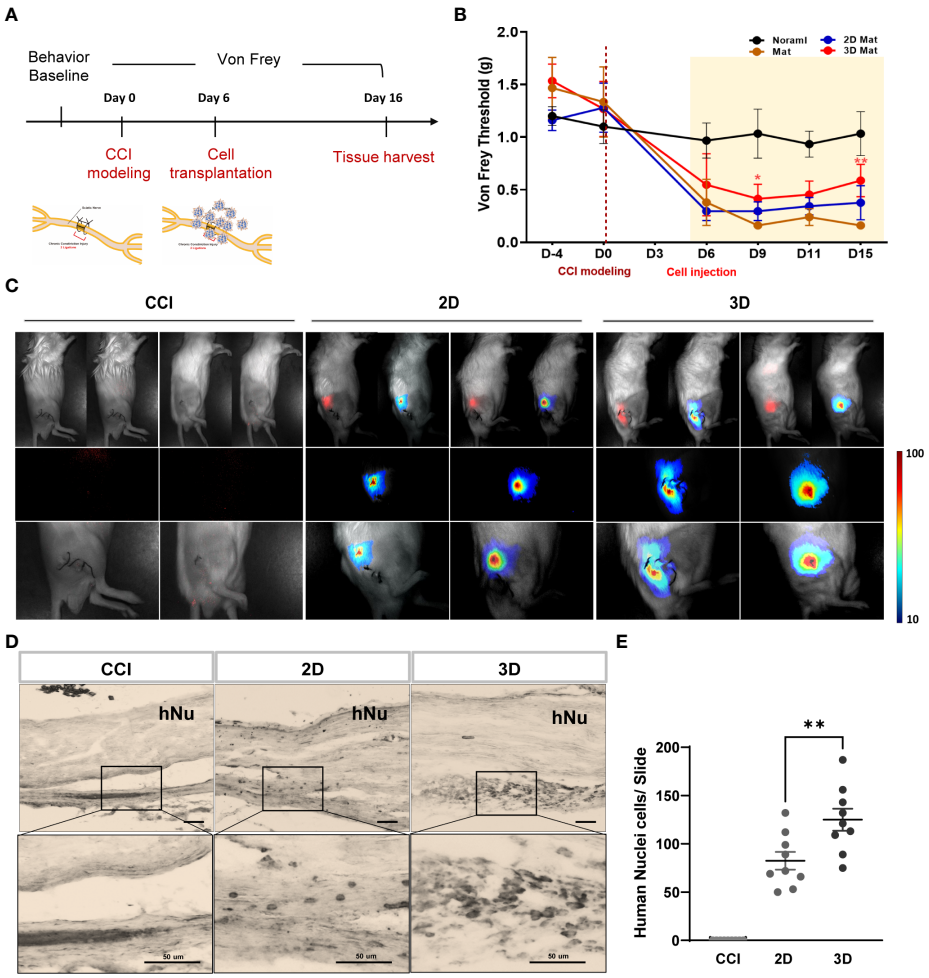

FIGURE 4
Von Frey test and grafted cell survival of MSC spheroids compared to monolayer cells. **(A)** Schematic overview of MSC spheroids and monolayer cell transplantation. All experimental groups are normal, vehicle, monolayer (2D MSC) and spheroid (3D MSC) (n = 6 mice for each group). Before CCI modeling (D-4), all groups were measured the baseline threshold of von Frey test. On day 6, vehicle and cell transplanted groups (1 X 10^6 cells/100ul for 2D and 100 spheroids/100ul for 3D) were transplanted into the injured hindlimb sciatic muscle region. All groups were performed the von Frey test every 3~4 days and sacrificed them on day 15. **(B)** Von Frey test of normal, vehicle, 2D MSC and 3D MSCs. **(C)** *In vivo* NIR-2 fluorescence whole body imaging. The grafted cells are brightly labeled and detected on the injured sciatic nerve area. DAB immunohistochemistry staining **(D)** and number of positive cells **(E)** using an antibody against human nuclei (hNu) in the sciatic nerve. Scale bar: 50 μm. Von Frey threshold curves were statistically analyzed by two-way ANOVA. Data are presented as mean ± SEM, $^{**}p < 0.01$. Statistical differences for the human nuclei cells were analyzed using one-way ANOVA, followed by Tukey's *post hoc* test, $^{**}p < 0.01$.

To further explore the molecular mechanism underlying the peripheral inflammation observed within the injured nerve after cell transplantation, we analyzed the expression of genes involved in the host inflammatory response (Supplementary Table 1). The injured sciatic nerves isolated from the CCI, 2D MSC, and 3D MSC groups, including the control and inflammatory response genes, were examined *via* qPCR screening analysis. Genetic heatmap analysis revealed that although 30 genes related to mouse-specific inflammatory signaling were upregulated in the CCI group compared to the normal group, these genes were significantly downregulated in both the 2D and 3D groups (Figure 6A). Moreover, human-specific inflammatory genes were not expressed in the CCI group, and a few of these genes were upregulated in the 2D and 3D groups (Figures 6A, C). Finally, a comparison of 3D and 2D MSCs revealed that the overall expression of mouse inflammatory genes decreased to a greater extent in the 3D condition (Figure 6B). The engrafting of 2D and 3D MSCs mainly reduced the expression of interleukin–cytokine signaling genes (*Ifng*, *Ilb*, and *Il22*), cytokine receptor genes (*Ccr7* and *Cxcr1*), and humoral immune response genes (*Ccl22*, *Il1b*, and *Nfkb1*) (Figure 6D). Interestingly, compared to other signaling,

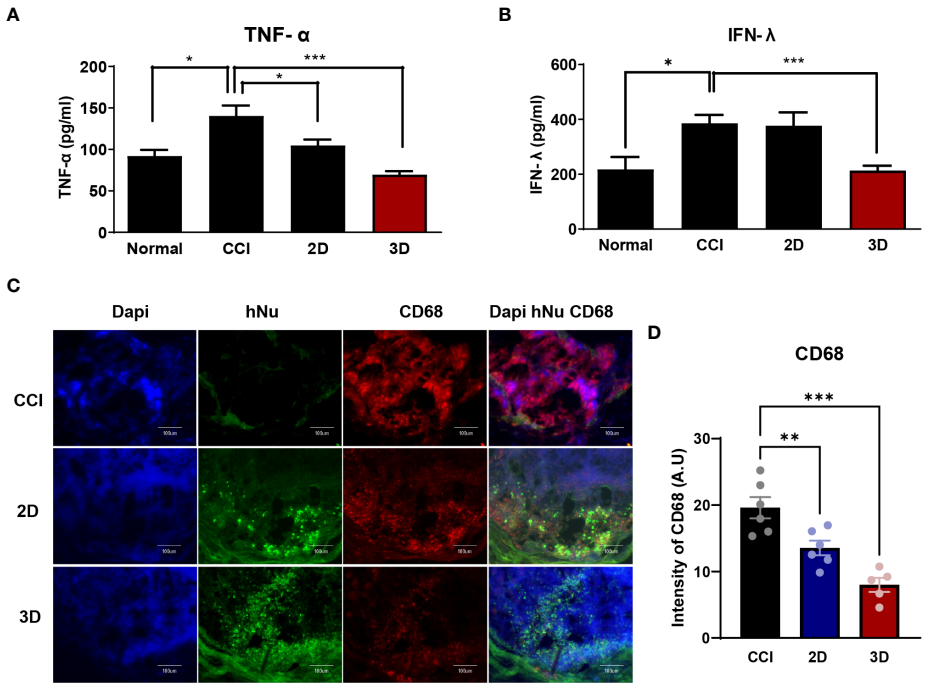

FIGURE 5
The expression of inflammatory factors in CCI, MSC 2D and 3D graft sites. Pro-inflammatory cytokine tumor necrosis factor- α (TNF-α) **(A)** and interferon- γ (IFN-r) **(B)** level in normal, CCI and cell transplantation groups. Cytokine concentrations are indicated as pg/ml (n = 6 per group). **(C)** Immunofluorescence staining of CD68 positive cells (Red) and human nuclei positive cells (hNu; Green) double staining with DAPI (Blue) in the cell transplantation region. Scale bar: 100 μm. **(D)** Quantification of CD68 intensity in the CCI and cell transplantation groups. (n = 6 per group). The area and fluorescent intensities of all CD68 positive cells in each cell grafted regions measured by Image J. Intensity is indicated as arbitrary unit (A.U.). Scale bar: 100 μm. Data are presented as mean ± SEM. Statistical differences for the intensities of CD68 expression were analyzed using one-way ANOVA, followed by Tukey's *post hoc* test, *p < 0.05, **p < 0.01, ***p < 0.005.

the main genes involved in the functional signaling of chronic inflammatory responses, such as *Ccl11/Eotaxin*, *Il1A*, *Lta*, and *Tnf*, were greatly downregulated under 3D groups. These finding indicates that, under conditions of neuropathic pain, the chronic inflammatory response genes may be key regulators to reduce the pain response and improve motor behavior after cell transplantation. Therefore, we suggest that transplantation of MSC spheroids has a sufficient effect in reducing and modulating inflammation-related genes triggered by nerve injury pain *via* chronic inflammatory response signaling.

Discussion

The CCI model is a simple means of replicating chronic pain, although the histological and behavioral results of nerve ligation vary (17–19). Therefore, it is necessary to examine the ligations regarding the effects of consistent compressive pain. To this end, we first investigated a suitable number of ligation knots on the sciatic nerve on the right hindlimb. All groups underwent von Frey testing, which measures the paw response to the application of filament force. The results showed that both two and four ligations induced hyperarlgesia and produced the CCI neuropathic pain phenotype. Consistent with the pain response, two ligations produced muscle atrophy/weakness and moderate nerve damage compared to one ligation, whereas four ligations resulted in severe nerve damage, such as sensory loss and hyper-inflammation. Therefore, we selected two ligation knots to generate a CCI neuropathic pain mouse model.

Many studies on the treatment of neuropathic pain using stem cells have shown promising results in preclinical research, with MSCs being the best tool for cell-based therapy (8–10). However, the important aspects of the mechanism and signaling underlying that occur the injured regions after MSC transplantation remain unclear. Next, for spheroid aggregation, we cultured MSCs using the hanging drop method, as shown in Figure 3A (24–26). Previous studies have reported the formation of spheroids of varying sizes using this method. Importantly, the aggregation size must be carefully

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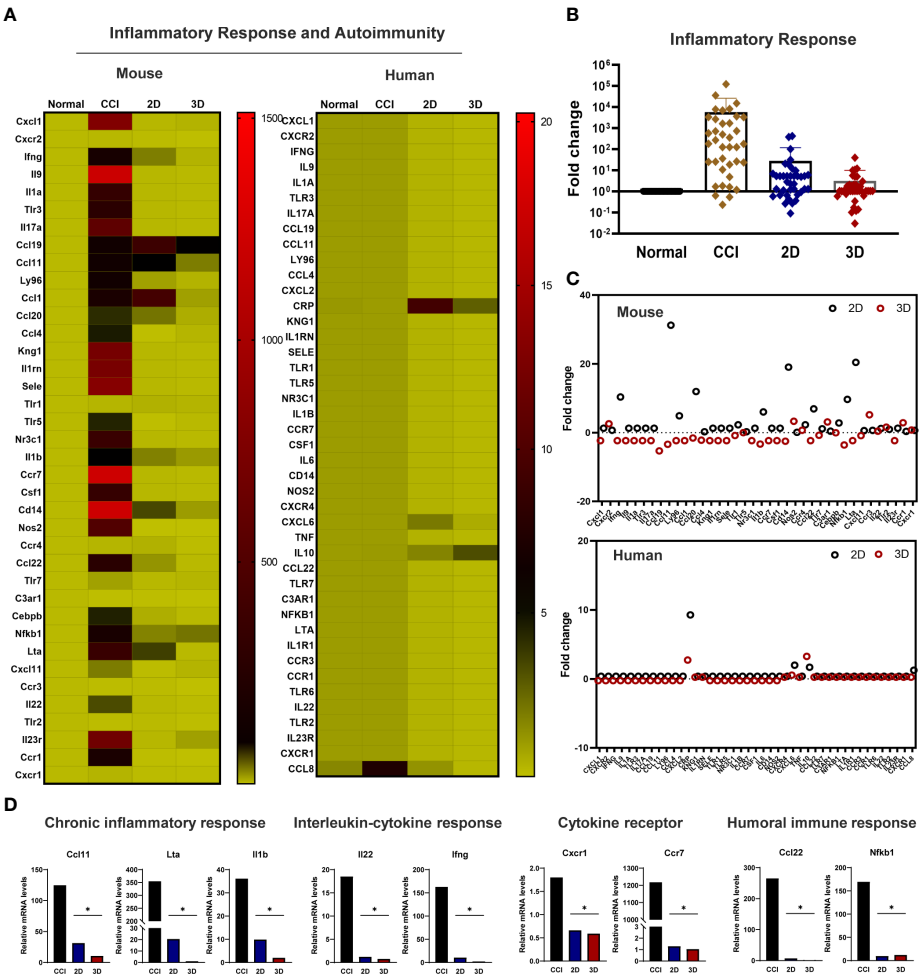

FIGURE 6
Modulation of the inflammatory response genes in the transplanted MSC spheroid and monolayer cells. **(A)** Genetic heatmap analysis revealed that 30 genes were associated with mouse- and human-specific inflammatory genes. Primers were obtained from Accutarget ™ qPCR Screening kit (Bioneer). Target gene expression was determined by normalization to endogenous GAPDH or actin using the comparative cycle time method. **(B)** Fold change of whole inflammatory response genes related to mouse-specific response in CCI and cell transplantation groups. **(C)** Scatter plot of mouse and human-specific inflammatory response genes. **(D)** In the comparison of 3D and 2D MSCs, mainly reduced the expression of chronic inflammatory response genes (*Ccl11* (Eotaxin), *Il1a* and *Lta*), interleukin–cytokine signaling genes (*Ifng*, *Il1b*, and *Il22*), cytokine receptor genes (*Ccr7* and *Cxcr1*), and humoral immune response genes (*Ccl22*, *Il1b*, and *Nfkb1*) (n = 4 per group). Data are presented as mean ± SEM. Statistical differences were analyzed using one-way ANOVA, followed by Tukey's *post hoc* test, $*p < 0.05$.

considered because of the limitations in the diffusive length of nutrient transport. Waslberg et al. showed that the core of spheroids with radii > 200 μm is vulnerable to hypoxia, with cell death and breaking, during application using a syringe and needle (15, 31–33). Thus, we examined the range of 1000 to 25000 cells per spheroid and observed the diameter of this range. In our experiment, spheroids of approximately 10000 cells had a mean radius of approximately 200 μm and were the most viable, as assessed based on caspase 3 activity, compared to spheroids of larger sizes. Moreover, when 10000 spheroids were passed through a 26-gauge syringe needle, their surface was maintained more consistently than that of the larger spheroids. Based on this result, we aggregated 3D spheroids at a density of 10000 cells and investigated their *in vivo* effects on CCI neuropathic pain.

MSC spheroids elicit neuroprotective and anti-inflammatory effects, which are known MSC therapies in disease models (11–14). However, the detailed mechanisms underlying the effects of spheroids on neuropathic pain remain unclear. For cell transplantation, spheroid MSCs and monolayer MSCs at a density of 1×10^6 cells were transplanted close to the sciatic nerve. The pain response was measured by the von Frey test. In the spheroid group, 3D MSCs exhibited a significantly improved pain response. Furthermore, we found that engrafted 2D MSCs

with phosphate-buffered saline (PBS) spread out to other regions and migrated to different target sites, whereas 3D MSCs with PBS persisted well at the grafting site (data not shown). This demonstrated that 3D spheroids have better survival at the target site than 2D MSCs and may alleviate hyperalgesia. To confirm this finding, we investigated the levels of proinflammatory cytokines triggered by inflammation derived from host immune cells and their effect on cell survival. The levels of TNF-α and IFN-γ released into the plasma were greatly reduced (by approximately 50%) in 3D MSCs, indicating that the inflammatory response is regulated at the engraftment site. Overall, immediately after nerve damage, immune cells are activated to release cytokines and chemokines, followed by macrophage infiltration into the damaged region (within hours to days) (34–37). Here, we identified 30 genes associated with the upregulation of the mouse-specific inflammatory response in the CCI group, as well as genes that were significantly downregulated in interleukin-cytokine signaling, cytokine signaling (38), and the humoral immune response in 2D and 3D MSCs (39). Importantly, we found that implantation of MSC spheroids was modulated by chronic inflammatory response signaling to reduce inflammation.

In this study, we established a suitable animal model of CCI neuropathic pain and observed the good survival potential afforded by MSC spheroid injection and the good effects of the injected MSC spheroids on the repair of pain defects compared to the monolayer cell condition. We demonstrated that MSC spheroids containing 10000 cells exhibited greater resistance to apoptosis and significantly reduced secretion of immune response factors induced by CCI. Furthermore, good graft survival of spheroids dramatically modulated proinflammatory cytokines and genes related to chronic inflammatory response, suggesting that MSC spheroids may enhance the alleviation of pain and motor function. These data provide strong evidence that spheroids have the potential to facilitate MSC-based cell therapies for the repair of induced neuropathic pain injury.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies using human tonsils were reviewed and approved by the Institution Review Board of Pusan National University Hospital (1801-033-062). The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by Institutional Animal Care and Use Committee of Pusan National University.

Author contributions

NL designed the study and wrote the manuscript. GP wrote the manuscript and performed the experiments. JL performed the experiments. EC, HM, DK, and SC analyzed the data. YS obtained the TMSCs. JK conceived and supervised the project. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.940258/full#supplementary-material

References

1. Marchettini P, Lacerenza M, Mauri E, Marangoni C. Painful peripheral neuropathies. *Curr Neuropharmacol* (2006) 4(3):175–81. doi: 10.2174/157015906778019536

2. Colloca L, Ludman T, Bouhassira D, Baron R, Dickenson AH, Yarnitsky D, et al. Neuropathic pain. *Nat Rev Dis Primers* (2017) 3:17002. doi: 10.1038/nrdp.2017.2

3. Hylands-White N, Duarte RV, Raphael JH. An overview of treatment approaches for chronic pain management. *Rheumatol Int* (2017) 37(1):29–42. doi: 10.1007/s00296-016-3481-8

4. Varshney V, Osborn J, Chaturvedi R, Shah V, Chakravarthy K. Advances in the interventional management of neuropathic pain. *Ann Transl Med* (2021) 9 (2):187. doi: 10.21037/atm-20-6190

5. Lowy DB, Makker PGS, Moalem-Taylor G. Cutaneous neuroimmune interactions in peripheral neuropathic pain states. *Front Immunol* (2021) 12:660203. doi: 10.3389/fimmu.2021.660203

6. Varrassi G, Müller-Schwefe G, Pergolizzi J, Orónska A, Morlion B, Mavrocordatos P, et al. Pharmacological treatment of chronic pain - the need for change. *Curr Med Res Opin* (2010) 26(5):1231–45. doi: 10.1185/03007991003689175

7. Asgharzadeh H, Shahmohammadi A, Varble N, Levy EI, Meng H, Borazjani I. A simple flow classification parameter can discriminate rupture status in intracranial aneurysms. *Neurosurgery* (2020) 87(5):E557–E64. doi: 10.1093/neuros/nyaa189

8. Joshi HP, Jo HJ, Kim YH, An SB, Park CK, Han I. Stem cell therapy for modulating neuroinflammation in neuropathic pain. *Int J Mol Sci* (2021) 22 (9):4853. doi: 10.3390/ijms22094853

9. Liu M, Li K, Wang Y, Zhao G, Jiang J. Stem cells in the treatment of neuropathic pain: Research progress of mechanism. *Stem Cells Int* (2020) 2020:8861251. doi: 10.1155/2020/8861251

10. Yoo SH, Lee SH, Lee S, Park JH, Lee S, Jin H, et al. The effect of human mesenchymal stem cell injection on pain behavior in chronic post-ischemia pain mice. *Korean J Pain* (2020) 33(1):23–9. doi: 10.3344/kjp.2020.33.1.23

11. Chen LC, Wang HW, Huang CC. Modulation of inherent niches in 3d multicellular msc spheroids reconfigures metabolism and enhances therapeutic potential. *Cells* (2021) 10(10):2747. doi: 10.3390/cells10102747

12. Noronha NC, Mizukami A, Caliári-Oliveira C, Cominal JG, Rocha JLM, Covas DT, et al. Priming approaches to improve the efficacy of mesenchymal stromal cell-based therapies. *Stem Cell Res Ther* (2019) 10(1):131. doi: 10.1186/s13287-019-1224-y

13. Sart S, Tsai AC, Li Y, Ma T. Three-dimensional aggregates of mesenchymal stem cells: Cellular mechanisms, biological properties, and applications. *Tissue Eng Part B Rev* (2014) 20(5):365–80. doi: 10.1089/ten.TEB.2013.0537

14. Jauković A, Abadjieva D, Trivanović D, Stoyanova E, Kostadinova M, Pashova S, et al. Specificity of 3d msc spheroids microenvironment: Impact on msc behavior and properties. *Stem Cell Rev Rep* (2020) 16(5):853–75. doi: 10.1007/s12015-020-10006-9

15. Schmehl J, Stoll H, Danalache M, Grözinger GC, Greiner TO, Leibfritz RF, et al. Intravascular application of labelled cell spheroids: An approach for ischemic peripheral artery disease. *Int J Mol Sci* (2021) 22(13):6831. doi: 10.3390/ijms22136831

16. Austin PJ, Wu A, Moalem-Taylor G. Chronic constriction of the sciatic nerve and pain hypersensitivity testing in rats. *J Vis Exp* (2012) 61):3393. doi: 10.3791/3393

17. Chen SH, Wu CC, Lin SC, Tseng WL, Huang TC, Yadav A, et al. Investigation of neuropathology after nerve release in chronic constriction injury of rat sciatic nerve. *Int J Mol Sci* (2021) 22(9):4746. doi: 10.3390/ijms22094746

18. Chen SH, Huang TC, Wang JY, Wu CC, Hsueh YY. Controllable forces for reproducible chronic constriction injury mimicking compressive neuropathy in rat sciatic nerve. *J Neurosci Methods* (2020) 335:108615. doi: 10.1016/j.jneumeth.2020.108615

19. Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* (1988) 33(1):87–107. doi: 10.1016/0304-3959(88)90209-6

20. Mogil JS, Graham AC, Ritchie J, Hughes SF, Austin JS, Schorscher-Petcu A, et al. Hypolocomotion, asymmetrically directed behaviors (Licking, lifting, flinching, and shaking) and dynamic weight bearing (Gait) changes are not measures of neuropathic pain in mice. *Mol Pain* (2010) 6:34. doi: 10.1186/1744-8069-6-34

21. Urban R, Scherrer G, Goulding EH, Tecott LH, Basbaum AI. Behavioral indices of ongoing pain are largely unchanged in Male mice with tissue or nerve injury-induced mechanical hypersensitivity. *Pain* (2011) 152(5):990–1000. doi: 10.1016/j.pain.2010.12.003

22. Ravi M, Paramesh V, Kaviya SR, Anuradha E, Solomon FD. 3d cell culture systems: Advantages and applications. *J Cell Physiol* (2015) 230(1):16–26. doi: 10.1002/jcp.24683

23. McKee C, Chaudhry GR. Advances and challenges in stem cell culture. *Colloids Surf B Biointerfaces* (2017) 159:62–77. doi: 10.1016/j.colsurfb.2017.07.051

24. Huang SW, Tzeng SC, Chen JK, Sun JS, Lin FH. A dynamic hanging-drop system for mesenchymal stem cell culture. *Int J Mol Sci* (2020) 21(12):4298. doi: 10.3390/ijms21124298

25. Todorov L, VadeBoncouer T. Etiology and use of the "Hanging drop" technique: A review. *Pain Res Treat* (2014) 2014:146750. doi: 10.1155/2014/146750

26. Frey O, Misun PM, Fluri DA, Hengstler JG, Hierlemann A. Reconfigurable microfluidic hanging drop network for multi-tissue interaction and analysis. *Nat Commun* (2014) 5:4250. doi: 10.1038/ncomms5250

27. Lu L, Li B, Ding S, Fan Y, Wang S, Sun C, et al. Nir-ii bioluminescence for *in vivo* high contrast imaging and in situ atp-mediated metastases tracing. *Nat Commun* (2020) 11(1):4192. doi: 10.1038/s41467-020-18051-1

28. Chen C, Chen F, Yao C, Shu S, Feng J, Hu X, et al. Intrathecal injection of human umbilical cord-derived mesenchymal stem cells ameliorates neuropathic pain in rats. *Neurochem Res* (2016) 41(12):3250–60. doi: 10.1007/s11064-016-2051-5

29. Aso K, Tsuruhara A, Takagaki K, Oki K, Ota M, Nose Y, et al. Adipose-derived mesenchymal stem cells restore impaired mucosal immune responses in aged mice. *PloS One* (2016) 11(2):e0148185. doi: 10.1371/journal.pone.0148185

30. Qadura M, Terenzi DC, Verma S, Al-Omran M, Hess DA. Concise review: Cell therapy for critical limb ischemia: An integrated review of preclinical and clinical studies. *Stem Cells* (2018) 36(2):161–71. doi: 10.1002/stem.2751

31. Smith EJ, Stroemer RP, Gorenkova N, Nakajima M, Crum WR, Tang E, et al. Implantation site and lesion topology determine efficacy of a human neural stem cell line in a rat model of chronic stroke. *Stem Cells* (2012) 30(4):785–96. doi: 10.1002/stem.1024

32. Amer MH, Rose F, Shakesheff KM, Modo M, White LJ. Translational considerations in injectable cell-based therapeutics for neurological applications: Concepts, progress and challenges. *NPJ Regener Med* (2017) 2:23. doi: 10.1038/s41536-017-0028-x

33. Wahlberg B, Ghuman H, Liu JR, Modo M. *Ex vivo* biomechanical characterization of syringe-needle ejections for intracerebral cell delivery. *Sci Rep* (2018) 8(1):9194. doi: 10.1038/s41598-018-27568-x

34. Schomberg D, Ahmed M, Miranpuri G, Olson J, Resnick DK. Neuropathic pain: Role of inflammation, immune response, and ion channel activity in central injury mechanisms. *Ann Neurosci* (2012) 19(3):125–32. doi: 10.5214/ans.0972.7531.190309

35. Moalem G, Tracey DJ. Immune and inflammatory mechanisms in neuropathic pain. *Brain Res Rev* (2006) 51(2):240–64. doi: 10.1016/j.brainresrev.2005.11.004

36. Kuffler DP. Injury-induced effectors of neuropathic pain. *Mol Neurobiol* (2020) 57(1):51–66. doi: 10.1007/s12035-019-01756-w

37. Hung AL, Lim M, Doshi TL. Targeting cytokines for treatment of neuropathic pain. *Scand J Pain* (2017) 17:287–93. doi: 10.1016/j.sjpain.2017.08.002

38. Vanderwall AG, Milligan ED. Cytokines in pain: Harnessing endogenous anti-inflammatory signaling for improved pain management. *Front Immunol* (2019) 10:3009. doi: 10.3389/fimmu.2019.03009

39. Baral P, Udit S, Chiu IM. Pain and immunity: Implications for host defence. *Nat Rev Immunol* (2019) 19(7):433–47. doi: 10.1038/s41577-019-0147-2

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Tryptophan metabolism: Mechanism-oriented therapy for neurological and psychiatric disorders

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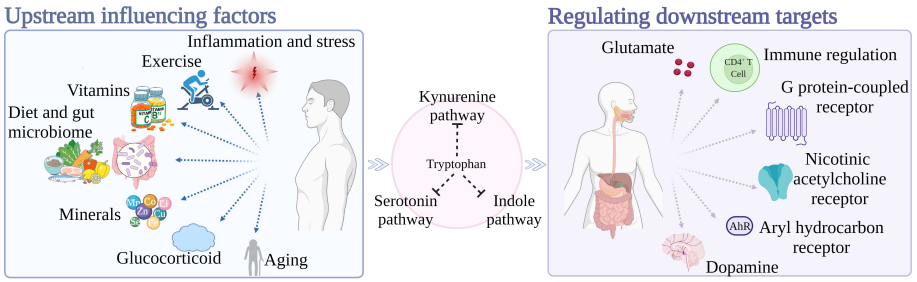
Neurological and psychiatric disorders are a category of chronic diseases that are widespread and pose serious mental and physical health problems for patients. The substrates, products, and enzymes of Tryptophan metabolism all contribute to the development of neurological and psychiatric disorders. This paper deals with three metabolic pathways of tryptophan that produce a series of metabolites called tryptophan Catabolics (TRYCATs). These metabolites are involved in pathological processes such as excitotoxicity, neuroinflammation, oxidative stress, and mitochondrial damage and are closely associated with neurological and psychiatric disorders such as Alzheimer's disease and depression. Here, we review the elements that affect how tryptophan metabolism is regulated, including inflammation and stress, exercise, vitamins, minerals, diet and gut microbes, glucocorticoids, and aging, as well as the downstream regulatory effects of tryptophan metabolism, including the regulation of glutamate (Glu), immunity, G-protein coupled receptor 35 (Gpr35), nicotinic acetylcholine receptor (nAChR), aryl hydrocarbon receptor (AhR), and dopamine (DA). In order to advance the general understanding of tryptophan metabolism in neurological and psychiatric disorders, this paper also summarizes the current situation and effective drugs of tryptophan metabolism in the treatment of neurological and psychiatric disorders and considers its future research prospects.

KEYWORDS

tryptophan metabolism, neurotoxicity, neuroprotection, influence factor, neurological, psychiatric disorders



Drug treatment and current situation of diseases

Alzheimer's disease	Depression	Anxiety disoder
Parkinson's disease	Multiple sclerosis	Amyotrophic lateral sclerosis
Huntington's disease	Schizophrenia	Autism spectrum disorder
	Epilepsy	

GRAPHICAL ABSTRACT

1 Introduction

According to the World Health Organization (WHO), approximately 6.8 million people worldwide die each year from a variety of neurological and psychiatric disorders, including Stroke, Alzheimer's disease (AD), and Parkinson's disease (PD). Not only are neurological and psychiatric disorders expensive to treat, but patients also experience significant stigma, social exclusion, and poor quality of life (1). Neurological and psychiatric disorders can be caused by external stresses and internal genetic factors. Initial mood disorders, if left untreated, can gradually cause changes in brain physiological activity and eventually develop into neurological and psychiatric disorders (2). The prevalence of mental disorders is increasing worldwide year by year, and the disease burden of mental disorders ranks first among chronic diseases and is one of the three leading causes of disability (3). In the face of the severe personal and social burden caused by neurological and psychiatric disorders, efforts should be made to provide solutions to benefit patients. In order to fulfill the demand for disease-oriented therapies, mechanism-oriented therapies must be implemented on the supply side (4). Mechanism-oriented therapy is the basis and the means, while disease-oriented therapy is the end and the way out, and they are not contradictory. At the same time, focusing on mechanism-oriented therapy can address the co-morbidity of diseases and ultimately achieve the goal of meeting clinical needs.

Currently, the tryptophan metabolic pathway is considered to be a major pathway connecting multiple systems such as the immune inflammatory response of the nervous system, involving stress, inflammation, the kynurenine pathway, 5-HTergic, and glutamatergic neurotransmission (5). Various enzymes or products of the tryptophan metabolic process are extremely closely related to neurological disorders (Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic lateral sclerosis, Multiple sclerosis, Autism, Epilepsy) and psychiatric disorders (Depression, Schizophrenia, Bipolar disorder, Anxiety) with a high degree of co-morbidity (6). The study of its effect on upstream and downstream is conducive to a holistic understanding of the mechanism of action of this pathway, and provides support for the realization of drug development from mechanism-oriented therapy to disease-oriented therapy. To this end, we outline several metabolic pathways and metabolites of tryptophan, as well as the upstream and downstream influences on tryptophan metabolism. Finally, effective drugs and research advances in tryptophan metabolism in neuropsychiatric disorders are summarized.

2 Tryptophan

Tryptophan (TRP), an alfa-amino acid with the chemical name 2-amino-3-(1H-indol-3-yl) propanoic acid, may exist as L, D, or DL isomers (7). It is an essential amino acid for protein synthesis, which is the least abundant in cells and proteins (8). It can only be obtained through diet and is the substrate of several bioactive compounds with important physiological effects (9). TRP is metabolized to various bioactive compounds through

different metabolic pathways, which in turn participate in various physiological activities in the body and regulate body homeostasis. These TRYCATs include kynurenine(KYN), serotonin, melatonin, indole, tryptamine, vitamin B3, protein, etc (10). However, only free TRP can participate in metabolism and unbound TRP can be transported across the blood-brain barrier (BBB) *via* non-specific and competitive Large neutral amino acid transporter (LAT). As the only amino acid that can bind to albumin, TRP is first bound to plasma albumin and then released from albumin in the presence of free fatty acids to function (11). In general, 85-90% of TRP is present in the plasma in the bound form. By competitively binding to albumin, substances like free fatty acids can replace TRP and raise the concentration of free TRP in the body.

3 Tryptophan metabolic pathway

TRP is metabolized through three pathways, the kynurenine pathway (KP), the serotonin pathway (SP), and the indole pathway (IP). Imbalances in TRP metabolic pathways, particularly an excess or change in the ratio of metabolites of specific neuroactive features, are thought to be responsible for a variety of neurological and psychiatric disorders (Figure 1).

3.1 Kynurenine pathway

In the metabolism of TRP, 95-99% of TRP is metabolized toward the KP. Through signaling cascade reaction, TRP is catalyzed by indoleamine 2, 3-dioxygenase (IDO) and tryptophan 2, 3-dioxygenase (TDO), the first rate-limiting enzymes of the tryptophan metabolic pathway, to produce N-formyl-kynurenine, followed by the formation of KYN in the presence of formamidase (12, 13). Not only is KYN the first stable product of KP, but it is also the central point of KP. 60% of KYN in the central nervous system (CNS) is derived from peripheral KYN carried by LAT across the BBB and degraded in the CNS *via* two cellular pathways, astrocytes and microglia (14). This pathway is involved in inflammation, immune response, and excitatory neurotransmission, and is associated with a variety of neurological and psychiatric disorders, as well as with liver and kidney dysfunction, cataracts, diabetes, and various chronic malignant diseases and abnormal pregnancies (15). Modern pharmacological studies have revealed that metabolites of KYN can modulate glutamatergic neurotransmission. In particular, they exert neurotoxic/neuroprotective effects through the antagonist/agonist activity of the N-methyl-D-aspartate receptor (NMDAR). Therefore, KP is considered to be a bridge between systemic inflammation and tissue and organ function.

TDO is found in eukaryotes and bacteria, but IDO is only found in mammals and yeast, although mammals can express both IDO and TDO (16). IDO (IDO-1, IDO-2) is considered to be the main catalytic enzyme for KYN production under inflammation or stress, and can be activated by various pro-inflammatory factors such as interferon (IFN-γ), lipopolysaccharide (LPS), and tumor necrosis factor (TNF), which in turn accelerate KP (17). Among them, IDO-1 is widely expressed in immune and non-immune tissues other than the liver, including in immune cells such as microglia, astrocytes, and macrophages (11). Activation of IDO-1

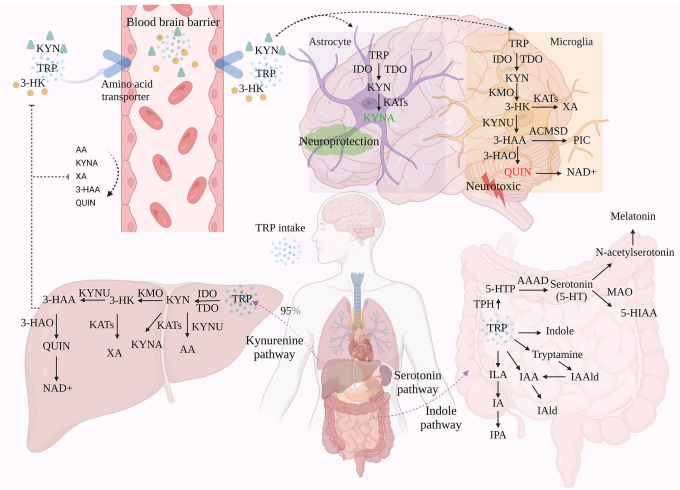


FIGURE 1
The three metabolic pathways of TRP. TRP is metabolized through three different pathways: KP, SP, and IP, with KP accounting for around 95% of TRP metabolism in the liver. Under the action of a series of enzymes, TRP generates metabolites such as KYN and KYNA *via* KP. Of these, only TRP, KYN, and 3-HK can enter the brain through the BBB, which in turn is involved in the neurotoxic and neuroprotective branches of TRP. A small proportion of TRP is metabolized in the gastrointestinal tract *via* SP and IP.

may be associated with activation of NF-κB and MAPK signaling pathways by inflammatory factors and release of IFN-γ by activated $CD4^+$ T cells (18). IDO-2 is mainly expressed in the liver, kidney, and epididymis of mice and catalyzes the same substrates as IDO-1, but its activity is much lower than IDO-1. TDO, on the other hand, is present in large amounts in the liver and is preferentially activated by glucocorticoids, but also by low concentrations of reactive oxygen species (ROS), however, accumulation of ROS inactivates TDO. Overall, TDO is only responsible for the metabolism of KP in normal physiological situations, while IDO acts more in pathological situations.

It is generally accepted that TRP is metabolized to quinolinic acid (QUIN) in microglia and macrophages, or kynurenic acid (KYNA) in astrocytes. Depending on the activity of the metabolite, this reaction is considered to be "neuroprotective" in astrocytes and "neurotoxic" in microglia and macrophages, thus achieving a duality in terms of disease. Therefore, QUIN levels and the QUIN/TRP ratio are often used as important indicators of pathological changes in neurocentral diseases. Among them, QUIN is an important metabolite of the KP pathway that severely contributes to neuronal cell death and chronic dysfunction through at least nine different mechanisms, including ROS production, disruption of the BBB, glutamate (Glu) excitotoxicity, cytoskeletal instability, mitochondrial dysfunction, promotion of tau protein phosphorylation, and disruption of autophagy (19, 20).In conclusion, KYN has three different metabolic pathways due to different enzymes, conversion to 3-hydroxykynurenine (3-HK) *via* kynurenine 3-monooxygenase (KMO), dehydrogenation to KYNA *via* kynurenine aminotransferase (KAT), and degradation of kynureninase (KYNU) to anthranilic acid (AA), the ratio of different kynurenine metabolite concentrations has been widely used as an indicator of the enzymatic activity of different pathways (21). Currently, TRP, KYN, and 3-HK have been shown to enter the brain *via* the BBB and subsequently produce kynurenine metabolites in activated macrophages and microglia (21). The rate of their transport through the BBB depends on the concentration of LAT in the blood.

3.1.1 Neurotoxic branch

KMO (located in the outer mitochondrial membrane), the main synthetase of the QUIN pathway, converts kynurenine to 3-HK. This component crosses the BBB and increases the substrate required for QUIN production, producing neuronal apoptosis, free radical generation, and oxidative stress damage in the brain at nanomolar concentrations, leading to elevated metal toxicity in rat astrocyte cultures and effectively synergizing the neurotoxic effects of QUIN (22). Subsequently, 3-HK is formed into 3-hydroxyanthranilic acid (3-HAA) by the action of KYNU. It is then converted to QUIN by the action of 3-hydroxyanthranilic acid dioxygenase (3-HAO) and finally degraded to nicotinamide adenine dinucleotide (NAD+) (23). In addition, 3-HK can produce Xanthurenic acid (XA) in the presence of KAT, and 3-HAA can produce picolinic acid (PIC) in the presence of 2-amino3-carboxymuconate-6-semialdehydedecarboxylase (ACMSD). XA is a regulator of glutamatergic synaptic transmission, which can directly or indirectly regulate metabolic Glu receptors (24). PIC is a non-selective metal ion chelator and neuroprotective agent, which can inhibit QUIN induced neurotoxicity, but the inhibitory effect is weaker than KYNA (25).

When QUIN is in a high expression state, microglia and neurons are restricted in their catabolism of NAD+, which in turn leads to cumulative neurotoxicity of QUIN, manifested in recurrent major depression patients having higher levels of KYN metabolites (26). QUIN is an NMDAR activator that enhances Glu release from neurons, leading to excess microenvironmental Glu concentrations and neurotoxicity, and can also induce selective apoptosis of novel glial cells. Meanwhile, prolonged exposure of neurons to elevated QUIN disrupts the structure of dendrites and reduces the immunoreactivity of microtubule-associated protein 2, thereby perpetuating neurodegenerative disease (27). In addition to this, QUIN-induced free radical generation and oxidative stress may also produce neurotoxicity. Its induction of lipid peroxidation is regulated by interaction with Fe^{2+}, forming QUIN-Fe^{2+} complexes that promote ROS production (21).

3.1.2 Neuroprotection branch

KYN generates KYNA in the mammalian CNS in the presence of KAT. among the four KATs, KAT II is mainly expressed in astrocytes and is responsible for most KYNA synthesis. KYNA exerts a neuroprotective effect against the excitotoxic and apoptotic effects of NMDAR due to its ability to antagonize this receptor (28). In addition, KYNA can antagonize α7-nicotinic acetylcholine receptor (α7-nAChR), reducing extracellular Glu and dopamine (DA) levels (29). And it plays a direct immunomodulatory role through the role of G-protein coupled receptor 35 (GPR35) and Aryl hydrocarbon receptor (30). However, high levels of KYNA can be detrimental, leading to low Glu levels that interfere with cognitive function and are associated with psychiatric disorders such as Schizophrenia, including memory deficits and reduced dopaminergic and glutamatergic neurotransmission (26).

3.1.3 Relationship between quinolinic acid and kynurenic acid

There is a dynamic balance between activation and antagonism of NMDA receptors by QUIN and KYNA, and this balance ensures normal calcium and sodium influx into the glutamatergic postsynapse, thereby increasing synaptic plasticity and cell survival. QUIN and KYNA cannot pass through the BBB, which can indicate whether the integrity or function of the patient's BBB is damaged. In addition, QUIN production is

excessive under disease conditions, while KYNA cannot fully block QUIN. Moreover, LPS induced the expression of KMO rather than KATII, which proved that systemic inflammation stimulated the transfer of KP to the neurotoxicity branch (17). The agonistic and antagonistic metabolites produced by KP metabolism are balance. Once disturbed, it will cause functional and structural damage to the CNS. However, the over activation of KP will regulate the disorder of balance through negative feedback. Therefore, KP usually has both pathological mechanisms and compensatory mechanisms (31).

3.2 Serotonin pathway

About 1-2% of TRP is produced as 5-hydroxytryptophan (5-HTP) by tryptophan hydroxylase (TPH) and metabolized to serotonin (5-HT), an important monoamine neurotransmitter, by Aromatic Amino Acid Decarboxylase (AAAD), for which TRP is the only precursor substance. Serotonin reuptake transporter (SERT) is the only efficient transporter of 5-HT in a physiological state. Re-absorption of 5-HT in interstitial space can maintain the 5-HT system homeostasis by transferring 5-HT into intracellular inactivation. In addition, 5-HT is metabolized in two directions:(1 it is transformed into 5-hydroxyindoleacetic acid (5-HIAA) under the action of monoamine oxidase (MAO), which is a biomarker of low-level 5-HT in the brain; (2 it is metabolized into N-acetylserotonin (NAS) and melatonin.

There are two subtypes of TPH, including TPH1 and TPH2. TPH1 mainly exists in enterochromaffin cells (ECS), spleen, pineal gland, and thymus, and TPH2 completely exists in central neuronal cells (32). Up to 90% of the total 5-HT production comes from ECs in the gut and, to a lesser extent, from 5-HTergic neurons in the enteric nervous system (ENS) (33). 5-HT is related to cognition, emotion, feeding, and so on. it is critical for planning and decision-making, and plays an important role centrally and peripherally. Melatonin is a genetic susceptibility factor and an inhibitor of the increase of intestinal permeability induced by alcohol. The decrease of melatonin may mediate some diseases by changing the intestine. In addition, melatonin can also optimize mitochondrial function and drive the regulation of circadian rhythm, which is related to the changes in mental diseases (34).

3.3 Indole pathway

Most bacteria, fungi, and protozoa in the gastrointestinal tract are affected by the availability of TRP and can directly convert TRP into various molecules such as indole and their derivatives. Including tryptamine, indoleacetic-3-acid (IAA), indolepropionic-3-acid (IPA), indolelactic acid (ILA), indole-3-aldehyde (IAld), indoleacrylic acid (IA), indole-3-acetaldehyde (IAAld), etc (35). Most of these metabolites are ligands of AHR. Indole is formed by tryptophanase (TNAA), which is expressed in many Gram-negative and Gram-positive bacterial species, including *Escherichia coli, Clostridium* spp., *and Bacteroides spp* (36). Under the action of indole, the expression of pro-inflammatory cytokines and anti-inflammatory cytokines can promote intestinal health and regulate the coordinated changes of intestinal homeostasis. Trp decarboxylation to produce tryptamines is extremely rare in bacteria. Only *Xenorhabdus nematophilus*, *Bacillus atrophaeus*, and *Lactobacillus bulgaricus* are considered to produce tryptamine (37). Tryptamine is a ligand of Trace amine associated receptors (TAARs) and sigma-2 receptors, and can induce ECs to release 5-HT. In addition, Interleukin-4-induced gene 1 (IL4I1) is a key enzyme of IP (38). It is highly expressed in mature dendritic cells (DCs) and can catalyze Trp to produce IAA and IALD (39). IL4I1 is a potent agonist of AHR because it can mediate the production of indole metabolites (31).

4 Factors regulating tryptophan metabolism

Due to complex regulatory mechanisms, tryptophan metabolism is involved in various physiological mechanisms such as stress, inflammation, and KP metabolism. Therefore, understanding the multilevel control of this pathway can help predict susceptibility to related diseases and achieve multilevel effects from “mechanism-oriented therapy” to “disease-oriented therapy” (Figure 2).

4.1 Inflammation and stress

Pro-inflammatory cytokines are primarily produced by macrophages, but astrocytes, microglia, and neurons can also produce them in the brain. They can pass freely through the BBB by a variety of mechanisms, including passive diffusion through the leaky regions of the BBB, active transport, or *via* nerve fibers such as the vagus or trigeminal nerves (40). Pro-inflammatory cytokines are known to regulate KP enzymes. IDO-1 expression can be induced in several cell types by cytokines such as LPS, TNF, interleukin-1 (IL-1), and interleukin 2 (IL-2). Ibuprofen, a painkiller, boosted levels of the anti-inflammatory markers Arg-1 and YM-1, while decreasing the expression of TDO-2 in the process (41). In particular, IFN-γ released by activated $CD4^+$T cells induced the expression of IDO-1, promoted the catabolism of TRP, and reduced the synthesis of central 5-HT (42). Not only that, but cytokines, such as IFN-γ also affect the binding of the central receptors 5-HT1a and 5-HT2 and increase the release of Glu, resulting in psychiatric disorders (43). Additionally, brain damage and high levels of oxidative stress might trigger the expression of pro-inflammatory cytokines. According to studies, chronic stress can significantly increase levels of interleukin 6

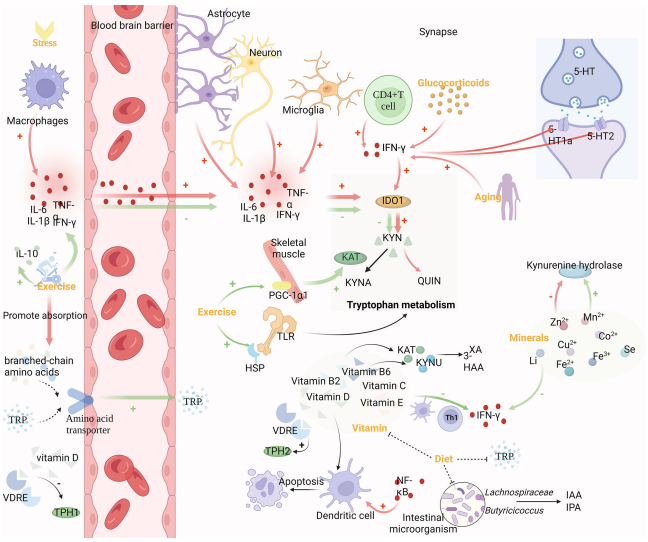


FIGURE 2
Mechanism of upstream regulation of TRP metabolism. Inflammation and stress, exercise, vitamins, diet and gut microbes, minerals, glucocorticoids and aging can modulate tryptophan metabolism. Pro-inflammatory cytokines produced by macrophages can cross the BBB and regulate IDO-1 expression together with pro-inflammatory cytokines produced by astrocytes, microglia, neurons, and $CD4^+$ T cells in the brain. The binding of central 5-HT1a and 5-HT2 receptor sites is also influenced by cytokines like IFN-γ, which affects Glu release. Glucocorticoids and aging can enhance the activity of IFN-γ. Exercise decreases IDO-1 activity *via* anti-inflammatory effects and lessens branched-chain amino acids' competitive effect on TRP entrance into the brain through the BBB, both of which result in an increase in TRP concentrations in the brain. Vitamins are directly involved in regulating in TRP metabolism as cofactors and coenzymes. Gut microbes promoting gut homeostasis, which also contributes to TRP metabolites and ω-3-fatty acids. Vitamins and gut microbes, which jointly govern tryptophan metabolism, can both be controlled by dietary changes. Additionally, minerals are cofactors and enzymes of KP, and KP enzymes are very sensitive to them.

(IL-6) and TNF-α, which in turn results in large drops in TRP, 3-HAA, and indole, suggesting metabolic abnormalities in KP and IP (44).

4.2 Exercise

Aerobic exercise can reduce KYN levels in the circulation and central nervous system through muscle and other means. Long-term exercise has been demonstrated to prevent cognitive deterioration, reduce the level of pro-inflammatory cytokine IL-1β, and increase the level of anti-inflammatory cytokine IL-10 in brain tissue of mice with craniocerebral injury (45). Furthermore, IDO activity is decreased, the content of metabolic product KYN in the TRP/KYN pathway in the brain is reduced, and the concentration of free TRP is increased, so that it can enter the brain more through BBB (46). However, the transport of TRP through BBB depends on the ratio of TRP to branched-chain amino acids, which are more competitive than TRP in trans-BBB transport. Exercise alleviates this competition by increasing muscle absorption of branched-chain amino acids, thereby increasing TRP utilization in the brain (47). In addition, through exercise, skeletal muscle increases KAT expression and pushes the peripheral KP toward KYNA production (33). This effect may be related to the exercise-induced elevated expression of the PGC-1α1 in skeletal muscle cells. Pgc-1α1 effectively regulates KAT activity and protects the brain from the harmful effects of KYN accumulation (48). However, changes in TRYCATs could be detected after the toll-like receptor (TLR) family was activated (49). After acute exercise, the expression of heat shock proteins (HSPs) is upregulated, which may be due to the cross-tolerance of TLR4 and induce the decreased expression of TLR4 in tissue cells (50). Therefore, exercise can also affect TRP metabolism by regulating the TLR family.

4.3 Vitamins

Vitamins, which are necessary for cell growth and metabolism, operate as cofactors and coenzymes in the regulation of TRP metabolism and have an impact on the 5-HT receptor's binding properties in adult rat brains (51). Studies have shown that vitamin D can affect TRP metabolism by affecting TPH1/2 (52). Two different vitamin D response elements (VDRE) exist in the regulatory regions of TPH1 and TPH2, which are responsible for converting TRP into 5-HT. Since VDRE responds differently to vitamins, vitamin D activates TPH2 in the brain and inhibits TPH1

outside of BBB (47). The study found that 58% of people who attempted suicide were vitamin D deficient, and their vitamin D levels were significantly lower than those of healthy individuals and depressed people who were not suicidal (53). Moreover, DCs can be activated by several pathways with NF-κB as the core. Vitamin D can inhibit the differentiation and maturation of immature DCs, inhibit the upregulation of CD40, CD80, and CD86, and promote the spontaneous apoptosis of mature DCs (54). In addition, vitamins B2 and B6 are decisive factors of the KP pathway, have a profound influence on the enzymes of KP, and are also markers of IFN-γ -mediated immune activation (55). Vitamin B2 is also a cofactor of KP, and the concentrations of XA and 3-HAA in plasma are positively correlated with vitamin B2 (56). Vitamin B6 is a cofactor of KYNU and KAT. In the KP process, except for 3-HK, all metabolites depend on vitamin B6 to produce, including 5-HT and melatonin (57–59). Vitamin B3 deficiency can lead to mental disorders, and this effect is mostly related to TRP metabolism. Mitogen can induce cell production of IFN-γ and further degradation of TRP. Vitamin C and vitamin E inhibit IFN-γ formation and release in a dose-dependent manner (60). This effect may be achieved by influencing the release of TH1-type cytokines by Tregs and DCs (61).

4.4 Diet and gut microbes

TRP comes from exogenous intake of protein-rich foods, including chicken, tuna, oats, peanuts, bananas, milk, cheese, and chocolate. Vitamin B6, on the other hand, is water-soluble and its intake can also be adjusted through food (58). Thus, the metabolic pathways are influenced by adjusting food intake to affect the source of metabolic pathway precursors and the activity of related enzymes. It was shown that a high protein diet enhances rat liver TDO activity in a dose-dependent manner, while a high carbohydrate diet increases brain TRP availability and a high-fat diet also inhibits rat liver TDO activity and promotes 5-HT synthesis by increasing brain TRP (55). In addition, changes in the microbiota regulate the host immune system by modulating TRP metabolism, and diet can influence the composition of the gut microbiota. Clinical trials have found that after a mediterranean diet (rich in plant-based foods such as fruits, vegetables, nuts, legumes, seeds, and grains, as well as olive oil, dairy, fish, and poultry), fiber-fermenting bacteria such as *Lachnospiraceae* and *Butyricicoccus* were added, and IAA and IPA (beneficial to nerve cells) generated by bacteria were significantly increased (62). With the intake of more dietary fiber, the anaerobic gut microbes produces more short-chain fatty acids (SCFA), promoting gut homeostasis, which also contributes to TRP metabolites and ω-3-fatty acids (63). And there is an association between higher levels of omega-3 fatty acids and lower levels of anxiety (64).

4.5 Minerals

Minerals such as Mn^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Li, and Se are cofactors and coenzymes of KP, to which the enzymes of this pathway are highly sensitive. It was found that Zn^{2+} inhibits kynurenine hydrolase, while Mn2+ activates this enzyme. Both metal ions activate KAT, while Cu^{2+} and Co^{2+} inhibit it, and these inhibitory effects are due to the blocking and inactivation of the sulfhydryl group of the enzyme (65). In addition, in the 5-HT pathway, TPH is strictly dependent on Fe^{2+} and is inactivated by Fe^{3+}. Also, it has been found that the induction of the KP pathway by IFN-γ through increased IDO-1 expression can be inhibited by Li (66).

4.6 Glucocorticoids

Factors detrimental to mental health, such as stress, social isolation, sleep deprivation and lack of physical activity, raise circulating glucocorticoid levels in humans and non-human social mammals. Whereas glucocorticoids can enhance IDO-1 expression by enhancing INF-γ activity, not only that, glucocorticoids can also enhance TDO activity (67, 68). The researchers detected an increase, decrease, and increase in the ratios of KYN/TRP, KYN/QUIN, and KYN/KYNA in the serum of rats after administration of hydrocortisone. The data suggest that the administration of glucocorticoids resulted in the promotion of KP in rats and a shift of KP toward the production of QUIN (69).

4.7 Aging

Currently, studies on model organisms such as yeast, worms, flies and mice have found a relationship between TRP metabolism and aging and age-related diseases including Alzheimer's disease, Parkinson's disease and Huntington's disease. Studies have found that the ratio of KYN/TRP increases in the elderly, and the degradation rate of TRP increases. IDO in the brain increases with age, and the elderly with higher KYN/TRP ratio have higher mortality (16). Aging alters KP metabolism and TRP availability, thereby increasing susceptibility to age-dependent neurological diseases (70). However, IDO activity in the liver and kidney decreased with age. The increase in inflammation observed during aging is the driving force of KP activity and leads to the overproduction of QUIN. In addition, the activity of IFN-γ increased with age, suggesting that increasing age may modulate IDO activity because IFN-γ can activate IDO. These data suggest that TRP metabolism is a powerful metabolic regulator of aging and age-related diseases, providing a new approach to disease intervention (71).

5 Regulation of TRP metabolism on downstream targets

Tryptophan metabolism has numerous downstream targets that have been demonstrated. KYNA, for example, can affect the antagonism of NMDAR, α7-nAChR, AMPA, Kainate receptors, and activation of Gpr35. At high concentrations, KYNA antagonizes AMPA and Kainate receptors, and at low concentrations, inhibits α7-nAChR (72)(Figure 3).

5.1 Glutamate regulation s

Glutamate (Glu) is an excitatory neurotransmitter, and about 70-80% of neurons in the cerebral cortex are glutaminergic neurons. Glu is not only a direct precursor of the inhibitory neurotransmitter γ-aminobutyric acid, but also a potential neurotoxin in the brain. Excessive amounts can lead to neuronal death or brain damage, while low levels are associated with the development of Schizophrenia, suggesting that maintenance of Glu homeostasis is critical for neural activity (73). In addition, Glu regulates synaptic efficiency and controls the release of various biomolecules, including cytokines. Therefore, Glu may cause a variety of physiological/pathological conditions (74).

Glu is involved in the body's physiological activities by acting on ionic receptors and metabolic receptors, among which ionic receptors include NMDA receptors, AMPA receptors, and kainate receptors, which mediate the excitatory transmission of Glu. The massive activation of Glu receptors leads to the imbalance of Ca^{2+} in cells, which activates a large number of proteases, such as nitric oxide synthase (NOS) and protein kinase A (PKA), and activates relevant signal pathways to mediate the excitatory toxicity of cells (75). QUIN and KYNA can affect Glu signal transduction in the brain. The abnormal production of these metabolites is related to neurodegeneration and other neurological diseases, including Depression, Bipolar disorder, Addiction and Schizophrenia.

5.1.1 N-methyl-D-Aspartic acid

N-methyl-D-Aspartic acid receptor (NMDAR) is a subtype of ionic glutamate receptor. It is composed of NR1, NR2 (A, B, C, D), and NR3 (A, B), among which NR1 is the basic subunit constituting the ion channel (76). NMDAR activity follows a hormetic dose-response curve, meaning that too much or too little NMDA activity is harmful to neurons (77). Activation of this receptor is dependent on Glu binding to the NR2 subunit and glycine binding to the NR1 or NR3 subunit (78).

KYNA can competitively bind to Glu and glycine sites on the NMDAR, with a stronger affinity for the latter, such as the Gly-B site (Glycine site B) (79, 80). Moreover, the inhibition is reversible. KYNA is the only endogenous NMDAR antagonist, while most of the other KYN metabolites are NMDAR agonists (81). After NMDAR activation, QUIN leads to extracellular excess of Glu by inhibiting Glu reuptake, and further NMDAR excitation is induced (82). The activation of NMDAR leads to

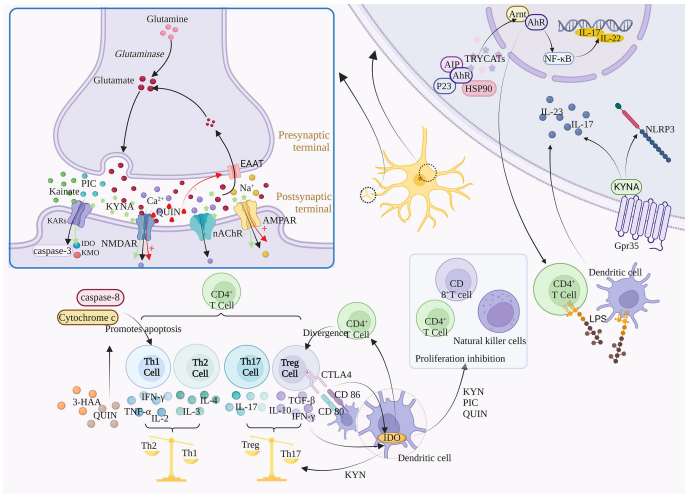


FIGURE 3
Regulatory mechanism of TRP metabolism on downstream signals. TRYCATs can achieve the regulation of Glu by acting on NMDAR, AMPAR, GABA, and KARs. For example, L-KYN, PIC and QUIN can exert immunosuppressive effects through different mechanisms, inhibiting the proliferation of $CD4^+$ T cells, $CD8^+$ T cells and natural killer (NK) cells. The specific activation of Gpr35 by KYNA can exert anti-inflammatory effects through negative regulation of NLRP3 and release of IL-23 and IL-17. α7-nACh receptors mediate large amounts of Ca^{2+} into neurons at resting or hyperpolarized membrane potentials, whereas NMDAR directs Ca^{2+} into neurons in a depolarized state. In addition, TRYCATs are the major endogenous ligands for AHR, which are released from the HSP90 complex and translocated to the nucleus when AHR binds to the ligand.

the blocking of the elongation phase of protein synthesis, thus inhibiting protein translation (83). In addition, excessive activation of NMDAR leads to pathologically open ligand-gated Ca^{2+} channels, resulting in massive extracellular Ca^{2+} influx and intracellular Ca^{2+} overload. The direct consequence of increased intracytoplasmic free Ca^{2+} concentration is the activation of various enzyme systems regulated by calcium under normal conditions, including proteases, oxidase systems, protein kinases, phospholipases, endonucleases, etc. It aggravates the damage and energy crisis of biofilm, and is closely related to the generation and damage of free radicals (84). In addition, activation of NMDAR leads to Na^+, and K^+ influx into cells, which then activates downstream signaling pathways and secondary messenger molecules, ultimately leading to various synaptic changes. Although QUIN and Glu have similar affinity for NMDAR, QUIN has a greater excitotoxicity potential due to its lower reuptake efficiency and longer retention time in the synaptic cleft (26).

5.1.2 α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid

α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPAR) is one of the main Glu receptors in the brain and is expressed in all glial cells. It is a tetramer composed of four subunits (GLIA1-GLIA4) that plays a key role in regulating excitatory synaptic transmission. The GliA2 subunit is a decisive factor in the calcium permeability of AMPA, and the AMPA receptor lacking GliA2 is mainly expressed in inhibitory intermediate neurons (85). Microglia and macrophages also express AMPA receptors, whose activation contributes to the release of pro-inflammatory cytokines. KYNA plays a dual role in the dose-dependent regulation of AMPA receptors, which are activated at low concentrations and neuroinhibitory at high concentrations (86). In addition, activation of AMPA receptors increased the firing rate of 5-HT neurons and the release of 5-HT in both local and terminal regions, and both 5-HT neurons showed concentration-dependent excitatory AMPA responses (87).

5.1.3 γ-aminobutyric acid

GABAergic interneurons are inhibitory neurons, accounting for 20% of all cortical neurons, and inhibit the excitation of downstream glutamatergic neurons. The antagonistic effect of NMDAR prevents the activation of GABA neurons and leads to the disinhibition and Glu surge of downstream glutamatergic neurons, which activates the postsynaptic AMPAR and enhances brain-derived neurotrophic factor/tyrosine receptor kinase B (BDNF/TrkB) signaling pathway, ultimately increasing synaptic plasticity and synaptic strength (88). Submicromolar concentrations of KYNA antagonize nicotinic receptor function in the prefrontal cortex, thereby inhibiting GABAergic neurons. In contrast, the inhibitory effect of KYNA on GABA could be reversed by KAT II inhibitor, showing that KAT II activity could regulate GABA (89).

5.1.4 Kainate receptors

Kainate (KA) is an agonist of Kainate receptors (KARs), and also acts as a non-desensitizing agonist of AMPA receptors. KARs, ligand-gated channel ionic receptors, exist in the postsynaptic membrane and mediate a small portion of ionic synaptic responses, playing a key role in synaptic integration (90). KARs is a tetramer composed of five subunits, including GluK1-5, of which GluK2 subunit is a key determinant of KARs properties. KA has excitatory toxicity, can activate caspase-3, and induce DNA fragments in hippocampal CA3 and CA1 regions, which is consistent with the occurrence of apoptotic cell death. However, inhibition of certain aspects of neuroinflammatory response ameliorates KA-induced neurotoxicity (91). Administration of KA induces the expression of IDO and KMO, but treatment with KMO inhibitors ameliorates KA-induced neurotoxicity (92). It was found that elevated KYNA levels antagonized the neurotoxicity of KA, while PIC modulated the KA-induced striatum Glu release (25).

5.2 Immune regulation

Th1, Th2, and Th17 cells belong to different Th cell subpopulations differentiated from $CD4^+$T cells. Th1 cells can differentiate from $CD4^+$T cells under the induction of cytokines such as IL-12 and mainly secrete IFN-γ, TNF-α, and IL-2 to exacerbate inflammation (93). In addition, Th2 can exert anti-inflammatory effects by secreting IL-4 and IL-3, and Th17 can secrete pro-inflammatory factors such as IL-17 to promote inflammation (94). Studies show that IDO is involved in immune tolerance and Th1/Th2 regulation, IDO-TRP metabolism, and Treg positively regulate one another, while Treg can release IL-10, and TGF-β to suppress inflammation (94). Tregs may induce IDO expression in DCs through the interaction between CTLA4 on Tregs and CD80/CD86 on DCs, or through cytokines secreted by Treg (e.g., IFN-γ). In contrast, IDO expression in DCs may induce differentiation of new Tregs from naive T cells. IDO was found to be a "switch" for the conversion of Th17 to Treg, inhibiting the differentiation of Th17 cells and increasing Treg, which may be related to KYN (95, 96). Kynurenines primarily induce a negative feedback loop and cell death in the Th1 cell population and promote upregulation of the Th2 cell population, which leads to a relative shift in the Th1-Th2 ratio toward Th2. In addition, 3-HAA and QUIN can induce selective apoptosis in Th1 cells through activation of caspase-8 and release of cytochrome c from mitochondria (97).

In addition, KP can affect T cell proliferation, and activation of IDO-1 in DCs completely prevents clonal expansion of T cells (98). Indeed, the negative feedback provided by IDO expression in DCs after contact with activated T cells is a necessary immunoregulatory mechanism of T cell deactivation (99). The kynurenine metabolites L-KYN, PIC, and QUIN can exert immunosuppressive effects through different mechanisms, inhibiting the proliferation of $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, and NK cells. This T cell proliferation inhibition may be associated with a sustained cell cycle arrest, where IDO blocks cells in the mid-G1 phase, and this inhibitory effect is selective and only applies to cells that are being activated (100, 101). In contrast, activated $CD4^+$ T cells can release TNF-γ, which induces LATs to transport TRP across the blood-brain barrier to the cytoplasm, maximizing TRP consumption in IDO-1-expressing cells, a process that involves the positive feedback mechanism of KYN-AhR (10). In addition, 3-HAA can lead to dysfunction and cell death of activated Th2 cells by inhibiting NF-κB. 3-HAA has been shown to directly inhibit the activity of Th1 and Th17 cells and also indirectly reduce their activity by increasing the amount of TGF-β secreted by DCs, leading to an increase in the number of Treg cells produced by primitive $CD4^+$ cells.

5.3 G protein-coupled receptor

G protein-coupled receptors (GPCRs) are one of the largest families of genes that can be activated by a variety of ligands, most of which are metabolic intermediates, including 5-HT and melatonin produced by TRP metabolism. While orphanized G protein-coupled receptor 35 (Gpr35), an orphan receptor in GPCRs, can be specifically activated by its ligand KYNA and its expression is mainly associated with immune cells and the gastrointestinal tract (102). KYNA was found to have anti-inflammatory effects, and this effect was associated with its activation of GPR35. (1) promoting autophagy of NLRP3 inflammatory vesicles, thus exerting a negative regulatory effect on NLRP3 (103); and (2) inhibiting LPS-induced release of IL-23 and IL-17 from DCs and $CD4^+$ T cells (104). In addition, KYNA regulates energy homeostasis in adipose tissue and cytokine release from invariant NK cells by activating Gpr35, which affects Ca^{2+} release, ERK1/2 phosphorylation, and Pgc-1α1 activation (105).

5.4 Nicotinic acetylcholine receptor

Nicotinic acetylcholine receptor (nAChR) belong to the cys-loop receptor superfamily, a ligand-gated ion channel protein with two subtypes, N1 (α1, β1, δ, ϵ, γ) and N2 (α2-α10, β2-β4) (106). Due to the different distribution regions, the N1-type receptors are called neural nicotinic receptors and the N2-type are called muscle nicotinic receptors. nAChR, similar to NMDAR, is involved in regulating neuroplasticity. α7-nACh receptors mediate large amounts of Ca^{2+} into neurons at resting or hyperpolarized membrane potentials, whereas NMDAR directs Ca^{2+} into neurons in a depolarized state. Furthermore, α7-nAChRs have synergistic effects with NMDAR, and the interaction between the cholinergic and glutamatergic systems is necessary to induce synaptic plasticity and BDNF dependence. α7-nAChRs are present at the postsynaptic sites of glutamatergic synapses, and acetylcholine acting on α7-nAChRs may provide permissive depolarization of the postsynaptic membrane, thereby increasing the number of the responsive NMDAR (107). Among brain nAChRs, α4β2-nAChR and α7-nAChR are the most prevalent, whereas α7-nAChR protects against Glu neurotoxicity and prevents cell death (108). KYNA regulates α4β2-nAChR expression by noncompetitive inhibition of α7-nAChR, and α7-nAChR was more sensitive to KYNA inhibition than NMDAR. In addition to the effect of KP metabolites on nAChR, melatonin produced by SP can also activate α7-nAChR, thus enabling the optimization of mitochondrial function (109).

5.5 Aryl hydrocarbon receptor

The aryl hydrocarbon receptor (AhR), often referred to as the dioxin receptor, is a ligand-activated transcription factor that is widely expressed centrally and systemically. AHR signaling is thought to be an important component of the barrier site immune response and can regulate a variety of cellular processes, including immune regulation, cell development, differentiation, proliferation, survival, and apoptosis (110, 111). AhR forms a protein complex with dimer of the 90 kDa heat shock protein (HSP90), AhR-interacting protein (AIP), the co-chaperone p23, and the protein kinase SRC, which is expressed in the cytoplasm (112). TRYCATs are the major endogenous ligands of AHR, and when AHR binds to the ligand, it is released from the HSP90 complex and translocates to the nucleus, where it binds to the AhR nuclear translocator (Arnt) to form a heterodimer (109). As a key regulator associated with immunity and inflammation, AhR has been implicated in a variety of biological processes including regulation of immune responses, maintenance of mucosal barrier function, intestinal homeostasis, and tumor development. Kynurenine metabolites such as KYN, KYNA, QUIN, and 3-HAA act as ligands to activate AhR and stimulate the expression of the downstream target such as interleukin-22 (IL-22) and interleukin-17 (IL-17) (113). Activated AhR induces the proliferation of regulatory T cells (Tregs) $CD4^+$, $CD25^+$ and suppresses the immune function of activated T cells (114). The limitations of single IDO/TDO-

targeted drugs can be overcome by selective blockade of AhR. In addition, its activation is associated with activation of toll-like receptor 2 (TLR2) and signaling pathways such as NF-kB downstream and MAPKs, which can promote phosphorylation of p65/NF-kB, JNK/MAPK, p38/MAPK and ERK/MAPK pathways to regulate multiple inflammatory signaling pathways and further promote the production of pro-inflammatory mediators, including interleukin-1β (IL-1β) and interleukin-6 (IL-6) (10).

5.6 Dopamine

Dopamine (DA) is a neurotransmitter known to reduce KYNA in the brain.KYNA has two opposing effects on DA neurotransmissiom. (1 KYNA can achieve excitatory effects on DA neurons by blocking the glycine site of GABA afferents to NMDA receptors. Elevated levels of KYNA in the brain increase the activity of dopamine neurons in the ventral tegmental area (VTA) dopamine and increase the firing rate and burst firing activity of these neurons (115). (2 KYNA inhibits α7-nAChR-induced decrease in DA release on dopaminergic nerve endings (72).

6 TRP metabolism in neurological and psychiatric disorders

Alterations in TRP metabolism have been associated with neurological and psychiatric disorders, and TRYCATs alterations have also been found in MDD, cancer, diabetes, cardiovascular disease, autoimmune syndrome, and other diseases (Table 1). TRP metabolism demonstrates the greatest potential as the druggable target for neurological and psychiatric disorders. Therefore, this paper helps to guide the development of new drugs by summarizing current research on TRP metabolism in neurological disorders (Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic lateral sclerosis, Multiple sclerosis, Autism, Epilepsy) and psychiatric disorders (Depression, Schizophrenia, Anxiety, Bipolar disorder) (Figure 4).

6.1 Neurological diseases

Neurological diseases are mostly accompanied by neuroinflammation and neuronal pathological damage in the

TABLE 1 Neurological and psychiatric disorders and their risk factors are related to the changes in TRP catabolism and enzymes.

Disease	Sample	Alteration of metabolites	Sample	Alteration of enzymes	References
Alzheimer's disease	Plasma	TRP, KYNA decreased and QUIN increased	The prefrontal cortex, hippocampal	IDO increased	(116, 117)
Parkinson's disease	Plasma, cerebrospinal fluid	3-HK in plasma increased, 3-HAA and 5-HT/KYN decreased, KYNA and KYNA/KYN in cerebrospinal fluid decreased, and QUIN/KYNA increased	Plasma	KAT I and KAT II decreased	(118, 119)
Huntington's disease	Serum	KYN increases, KYNA/KYN decreases	Putamen	KAT I and KAT II decrease, IDO activation	(120, 121)
Depression	Hippocampu	Lower ratios of KYNA/KYN, KYNA/3-HK, and KYNA/QUIN	Serum, prefrontal cortex, hippocampus, and cerebral cortex	TPH2, KATII/KMO mRNA decreased, KMO, IDO-1 upregulated	(122–124)
Multiple sclerosis	Prefrontal cortex、Hippocampus、Spinal cord、Spleen	TRP, KYN, and KYN/TRP in the prefrontal cortex, hippocampus, spinal cord, and spleen increased; 3-HK and QUIN in the Prefrontal cortex and hippocampus increased, while KYNA decreased	Plasma	KAT I and KAT II decreased	(125, 126)
Schizophrenia	Plasma	TRP decreases, KYN and KYN/TRP increase	Cerebral cortex	KMO, 3-HAO reduced	(127, 128)
Anxiety disorder	Plasma	KYN, KYN/TRP elevated	Center seam core	TPH2 decreased	(129, 130)
Amyotrophic lateral sclerosis	Cerebrospinal fluid, cortical	QUIN, IDO, TRP, KYN increased	Motor cortex, spinal cord	TPH decreased, IDO increased	(131, 132)
Autism spectrum disorder	Serum	TRP and 5-HT increased and KYNA decreased	–	KMO expression	(133, 134)
Epilepsy	Plasma	TRP, KYN higher, KYNA, 3-HK, KYNA/KYN lower	–	IDO Expression	(135, 136)
Bipolar disorder	Serum	TRP, KYNA, PIC, QUIN/TRP, and PIC/QUIN elevated	Serum	IDO-1 expression is upregulated	(137)

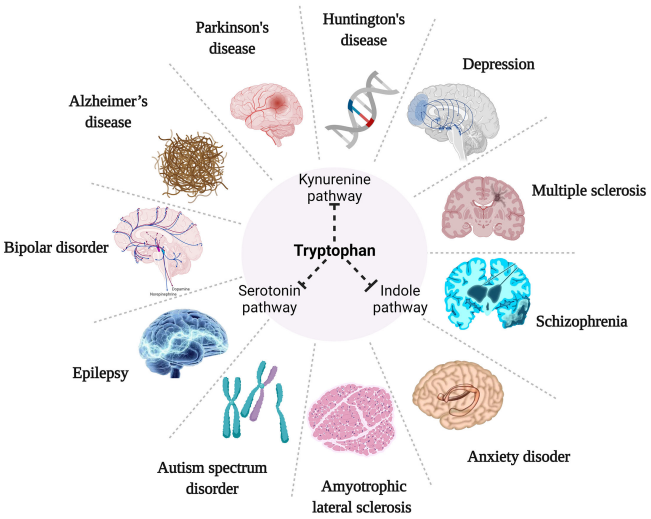


FIGURE 4
TRP metabolism for neurological and psychiatric disorders.

brain with certain motor and non-motor impairments (138). For example, early amyotrophic lateral sclerosis (ALS) mediates motor neuron degeneration through glutamatergic excitotoxicity causing hyperexcitability of motor cortex neurons (139). Pathological studies have revealed that Parkinson's patients are characterized by striatal dopaminergic neuron deficits and local neuroinflammation in the midbrain region, accompanied by chronic inflammation, oxidative stress, and other damage, and these events are usually associated with tryptophan metabolism (138). According to research, tryptophan metabolism is typically out of equilibrium when neurological illnesses like Alzheimer's, Parkinson's, Huntington's, multiple sclerosis, amyotrophic lateral sclerosis, autism, and epilepsy advance (131, 135, 140–143).

Alzheimer's disease (AD) neuropathology is characterized by progressive accumulation of beta-amyloid-beta (Aβ) oligomers and plaques in the brain, which leads to neurodegeneration in several regions of the brain (66). Aβ can induce the expression of inflammatory cytokines such as IL-1β, TNF-α, and enzymes in KP, including: IDO, KYNU, KAT I, KAT IV, etc. Following that, Aβ affects the expression of KYN metabolites such as 3-HK, 3-HAA, and QUIN, leading to neurological tissue damage, which later allows mechanisms such as Aβ re-accumulation, glial activation, and KP upregulation to further exacerbate neurodegenerative lesions (141). TRP/KYN and KYNA/TRP are significantly elevated in the frontal cortex, putamen, pars compacta of the substantia nigra, blood and cerebrospinal fluid of Parkinson's patients (PD), especially in the periphery, and their elevation was significantly associated with advanced Parkinson's (143, 144). In addition, studies have found that both KYN, as well as KYN/TRP in the urine of PD patients, increase as the disease progresses and can be used as a biomarker for PD (145). The collection of urine samples is also simpler than that of blood or cerebrospinal fluid, making urine detection a non-invasive and accessible method. Yilin Wang (111) found that TRP supplementation inhibited nuclear translocation of NF-κB in a PD model. However, With the use of AhR pathway inhibitors, TRP's neuroprotective effects and its suppression of NF-κB were reversed. In addition, increased KYNA levels in the brain protect nigrostriatal dopamine neurons from QUIN-induced excitotoxic damage, and the decrease in KYNA in PD patients was accompanied by a decrease in KAT-I and KAT-II activity (146, 147). In patients with Huntington's disease (HD), KYNA was found to be reduced, while 3-HK and QUIN were elevated. The susceptibility to NMDA-mediated neurotoxicity in HD was linked to KP activity by inhibition of IDO and TDO activity, which decreased 3-HK and QUIN production (142). In addition, ameliorates neurodegeneration in the drosophila model of HD by inhibiting KMO that increasing KYNA levels, while their effect on neurodegeneration was also demonstrated by direct feeding of KYNA and 3-HK (148).

In addition, TRP levels were reduced in serum and cerebrospinal fluid of patients with multiple sclerosis (MS). After the administration of INF-β, an increase in the L-KYN/TRP ratio was seen, indicating that the pathophysiology of MS may entail the impact of activated IDO-1 on TRP metabolism (140). Meanwhile, the impaired immunosuppressive activity of Treg cells against Th1 and Th17 cells has been shown to play a role in the pathogenesis of MS. The neurotoxicity mechanisms of QUIN are highly overlapping with those of amyotrophic lateral sclerosis (ALS) and motor neuro death. QUIN was significantly

elevated in neurons and microglia in the cerebrospinal fluid, motor cortex, and spinal cord of ALS patients, while cerebrospinal fluid IDO, TRP, and KYN were significantly elevated, and the 3-HK/KYNA and QUIN/KYNA ratios tended to be elevated (131). Since QUIN and 3-HK may have neurotoxic and KYNA neuroprotective effects, these findings may reflect the impairment of neurotoxic compounds by KP (149). It has been found that both reduced and elevated NMDAR functions are associated with autism spectrum disorder (ASD), and its deviation in either direction leads to ASD, demonstrating the importance of a normal range of NMDAR function. NMDAR modulators can alleviate repetitive or hyperactive behaviors in addition to social behaviors (150). TRYCATs, including KYNA and QUIN, can exert regulatory effects on both AMPAR and NMDAR to achieve effects on ASD. The researchers found that IDO-1 levels, KYN/TRP ratio and pro-inflammatory cytokine levels were elevated in the serum and hippocampus of mice with epilepsy in both the acute and chronic phases following status epilepticus (SE), and this was reversed in IDO-1 knockout mice. This demonstrated that reducing the production of IDO-1dependent neurotoxic metabolites could suppress epilepsy, attenuate neuronal damage, and ultimately inhibit glial cell activation and pro-inflammatory cytokine production (135). Not only that, but kynurenine metabolites can affect epilepsy by modulating enhanced excitatory neurotransmission mediatesd to glutamate receptors, including NMDAR and AMPAR, a well-established hypothesis for the pathogenesis of epilepsy (151). In addition, vagal stimulation increases AA levels by modulating tryptophan metabolism, which serves to reduce the number and frequency of seizures, and the increased AA levels are associated with improved mood (152).

6.2 Psychiatric disorders

Psychiatric disorders are often accompanied by pathological features of abnormal neurometabolic, dysfunction of the dopaminergic system, and hypo glutaminergic function, and tryptophan metabolism is strongly associated with all three abnormal states. It has been found that the course of depression, schizophrenia, anxiety, and bipolar disorder is often accompanied by imbalances in tryptophan metabolism (26, 127, 129, 137).

Studies have shown that TRP metabolism may affect depression in two ways: (1) TRP depletion leading to 5-HT underproduction and (2) neurotoxicity of KYN metabolites. Currently, 5-HT insufficiency is one of the currently recognized mechanisms in the pathogenesis of depression (153). The researchers propose that the lack of 5-HT in depression is caused by a shunt of TRP metabolism from 5–HT formation to KYN formation. TPH2 expression is significantly reduced and IDO-1 expression is upregulated in serum, prefrontal cortex and hippocampus of depressed mice, showing a shift of TRP metabolism toward KYN, while SP is somewhat inhibited (81). In contrast, administration of 1-methyl-tryptophan (1-MT), a competitive inhibitor of IDO enzymes in mice, significantly reversed the depressive behavior (122). In addition, KYNA/KYN, KYNA/3-HK and KYNA/QUIN ratios were reduced in depressed patients, while KYNA/QUIN ratio was positively correlated with hippocampal volume and negatively correlated with the severity of depression. These data show an imbalance of neuroprotection and neurotoxicity in depressed patients (26). Clinical studies have found that plasma TRP levels are significantly lower in schizophrenic patients (Schizophrenia, SZ), while KYNA and KYN/TRP are significantly higher, KATII activity is increased, KYNA is elevated, and lower TRP levels are accompanied by lower white matter integrity (127). However, KYNA is involved in cognitive processes and excitatory neural network formation through its hype inhibitory effects on NMDAR and α7-nAChR, involving physiological processes such as learning, memory, and synaptic plasticity, triggering psychiatric symptoms and cognitive deficits (154, 155). There are limited clinical studies on TRP metabolism and anxiety disorders, Mary I. Butler (129) studied the plasma levels of TRP metabolites, including KYN, TRP, KYNA, KYN/TRP, and KYNA/KYN, in patients with social anxiety disorder (SAD). Increased activation of KP was found, and differences in metabolite concentrations may be associated with abnormal pro-inflammatory cytokines. Whereas the activation of KP in SAD seems to be preferentially directed towards KYNA synthesis, patients exposed to chronic stress due to repeated social interactions can shift downstream metabolism towards KYNA production not only by stimulating the conversion of TRP to KYN but also. In addition to this, tryptophan metabolism has a contribution to bipolar disorder (Bipolar disorder, BD). The importance of TRYCAT pathways in BD is at least partly as a result of their integration of peripheral inflammatory and IDO changes with changes in central neuronal regulation, driven by changes in glia responses and TRYCAT fluxes. Patients with BD had elevated IL-6, IL-1β, TNF-α, and IFN-γ. The concentrations of TRP, KYNA, and PIC in the cerebrospinal fluid and the ratios of QUIN/TRP and PIC/QUIN were significantly higher than those in healthy controls, and QUIN/TRP indicated elevated IDO-1 activity (137). And the cerebrospinal fluid PIC levels were lower in BD patients with a history of suicidal behavior than in patients without suicidal behavior (156).

7 Interventions for neurological and psychiatric disorders through TRP metabolism

There is a growing body of research on TRP metabolism, especially on the involvement of TRYCATs and enzymes in the physiopathological processes of neurological and psychiatric

disorders. Although the specific mechanism of action is not yet fully understood, the regulation of key enzymes such as IDO and TDO to interfere with their enzymatic reactions may provide effective therapeutic strategies for the corresponding neurological and psychiatric disorders and provide theoretical guidance and experimental basis for the development of new drugs (157). Therefore, this paper summarizes the current drugs based on TRP metabolism for the treatment of neurological and psychiatric disorders, including natural drug extracts, prescriptions, biologics and other drugs (Supplementary Table 1).

8 Conclusions

Neurological and neurological disorders are important frontiers of neuroscience in this century, and the science of targeting TRP metabolic pathways has tremendous research potential. It can be modulated at multiple levels to exert neurological and psychoprotective effects, opening up possibilities and posing challenges for the development of medications for a variety of disorders.

TRP has many metabolic directions and complex regulatory mechanisms. Its metabolites also participate in many pathological processes, such as excitotoxicity, neuroinflammation, oxidative stress, mitochondrial damage, and so on, forming a complex regulatory network. However, the current understanding of this network is only the tip of the iceberg. In this paper, we try to summarize the network and its development in neurological and psychiatric diseases, and promote the subsequent more comprehensive study of the TRP metabolic network. Micro-regulation of the TRP pathway in the CNS is a key challenge for targeted therapies. This paper summarizes the factors regulating TRP metabolism (inflammation and stress, exercise, vitamins, minerals, diet and gut microbes, glucocorticoids, and aging) and the downstream (regulation of glutamate, immunity, Gpr35, nAChR, AhR, DA) role of TRP metabolism, and intends to provide a systematic introduction to its multi-linked regulation. However, there are multiple enzymes regulated in the tryptophan metabolic pathway, including IDO, TDO, KMO, KAT, KYUN, etc. Although there are a large number of preclinical studies on the treatment of neurological and psychiatric disorders by regulating TRP metabolism, most of them focus on the metabolite levels of 5-HT, TRP, and KYN, the expression of IDO, etc., and fewer studies on complex enzymes. As an important regulator of the TRP pathway, future efforts will require numerous researchers to conduct comprehensive studies on it using multidisciplinary techniques from molecular, cellular, and tissue to assess the role of enzymes in TRP metabolism.

Author contributions

NL, RA and DL designed the study; SY, YL, AS, JD, YM, JiW, XQL, SYL, YLZ, JYW supplied materials and analytic tools; DL, SY and YL wrote the paper. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.985378/full#supplementary-material

References

1. Jee HJ, Lee SG, Bormate KJ, Jung Y-S. Effect of caffeine consumption on the risk for neurological and psychiatric disorders: Sex differences in human. *Nutrients* (2020) 12(10):3080. doi: 10.3390/nu12103080

2. Lu Z. *Nano-aromatic drugs for the treatments of neuropsychiatric disorders: University of Chinese academy of sciences.* (2021).

3. Zhou M, Wang H, Zeng X, Yin P, Zhu J, Chen W, et al. Mortality, morbidity, and risk factors in China and its provinces, 1990-2017: a systematic analysis for the global burden of disease study 2017. *Lancet (London England)* (2019) 394 (10204):1145–58. doi: 10.1016/S0140-6736(19)30427-1

4. Li D, Long Y, Yu S, Shi A, Wan J, Wen J, et al. Research advances in cardio-cerebrovascular diseases of hort. *Front In Pharmacol* (2021) 12:832673. doi: 10.3389/fphar.2021.832673

5. Florensa-Zanuy E, Garro-Martínez E, Adell A, Castro E, Díaz Á, Pazos Á, et al. Cannabidiol antidepressant-like effect in the lipopolysaccharide model in

mice: Modulation of inflammatory pathways. *Biochem Pharmacol* (2021) 185:114433. doi: 10.1016/j.bcp.2021.114433

6. Chen B, Li J, Xie Y, Ming X, Li G, Wang J, et al. Cang-ai volatile oil improves depressive-like behaviors and regulates DA and 5-HT metabolism in the brains of CUMS-induced rats. *J Ethnopharmacol* (2019) 244:112088. doi: 10.1016/j.jep.2019.112088

7. Zhang X, Li R, Wang J, Huang S, Li G, Wang S, et al. Advances in the regulation of tryptophan on animal physiological function. *Special Wild Economic Anim Plant Res* (2019) 41(01):104–8. doi: 10.16720/j.cnki.tcyj.2019.01.023

8. Alkhalaf LM, Ryan KS. Biosynthetic manipulation of tryptophan in bacteria: pathways and mechanisms. *Chem Biol* (2015) 22(3):317–28. doi: 10.1016/j.chembiol.2015.02.005

9. Qin X, An Y. Research progress of tryptophan-kynurenine metabolic pathways and neurodegenerative diseases with intervention of traditional Chinese medicine. *Tianjin J Traditional Chin Med* (2019) 36(10):1031–6. doi: 10.11656/j.issn.1672-1519.2019.10.22

10. Ramprasath T, Han Y-M, Zhang D, Yu C-J, Zou M-H. Tryptophan catabolism and inflammation: A novel therapeutic target for aortic diseases. *Front In Immunol* (2021) 12:731701. doi: 10.3389/fimmu.2021.731701

11. Kong H, Zhu D. Tryptophan / kynurenine metabolic pathway and cardiovascular disease. *Chin J Hypertension* (2012) 20(05):433–5. doi: 10.16439/j.cnki.1673-7245.2012.05.010

12. Tanaka M, Tóth F, Polyák H, Szabó Á, Mándi Y, Vécsei L. Immune influencers in action: Metabolites and enzymes of the tryptophan-kynurenine metabolic pathway. *Biomedicines* (2021) 9(7):734. doi: 10.3390/biomedicines9070734

13. Wirthgen E, Hoeflich A, Rebl A, Günther J. Kynurenic acid: The janus-faced role of an immunomodulatory tryptophan metabolite and its link to pathological conditions. *Front Immunol* (2017) 8:1957. doi: 10.3389/fimmu.2017.01957

14. Gál EM, Sherman AD. L-kynurenine: its synthesis and possible regulatory function in brain. *Neurochemical Res* (1980) 5(3):223–39. doi: 10.1007/BF00964611

15. Li X. *Research of the exchange of blood serum Kyn/Trp ratio in post-hypertensive disorder complicating pregnancy*. Zunyi Medical University (2014).

16. van der Goot AT, Nollen EAA. Tryptophan metabolism: entering the field of aging and age-related pathologies. *Trends In Mol Med* (2013) 19(6):336–44. doi: 10.1016/j.molmed.2013.02.007

17. Connor TJ, Starr N, O'Sullivan JB, Harkin A. Induction of indolamine 2,3-dioxygenase and kynurenine 3-monooxygenase in rat brain following a systemic inflammatory challenge: a role for IFN-gamma? *Neurosci Lett* (2008) 441(1):29–34. doi: 10.1016/j.neulet.2008.06.007

18. Shibata Y, Hara T, Nagano J, Nakamura N, Ohno T, Ninomiya S, et al. The role of indoleamine 2,3-dioxygenase in diethylnitrosamine-induced liver carcinogenesis. *PLoS One* (2016) 11(1):e0146279. doi: 10.1371/journal.pone.0146279

19. Guillemin GJ. Quinolinic acid, the inescapable neurotoxin. *FEBS J* (2012) 279(8):1356–65. doi: 10.1111/j.1742-4658.2012.08485.x

20. Lim CK, Fernández-Gomez FJ, Braidy N, Estrada C, Costa C, Costa S, et al. Involvement of the kynurenine pathway in the pathogenesis of parkinson's disease. *Prog In Neurobiol* (2017) 155:76–95. doi: 10.1016/j.pneurobio.2015.12.009

21. Fujigaki H, Yamamoto Y, Saito K. L-tryptophan-kynurenine pathway enzymes are therapeutic target for neuropsychiatric diseases: Focus on cell type differences. *Neuropharmacology* (2017) 112(Pt B):264–74. doi: 10.1016/j.neuropharm.2016.01.011

22. Behl T, Kaur I, Sehgal A, Singh S, Bhatia S, Al-Harrasi A, et al. The footprint of kynurenine pathway in neurodegeneration: Janus-faced role in parkinson's disorder and therapeutic implications. *Int J Mol Sci* (2021) 22(13):6737. doi: 10.3390/ijms22136737

23. Myint A-M, Kim Y-K. Network beyond IDO in psychiatric disorders: revisiting neurodegeneration hypothesis. *Prog In Neuropsychopharmacol Biol Psychiatry* (2014) 48:304–13. doi: 10.1016/j.pnpbp.2013.08.008

24. Fazio F, Lionetto L, Curto M, Iacovelli L, Copeland CS, Neale SA, et al. Cinnabarinic acid and xanthurenic acid: Two kynurenine metabolites that interact with metabotropic glutamate receptors. *Neuropharmacology* (2017) 112(Pt B):365–72. doi: 10.1016/j.neuropharm.2016.06.020

25. Mor A, Tankiewicz-Kwedlo A, Krupa A, Pawlak D. Role of kynurenine pathway in oxidative stress during neurodegenerative disorders. *Cells* (2021) 10(7):1603. doi: 10.3390/cells10071603

26. Brown SJ, Huang X-F, Newell KA. The kynurenine pathway in major depression: What we know and where to next. *Neurosci Biobehav Rev* (2021) 127:917–27. doi: 10.1016/j.neubiorev.2021.05.018

27. Hunt C, Macedo E Cordeiro T, Suchting R, de Dios C, Cuellar Leal VA, Soares JC, et al. Effect of immune activation on the kynurenine pathway and depression symptoms - a systematic review and meta-analysis. *Neurosci Biobehav Rev* (2020) 118:514–23. doi: 10.1016/j.neubiorev.2020.08.010

28. Perkins MN, Stone TW. An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. *Brain Res* (1982) 247(1):184–7. doi: 10.1016/0006-8993(82)91048-4

29. Wang D, Wang S, Wei L, Zhao R, Liu X. Abnormal uric acid metabolic pathway and central nervous system diseases in dogs. *Prog Physiol Sci* (2016) 47(01):43–6.

30. Liu J-J, Movassat J, Portha B. Emerging role for kynurenines in metabolic pathologies. *Curr Opin In Clin Nutr Metab Care* (2019) 22(1):82–90. doi: 10.1097/MCO.0000000000000529

31. Joisten N, Ruas JL, Braidy N, Guillemin GJ, Zimmer P. The kynurenine pathway in chronic diseases: a compensatory mechanism or a driving force? *Trends In Mol Med* (2021) 27(10):946–54. doi: 10.1016/j.molmed.2021.07.006

32. Walther DJ, Peter J-U, Bashammakh S, Hörtnagl H, Voits M, Fink H, et al. Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Sci (New York N.Y.)* (2003) 299(5603):76. doi: 10.1126/science.1078197

33. Cervenka I, Agudelo LZ, Ruas JL. Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health. *Sci (New York N.Y.)* (2017) 357(6349):eaaf9794. doi: 10.1126/science.aaf9794

34. Anderson G, Maes M. Bipolar disorder: role of immune-inflammatory cytokines, oxidative and nitrosative stress and tryptophan catabolites. *Curr Psych Rep* (2015) 17(2):8. doi: 10.1007/s11920-014-0541-1

35. Agus A, Planchais J, Sokol H. Gut microbiota regulation of tryptophan metabolism in health and disease. *Cell Host Microbe* (2018) 23(6):716–24. doi: 10.1016/j.chom.2018.05.003

36. Roager HM, Licht TR. Microbial tryptophan catabolites in health and disease. *Nat Commun* (2018) 9(1):3294. doi: 10.1038/s41467-018-05470-4

37. Williams BB, Van Benschoten AH, Cimermancic P, Donia MS, Zimmermann M, Taketani M, et al. Discovery and characterization of gut microbiota decarboxylases that can produce the neurotransmitter tryptamine. *Cell Host Microbe* (2014) 16(4):495–503. doi: 10.1016/j.chom.2014.09.001

38. Sadik A, Somarribas Patterson LF, Öztürk S, Mohapatra SR, Panitz V, Secker PF, et al. IL4I1 is a metabolic immune checkpoint that activates the AHR and promotes tumor progression. *Cell* (2020) 182(5):1252–1270.e34. doi: 10.1016/j.cell.2020.07.038

39. Zhang X, Gan M, Li J, Li H, Su M, Tan D, et al. Endogenous indole pyruvate pathway for tryptophan metabolism mediated by IL4I1. *J Agric Food Chem* (2020) 68(39):10678–84. doi: 10.1021/acs.jafc.0c03735

40. Han Q-Q, Yu J. Inflammation: a mechanism of depression? *Neurosci Bull* (2014) 30(3):515–23. doi: 10.1007/s12264-013-1439-3

41. Golia MT, Poggini S, Alboni S, Garofalo S, Ciano Albanese N, Viglione A, et al. Interplay between inflammation and neural plasticity: Both immune activation and suppression impair LTP and BDNF expression. *Brain Behavior Immun* (2019) 81:484–94. doi: 10.1016/j.bbi.2019.07.003

42. Munn DH, Mellor AL. Indoleamine 2,3 dioxygenase and metabolic control of immune responses. *Trends Immunol* (2013) 34(3):137–43. doi: 10.1016/j.it.2012.10.001

43. Wichers M, Maes M. The psychoneuroimmuno-pathophysiology of cytokine-induced depression in humans. *Int J Neuropsychopharmacol* (2002) 5(4):375–88. doi: 10.1017/S1461145702003103

44. Li C-C, Gan L, Tan Y, Yan M-Z, Liu X-M, Chang Q, et al. Chronic restraint stress induced changes in colonic homeostasis-related indexes and tryptophan-kynurenine metabolism in rats. *J Proteomics* (2021) 240:104190. doi: 10.1016/j.jprot.2021.104190

45. Piao C-S, Stoica BA, Wu J, Sabirzhanov B, Zhao Z, Cabatbat R, et al. Late exercise reduces neuroinflammation and cognitive dysfunction after traumatic brain injury. *Neurobiol Dis* (2013) 54:252–63. doi: 10.1016/j.nbd.2012.12.017

46. Liu R, Qu H. Effect of aerobic exercise on hippocampal neuroinflammation and improvement of NF-κB,TNF-α/IDO/5-HT signal pathway against in CUMS depressive mice. *Chin J Immunol* (2021) 37(13):1563–70. doi: 10.3969/j.issn.1000-484X.2021.13.006

47. Patrick RP, Ames BN. Vitamin d and the omega-3 fatty acids control serotonin synthesis and action, part 2: relevance for ADHD, bipolar disorder, schizophrenia, and impulsive behavior. *FASEB J Off Publ Fed Am Societies For Exp Biol* (2015) 29(6):2207–22. doi: 10.1096/fj.14-268342

48. Pettersson-Klein AT, Izadi M, Ferreira DMS, Cervenka I, Correia JC, Martinez-Redondo V, et al. Small molecule PGC-1α1 protein stabilizers induce adipocyte Ucp1 expression and uncoupled mitochondrial respiration. *Mol Metab* (2018) 9:28–42. doi: 10.1016/j.molmet.2018.01.017

49. Fila M, Chojnacki J, Pawlowska E, Szczepanska J, Chojnacki C, Blasiak J. Kynurenine pathway of tryptophan metabolism in migraine and functional gastrointestinal disorders. *Int J Mol Sci* (2021) 22(18):10134. doi: 10.3390/ijms221810134

50. Yu F, Jia F, Xu S, Jiang H, Wang X. The research of neuroinflammatory-mediated exercise regulating the mechanism of alzheimer's disease. *J Nanjing Sports Institute* (2018) 1(04):33–40+74. doi: 10.15877/j.cnki.nsin.2018.04.006

51. Lou X, Shen J, Cai Y, Liao X, Zhang D. Effect of vitamins on production of l -tryptophan in escherichia coli.JN8. *J Food Sci Biotechnol* (2013) 32(09):921–6.

52. Wei K, Zhang X. Vitamin d:a potential new approach to premature ejaculation research and treatment. *Chin J Hum Sexuality* (2020) 29(07):13–5. doi: 10.3969/j.issn.1672-1993.2020

53. Grudet C, Malm J, Westrin A, Brundin L. Suicidal patients are deficient in vitamin d, associated with a pro-inflammatory status in the blood. *Psychoneuroendocrinology* (2014) 50:210–9. doi: 10.1016/j.psyneuen.2014.08.016

54. Penna G, Adorini L. 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. *J Immunol (Baltimore Md. 1950)* (2000) 164 (5):2405–11. doi: 10.4049/jimmunol.164.5.2405

55. Badawy AAB. Tryptophan availability for kynurenine pathway metabolism across the life span: Control mechanisms and focus on aging, exercise, diet and nutritional supplements. *Neuropharmacology* (2017) 112(Pt B):248–63. doi: 10.1016/j.neuropharm.2015.11.015

56. Theofylaktopoulou D, Ulvik A, Midttun Ø, Ueland PM, Vollset SE, Nygård O, et al. Vitamins B2 and B6 as determinants of kynurenines and related markers of interferon-γ-mediated immune activation in the community-based hordaland health study. *Br J Nutr* (2014) 112(7):1065–72. doi: 10.1017/S0007114514001858

57. Ciorba MA. Kynurenine pathway metabolites: relevant to vitamin b-6 deficiency and beyond. *Am J Clin Nutr* (2013) 98(4):863–4. doi: 10.3945/ajcn.113.072025

58. Shabbir F, Patel A, Mattison C, Bose S, Krishnamohan R, Sweeney E, et al. Effect of diet on serotonergic neurotransmission in depression. *Neurochem Int* (2013) 62(3):324–9. doi: 10.1016/j.neuint.2012.12.014

59. Zhu J, Hua X, Yang T, Guo M, Li Q, Xiao L, et al. Alterations in gut vitamin and amino acid metabolism are associated with symptoms and neurodevelopment in children with autism spectrum disorder. *J Autism Dev Disord* (2021) 52:3116–28. doi: 10.1007/s10803-021-05066-w

60. Winkler C, Schroecksnadel K, Schennach H, Fuchs D. Vitamin c and e suppress mitogen-stimulated peripheral blood mononuclear cells. *vitro. Int Arch Allergy Immunol* (2007) 142(2):127–32. doi: 10.1159/000096438

61. Tan PH, Sagoo P, Chan C, Yates JB, Campbell J, Beutelspacher SC, et al. Inhibition of NF-kappa b and oxidative pathways in human dendritic cells by antioxidative vitamins generates regulatory T cells. *J Immunol (Baltimore Md. 1950)* (2005) 174(12):7633–44. doi: 10.4049/jimmunol.174.12.7633

62. Zhu C, Sawrey-Kubicek L, Beals E, Rhodes CH, Houts HE, Sacchi R, et al. Human gut microbiome composition and tryptophan metabolites were changed differently by fast food and Mediterranean diet in 4 days: a pilot study. *Nutr Res (New York N.Y.)* (2020) 77:62–72. doi: 10.1016/j.nutres.2020.03.005

63. Calabrese CM, Valentini A, Calabrese G. Gut microbiota and type 1 diabetes mellitus: The effect of Mediterranean diet. *Front In Nutr* (2020) 7:612773. doi: 10.3389/fnut.2020.612773

64. Aucoin M, LaChance L, Naidoo U, Remy D, Shekdar T, Sayar N, et al. Diet and anxiety: A scoping review. *Nutrients* (2021) 13(12):4418. doi: 10.3390/nu13124418

65. el-Sewedy SM, Abdel-Tawab GA, el-Zoghby SM, Zeitoun R, Mostafa MH, Shalaby SM. Studies with tryptophan metabolites *in vitro.* effect of zinc, manganese, copper and cobalt ions on kynurenine hydrolase and kynurenine aminotransferase in normal mouse liver. *Biochem Pharmacol* (1974) 23(18):2557–65. doi: 10.1016/0006-2952(74)90178-6

66. Maitre M, Klein C, Patte-Mensah C, Mensah-Nyagan A-G. Tryptophan metabolites modify brain aβ peptide degradation: A role in alzheimer's disease? *Prog In Neurobiol* (2020) 190:101800. doi: 10.1016/j.pneurobio.2020.101800

67. Sanguino-Gómez J, Buurstede JC, Abiega O, Fitzsimons CP, Lucassen PJ, Eggen BJL, et al. An emerging role for microglia in stress-effects on memory. *Eur J Neurosci* (2021) 55(9–10):2491–518. doi: 10.1111/ejn.15188

68. Soichot M, Vaast A, Vignau J, Guillemin GJ, Lhermitte M, Broly F, et al. Characterization of functional polymorphisms and glucocorticoid-responsive elements in the promoter of TDO2, a candidate gene for ethanol-induced behavioural disorders. *Alcohol Alcoholism (Oxford Oxfordshire)* (2013) 48 (4):415–25. doi: 10.1093/alcalc/agt028

69. Li H, Li L, Wang S, Fan A, Yu X, Wang P, et al. Effects of peiyuan jieyu formula on TRP-KYN metabolic pathway and its neuron protection mechanism in depressed rats induced by glucocorticoid. *J Beijing Univ Traditional Chin Med* (2017) 40(12):1011–7. doi: 10.3969/j.issn.1006-2157.2017.12.008

70. Erhardt S, Schwieler L, Imbeault S, Engberg G. The kynurenine pathway in schizophrenia and bipolar disorder. *Neuropharmacology* (2017) 112(Pt B):297–306. doi: 10.1016/j.neuropharm.2016.05.020

71. Tang Y, Dong H. Advances in research on relationship between kynurenine pathway and type 2 diabetes mellitus. *Chin J Pathophysiol* (2019) 35(10):1909–14. doi: 10.3969/j.issn.1000-4718.2019.10.028

72. Linderholm KR, Andersson A, Olsson S, Olsson E, Snodgrass R, Engberg G, et al. Activation of rat ventral tegmental area dopamine neurons by endogenous kynurenic acid: a pharmacological analysis. *Neuropharmacology* (2007) 53(8):918–24. doi: 10.1016/j.neuropharm.2007.09.003

73. Liu X, Hong H. Progress in studies of glutamate-glutamine cycle in the pathogenesis of depression. *Pharm Today* (2022) 32(03):206–10. doi: 10.12048/j.issn.1674-229X.2022.03.008

74. Hanada T. Ionotropic glutamate receptors in epilepsy: A review focusing on AMPA and NMDA receptors. *Biomolecules* (2020) 10(3):464. doi: 10.3390/biom10030464

75. Yuan L, Gong J, Zhang H, Xu B, Qian S, Li G, et al. Research progress of relationship between kainate receptors and neurodegenerative diseases. *Chin J New Clin Med* (2014) 7(04):367–71. doi: 10.3969/j.issn.1674-3806.2014.04.33

76. Zhang G, Yang J. Research progress of NMDA receptor related antidepressants. *Chin Pharmacol Bull* (2018) 34(01):1–4. doi: 10.3969/j.issn.1001-1978.2018.01.001

77. Hardingham GE. Coupling of the NMDA receptor to neuroprotective and neurodestructive events. *Biochem Soc Trans* (2009) 37(Pt 6):1147–60. doi: 10.1042/BST0371147

78. Huang X, Chen H, Wei Y, Qin D, Liu P, Su J. Research progress in targeted antagonists of NMDA receptor. *Prog Physiol Sci* (2018) 49(03):212–6. doi: 10.3969/j.issn.0559-7765.2018.03.011

79. Mok MHS, Fricker A-C, Weil A, Kew JNC. Electrophysiological characterisation of the actions of kynurenic acid at ligand-gated ion channels. *Neuropharmacology* (2009) 57(3):242–9. doi: 10.1016/j.neuropharm.2009.06.003

80. Stone TW, Darlington LG. The kynurenine pathway as a therapeutic target in cognitive and neurodegenerative disorders. *Br J Pharmacol* (2013) 169(6):1211–27. doi: 10.1111/bph.12230

81. Oxenkrug G. Serotonin-kynurenine hypothesis of depression: historical overview and recent developments. *Curr Drug Targets* (2013) 14(5):514–21. doi: 10.2174/1389450111314050002

82. Maddison DC, Giorgini F. The kynurenine pathway and neurodegenerative disease. *Semin In Cell Dev Biol* (2015) 40:134–41. doi: 10.1016/j.semcdb.2015.03.002

83. Zanos P, Thompson SM, Duman RS, Zarate CA, Gould TD. Convergent mechanisms underlying rapid antidepressant action. *CNS Drugs* (2018) 32(3):197–227. doi: 10.1007/s40263-018-0492-x

84. Yan J, Wang C, Ye A, Chen Y. Effects of stress on behavior and glutamate content in some brain regions of rats. *Acta Psychologica Sin* (1995) 04):422–7.

85. Talos DM, Fishman RE, Park H, Folkerth RD, Follett PL, Volpe JJ, et al. Developmental regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor subunit expression in forebrain and relationship to regional susceptibility to hypoxic/ischemic injury. i. rodent cerebral white matter and cortex. *J Comp Neurol* (2006) 497(1):42–60. doi: 10.1002/cne.20972

86. Fujigaki H, Mouri A, Yamamoto Y, Nabeshima T, Saito K. Linking phencyclidine intoxication to the tryptophan-kynurenine pathway: Therapeutic implications for schizophrenia. *Neurochem Int* (2019) 125:1–6. doi: 10.1016/j.neuint.2019.02.001

87. Weber T, Vogt MA, Gartside SE, Berger SM, Lujan R, Lau T, et al. Adult AMPA GLUA1 receptor subunit loss in 5-HT neurons results in a specific anxiety-phenotype with evidence for dysregulation of 5-HT neuronal activity. *Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol* (2015) 40 (6):1471–84. doi: 10.1038/npp.2014.332

88. Qin Y, Song W, Liang Z, Gao M. Progress in research on signal pathway related to antidepressant action of ketamine. *Chin Pharm J* (2021) 56(22):1791–5. doi: 10.11669/cpj.2021.22.002

89. Beggiato S, Tanganelli S, Fuxe K, Antonelli T, Schwarcz R, Ferraro L. Endogenous kynurenic acid regulates extracellular GABA levels in the rat prefrontal cortex. *Neuropharmacology* (2014) 82:11–8. doi: 10.1016/j.neuropharm.2014.02.019

90. Henley JM, Nair JD, Seager R, Yucel BP, Woodhall G, Henley BS, et al. Kainate and AMPA receptors in epilepsy: Cell biology, signalling pathways and possible crosstalk. *Neuropharmacology* (2021) 195:108569. doi: 10.1016/j.neuropharm.2021.108569

91. Gleeson LC, Ryan KJ, Griffin EW, Connor TJ, Harkin A. The β2-adrenoceptor agonist clenbuterol elicits neuroprotective, anti-inflammatory and neurotrophic actions in the kainic acid model of excitotoxicity. *Brain Behavior Immun* (2010) 24(8):1354–61. doi: 10.1016/j.bbi.2010.06.015

92. Behan WM, Stone TW. Role of kynurenines in the neurotoxic actions of kainic acid. *Br J Pharmacol* (2000) 129(8):1764–70. doi: 10.1038/sj.bjp.0703250

93. Burkholder B, Huang R-Y, Burgess R, Luo S, Jones VS, Zhang W, et al. Tumor-induced perturbations of cytokines and immune cell networks. *Biochim Et Biophys Acta* (2014) 1845(2):182–201. doi: 10.1016/j.bbcan.2014.01.004

94. Yan W, Xue D, Jiang S, Ling X, Li N. Research progress on the role of CD4 +T cell polarization in inflammatory diseases. *Chin J Immunol*, 1–18.

95. Fan W, Zhong S, Hu Q, Zeng J, Liao W, Wang J. Tryptophan metabolites regulate Th17/Treg differentiation to alleviate airway inflammation in asthmatic mice receiving allergen-specific immunotherapy. *J Army Med Univ* (2018) 40 (08):658–65. doi: 10.16016/j.1000-5404.201709229

96. Sharma MD, Baban B, Chandler P, Hou D-Y, Singh N, Yagita H, et al. Plasmacytoid dendritic cells from mouse tumor-draining lymph nodes directly activate mature tregs *via* indoleamine 2,3-dioxygenase. *J Clin Invest* (2007) 117 (9):2570–82. doi: 10.1172/JCI31911

97. Fallarino F, Grohmann U, Vacca C, Bianchi R, Orabona C, Spreca A, et al. T Cell apoptosis by tryptophan catabolism. *Cell Death Differentiation* (2002) 9 (10):1069–77. doi: 10.1038/sj.cdd.4401073

98. Mellor AL, Baban B, Chandler P, Marshall B, Jhaver K, Hansen A, et al. Cutting edge: induced indoleamine 2,3 dioxygenase expression in dendritic cell subsets suppresses T cell clonal expansion. *J Immunol (Baltimore Md. 1950)* (2003) 171(4):1652–5. doi: 10.4049/jimmunol.171.4.1652

99. Godin-Ethier J, Pelletier S, Hanafi L-A, Gannon PO, Forget M-A, Routy J-P, et al. Human activated T lymphocytes modulate IDO expression in tumors through Th1/Th2 balance. *J Immunol (Baltimore Md. 1950)* (2009) 183(12):7752–60. doi: 10.4049/jimmunol.0901004

100. Frumento G, Rotondo R, Tonetti M, Damonte G, Benatti U, Ferrara GB. Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. *J Exp Med* (2002) 196(4):459–68. doi: 10.1084/jem.20020121

101. McGovern JL, Nguyen DX, Notley CA, Mauri C, Isenberg DA, Ehrenstein MR. Th17 cells are restrained by treg cells *via* the inhibition of interleukin-6 in patients with rheumatoid arthritis responding to anti-tumor necrosis factor antibody therapy. *Arthritis Rheumatism* (2012) 64(10):3129–38. doi: 10.1002/art.34565

102. Wang J, Simonavicius N, Wu X, Swaminath G, Reagan J, Tian H, et al. Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35. *J Biol Chem* (2006) 281(31):22021–8. doi: 10.1074/jbc.M603503200

103. Zheng X, Hu M, Zang X, Fan Q, Liu Y, Che Y, et al. Kynurenic acid/GPR35 axis restricts NLRP3 inflammasome activation and exacerbates colitis in mice with social stress. *Brain Behavior Immun* (2019) 79:244–55. doi: 10.1016/j.bbi.2019.02.009

104. Salimi Elizei S, Poormasjedi-Meibod M-S, Wang X, Kheirandish M, Ghahary A. Kynurenic acid downregulates IL-17/1L-23 axis in vitro. *Mol Cell Biochem* (2017) 431(1-2):55–65. doi: 10.1007/s11010-017-2975-3

105. Agudelo LZ, Ferreira DMS, Cervenka I, Bryzgalova G, Dadvar S, Jannig PR, et al. Kynurenic acid and Gpr35 regulate adipose tissue energy homeostasis and inflammation. *Cell Metab* (2018) 27(2):378–392.e5. doi: 10.1016/j.cmet.2018.01.004

106. Kong D, Yu Z, Zhou Q, Du G. a4β2-nicotine acetylcholine receptor: advances in relevant diseases and drugs. *Acta Pharm Sin* (2021) 56(01):1–8. doi: 10.16438/j.0513-4870.2020-0677

107. Koola MM. Alpha7 nicotinic-N-methyl-D-aspartate hypothesis in the treatment of schizophrenia and beyond. *Hum Psychopharmacol* (2021) 36(1):1–16. doi: 10.1002/hup.2758

108. Hilmas C, Pereira EF, Alkondon M, Rassoulpour A, Schwarcz R, Albuquerque EX. The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: physiopathological implications. *J Neurosci Off J Soc For Neurosci* (2001) 21 (19):7463–73. doi: 10.1523/JNEUROSCI.21-19-07463.2001

109. Anderson G, Maes M. Interactions of tryptophan and its catabolites with melatonin and the alpha 7 nicotinic receptor in central nervous system and psychiatric disorders: Role of the aryl hydrocarbon receptor and direct mitochondria regulation. *Int J Tryptophan Res IJTR* (2017) 10:1178646917691738. doi: 10.1177/1178646917691738

110. Modoux M, Rolhion N, Mani S, Sokol H. Tryptophan metabolism as a pharmacological target. *Trends In Pharmacol Sci* (2021) 42(1):60–73. doi: 10.1016/j.tips.2020.11.006

111. Wang Y, Chen S, Tan J, Gao Y, Yan H, Liu Y, et al. Tryptophan in the diet ameliorates motor deficits in a rotenone-induced rat parkinson's disease model *via* activating the aromatic hydrocarbon receptor pathway. *Brain Behav* (2021) 11(8): e2226. doi: 10.1002/brb3.2226

112. Rothhammer V, Quintana FJ. The aryl hydrocarbon receptor: an environmental sensor integrating immune responses in health and disease. *Nat Rev Immunol* (2019) 19(3):184–97. doi: 10.1038/s41577-019-0125-8

113. Marszalek-Grabska M, Walczak K, Gawel K, Wicha-Komsta K, Wnorowska S, Wnorowski A, et al. Kynurenine emerges from the shadows - current knowledge on its fate and function. *Pharmacol Ther* (2021) 225:107845. doi: 10.1016/j.pharmthera.2021.107845

114. Sun M, Ma N, He T, Johnston LJ, Ma X. Tryptophan (Trp) modulates gut homeostasis *via* aryl hydrocarbon receptor (AhR). *Crit Rev In Food Sci Nutr* (2020) 60(10):1760–8. doi: 10.1080/10408398.2019.1598334

115. Tufvesson-Alm M, Schwieler L, Schwarcz R, Goiny M, Erhardt S, Engberg G. Importance of kynurenine 3-monooxygenase for spontaneous firing and pharmacological responses of midbrain dopamine neurons: Relevance for schizophrenia. *Neuropharmacology* (2018) 138:130–9. doi: 10.1016/j.neuropharm.2018.06.003

116. Gulaj E, Pawlak K, Bien B, Pawlak D. Kynurenine and its metabolites in alzheimer's disease patients. *Adv In Med Sci* (2010) 55(2):204–11. doi: 10.2478/v10039-010-0023-6

117. Souza LC, Jesse CR, Antunes MS, Ruff JR, de Oliveira Espinosa D, Gomes NS, et al. Indoleamine-2,3-dioxygenase mediates neurobehavioral alterations induced by an intracerebroventricular injection of amyloid-β1-42 peptide in mice. *Brain Behavior Immun* (2016) 56:363–77. doi: 10.1016/j.bbi.2016.03.002

118. Hartai Z, Klivenyi P, Janaky T, Penke B, Dux L, Vecsei L. Kynurenine metabolism in plasma and in red blood cells in parkinson's disease. *J Neurological Sci* (2005) 239(1):31–5. doi: 10.1016/j.jns.2005.07.006

119. Heilman PL, Wang EW, Lewis MM, Krzyzanowski S, Capan CD, Burmeister AR, et al. Tryptophan metabolites are associated with symptoms and nigral pathology in parkinson's disease. *Movement Disord Off J Movement Disord Soc* (2020) 35(11):2028–37. doi: 10.1002/mds.28202

120. Forrest CM, Mackay GM, Stoy N, Spiden SL, Taylor R, Stone TW, et al. Blood levels of kynurenines, interleukin-23 and soluble human leucocyte antigen-G at different stages of huntington's disease. *J Neurochem* (2010) 112(1):112–22. doi: 10.1111/j.1471-4159.2009.06442.x

121. Jauch D, Urbańska EM, Guidetti P, Bird ED, Vonsattel JP, Whetsell WO, et al. Dysfunction of brain kynurenic acid metabolism in huntington's disease: focus on kynurenine aminotransferases. *J Neurological Sci* (1995) 130(1):39–47. doi: 10.1016/0022-510x(94)00280-2

122. Deng Y, Zhou M, Wang J, Yao J, Yu J, Liu W, et al. Involvement of the microbiota-gut-brain axis in chronic restraint stress: disturbances of the kynurenine metabolic pathway in both the gut and brain. *Gut Microbes* (2021) 13(1):1–16. doi: 10.1080/19490976.2020.1869501

123. Duda W, Curzytek K, Kubera M, Connor TJ, Fagan EM, Basta-Kaim A, et al. Interaction of the immune-inflammatory and the kynurenine pathways in rats resistant to antidepressant treatment in model of depression. *Int Immunopharmacol* (2019) 73:527–38. doi: 10.1016/j.intimp.2019.05.039

124. Marx W, McGuinness AJ, Rocks T, Ruusunen A, Cleminson J, Walker AJ, et al. The kynurenine pathway in major depressive disorder, bipolar disorder, and schizophrenia: a meta-analysis of 101 studies. *Mol Psychiatry* (2021) 26(8):4158–78. doi: 10.1038/s41380-020-00951-9

125. Hartai Z, Klivenyi P, Janaky T, Penke B, Dux L, Vecsei L. Kynurenine metabolism in multiple sclerosis. *Acta Neurologica Scandinavica* (2005) 112(2):93–6. doi: 10.1111/j.1600-0404.2005.00442.x

126. Zarzecki MS, Cattelan Souza L, Giacomeli R, Silva MRP, Prigol M, Boeira SP, et al. Involvement of indoleamine-2,3-Dioxygenase and kynurenine pathway in experimental autoimmune encephalomyelitis in mice. *Neurochemical Res* (2020) 45 (12):2959–77. doi: 10.1007/s11064-020-03144-6

127. Chiappelli J, Postolache TT, Kochunov P, Rowland LM, Wijtenburg SA, Shukla DK, et al. Tryptophan metabolism and white matter integrity in schizophrenia. *Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol* (2016) 41(10):2587–95. doi: 10.1038/npp.2016.66

128. Sathyasaikumar KV, Stachowski EK, Wonodi I, Roberts RC, Rassoulpour A, McMahon RP, et al. Impaired kynurenine pathway metabolism in the prefrontal cortex of individuals with schizophrenia. *Schizophr Bull* (2011) 37(6):1147–56. doi: 10.1093/schbul/sbq112

129. Butler MI, Long-Smith C, Moloney GM, Morkl S, O'Mahony SM, Cryan JF, et al. The immune-kynurenine pathway in social anxiety disorder. *Brain Behavior Immun* (2022) 99:317–26. doi: 10.1016/j.bbi.2021.10.020

130. Liu L, Fan P. Effects of tryptophan hydroxylase 2 gene knockout on male rat behavior. *J Northwest A F University(Natural Sci Edition)* (2022) 50(04):17–23. doi: 10.13207/j.cnki.jnwafu.2022.04.003

131. Chen Y, Stankovic R, Cullen KM, Meininger V, Garner B, Coggan S, et al. The kynurenine pathway and inflammation in amyotrophic lateral sclerosis. *Neurotoxicity Res* (2010) 18(2):132–42. doi: 10.1007/s12640-009-9129-7

132. Haney MM, Sinnott J, Osman KL, Deninger I, Andel E, Caywood V, et al. Mice lacking brain-derived serotonin have altered swallowing function. *Otolaryngology–head Neck Surg Off J Am Acad Otolaryngology-Head Neck Surg* (2019) 161(3):468–71. doi: 10.1177/0194599819846109

133. McFarlane HG, Kusek GK, Yang M, Phoenix JL, Bolivar VJ, Crawley JN. Autism-like behavioral phenotypes in BTBR T+tf/J mice. *Genes Brain Behav* (2008) 7(2):152–63. doi: 10.1111/j.1601-183X.2007.00330.x

134. Ormstad H, Bryn V, Verkerk R, Skjeldal OH, Halvorsen B, Saugstad OD, et al. Serum tryptophan, tryptophan catabolites and brain-derived neurotrophic factor in subgroups of youngsters with autism spectrum disorders. *CNS Neurological Disord Drug Targets* (2018) 17(8):626–39. doi: 10.2174/1871527317666180720163221

135. Deng N, Hu J, Hong Y, Ding Y, Xiong Y, Wu Z, et al. Indoleamine-2,3-Dioxygenase 1 deficiency suppresses seizures in epilepsy. *Front In Cell Neurosci* (2021) 15:638854. doi: 10.3389/fncel.2021.638854

136. Żarnowska I, Wróbel-Dudzińska D, Tulidowicz-Bielak M, Kocki T, Mitosek-Szewczyk K, Gasior M, et al. Changes in tryptophan and kynurenine pathway metabolites in the blood of children treated with ketogenic diet for refractory epilepsy. *Seizure* (2019) 69:265–72. doi: 10.1016/j.seizure.2019.05.006

137. Fellendorf FT, Gostner JM, Lenger M, Platzer M, Birner A, Maget A, et al. Tryptophan metabolism in bipolar disorder in a longitudinal setting. *Antioxidants (Basel Switzerland)* (2021) 10(11):1795. doi: 10.3390/antiox10111795

138. Sodhi RK, Bansal Y, Singh R, Saroj P, Bhandari R, Kumar B, et al. IDO-1 inhibition protects against neuroinflammation, oxidative stress and mitochondrial dysfunction in 6-OHDA induced murine model of parkinson's disease. *Neurotoxicology* (2021) 84:184–97. doi: 10.1016/j.neuro.2021.03.009

139. Guo J, Kang K, Li H, Bai Y, Lu X, Li Z, et al. Research progress on pathological mechanism and treatment of amyotrophic lateral sclerosis. *Modern Chin Med* (2021) 41(06):6–10. doi: 10.13424/j.cnki.mtcm.2021.06.002

140. Amirkhani A, Rajda C, Arvidsson B, Bencsik K, Boda K, Seres E, et al. Interferon-beta affects the tryptophan metabolism in multiple sclerosis patients. *Eur J Neurol* (2005) 12(8):625–31. doi: 10.1111/j.1468-1331.2005.01041.x

141. Lv W, Han Y, Guo X, Wang R, Wang Z. Research progress of kynuren ine pathway in alzheime's disease. *J Hainan Med Univ* (2020) 26(09):712–716+720. doi: 10.13210/j.cnki.jhmu.20200212.007

142. Mazarei G, Leavitt BR. Indoleamine 2,3 dioxygenase as a potential therapeutic target in huntington's disease. *J Huntington's Dis* (2015) 4(2):109–18. doi: 10.3233/JHD-159003

143. Ogawa T, Matson WR, Beal MF, Myers RH, Bird ED, Milbury P, et al. Kynurenine pathway abnormalities in parkinson's disease. *Neurology* (1992) 42 (9):1702–6. doi: 10.1212/WNL.42.9.1702

144. Widner B, Leblhuber F, Fuchs D. Increased neopterin production and tryptophan degradation in advanced parkinson's disease. *J Neural Transm (Vienna Austria 1996)* (2002) 109(2):181–9. doi: 10.1007/s007020200014

145. Bai J-H, Zheng Y-L, Yu Y-P. Urinary kynurenine as a biomarker for parkinson's disease. *Neurological Sci Off J Ital Neurological Soc Ital Soc Clin Neurophysiol* (2021) 42(2):697–703. doi: 10.1007/s10072-020-04589-x

146. Miranda AF, Boegman RJ, Beninger RJ, Jhamandas K. Protection against quinolinic acid-mediated excitotoxicity in nigrostriatal dopaminergic neurons by endogenous kynurenic acid. *Neuroscience* (1997) 78(4):967–75. doi: 10.1016/s0306-4522(96)00655-0

147. Silva-Adaya D, Pérez-De La Cruz V, Villeda-Hernández J, Carrillo-Mora P, González-Herrera IG, García E, et al. Protective effect of l-kynurenine and probenecid on 6-hydroxydopamine-induced striatal toxicity in rats: implications of modulating kynurenate as a protective strategy. *Neurotoxicol Teratology* (2011) 33 (2):303–12. doi: 10.1016/j.ntt.2010.10.002

148. Campesan S, Green EW, Breda C, Sathyasaikumar KV, Muchowski PJ, Schwarcz R, et al. The kynurenine pathway modulates neurodegeneration in a drosophila model of huntington's disease. *Curr Biol CB* (2011) 21(11):961–6. doi: 10.1016/j.cub.2011.04.028

149. Alarcan H, Chaumond R, Emond P, Benz-De Bretagne I, Lefèvre A, Bakkouche S-E, et al. Some CSF kynurenine pathway intermediates associated with disease evolution in amyotrophic lateral sclerosis. *Biomolecules* (2021) 11 (5):691. doi: 10.3390/biom11050691

150. Lee E-J, Choi SY, Kim E. NMDA receptor dysfunction in autism spectrum disorders. *Curr Opin In Pharmacol* (2015) 20:8–13. doi: 10.1016/j.coph.2014.10.007

151. Shao H, Yang Y, Mi Z, Zhu G-X, Qi A-P, Ji W-G, et al. Anticonvulsant effect of rhynchophylline involved in the inhibition of persistent sodium current and NMDA receptor current in the pilocarpine rat model of temporal lobe epilepsy. *Neuroscience* (2016) 337:355–69. doi: 10.1016/j.neuroscience.2016.09.029

152. Klinkenberg S, van den Borne CJH, Aalbers MW, Verschuure P, Kessels AG, Leenen L, et al. The effects of vagus nerve stimulation on tryptophan metabolites in children with intractable epilepsy. *Epilepsy Behav E&B* (2014) 37:133–8. doi: 10.1016/j.yebeh.2014.06.001

153. Song H, Wu Z, Chauktu S, Luo T, Zhao J. Effects of chronic stress on KYN, IDO and TRP in the hippocampus of rats and the intervention effect of electroacupuncture. *Int Med Health Guidance News* (2019) 23):3833–5. doi: 10.3760/cma.j.issn.1007-1245.2019.23.004

154. Fu X, Hong L, Wang Y, Guo M, Liu W. Role of tryptophan-2, 3-dioxygenase in central nervous system diseases and potential for associated therapeutic strategies. *Chin J Comp Med* (2018) 28(10):117–21. doi: 10.3969/j.issn.1671-7856.2018.10.020

155. Plitman E, Iwata Y, Caravaggio F, Nakajima S, Chung JK, Gerretsen P, et al. Kynurenic acid in schizophrenia: A systematic review and meta-analysis. *Schizophr Bull* (2017) 43(4):764–77. doi: 10.1093/schbul/sbw221

156. Trepci A, Sellgren CM, Pålsson E, Brundin L, Khanlarkhani N, Schwieler L, et al. Central levels of tryptophan metabolites in subjects with bipolar disorder. *Eur Neuropsychopharmacol J Eur Coll Neuropsychopharmacol* (2021) 43:52–62. doi: 10.1016/j.euroneuro.2020.11.018

157. Long Y, Yang Q, Xiang Y, Zhang Y, Wan J, Liu S, et al. Nose to brain drug delivery - a promising strategy for active components from herbal medicine for treating cerebral ischemia reperfusion. *Pharmacol Res* (2020) 159:104795. doi: 10.1016/j.phrs.2020.104795

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Neuroinflammation in Huntington's disease: From animal models to clinical therapeutics

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Huntington's disease (HD) is a progressive neurodegenerative disease characterized by preferential loss of neurons in the striatum in patients, which leads to motor and cognitive impairments and death that often occurs 10-15 years after the onset of symptoms. The expansion of a glutamine repeat (>36 glutamines) in the N-terminal region of huntingtin (HTT) has been defined as the cause of HD, but the mechanism underlying neuronal death remains unclear. Multiple mechanisms, including inflammation, may jointly contribute to HD pathogenesis. Altered inflammation response is evident even before the onset of classical symptoms of HD. In this review, we summarize the current evidence on immune and inflammatory changes, from HD animal models to clinical phenomenon of patients with HD. The understanding of the impact of inflammation on HD would help develop novel strategies to treat HD.

KEYWORDS

Huntington's disease, immune response, inflammation, animal models, immune therapies

1 Introduction

Huntington's disease (HD) is a devastated neurodegenerative disorder caused by a trinucleotide CAG repeat expansion in exon 1 of HD gene encoding for huntingtin (HTT) protein (1–3). The normal *HTT* gene contains less than 36 CAG repeats, whereas mutant *HTT* carriers 40 or more CAG repeats with complete penetrance. Expansions varying from 36 to 39 CAG repeats may also result in HD, but with incomplete penetrance (1). The CAG repeat expansion encodes an expanded polyglutamine (polyQ) tract in mutant HTT that elicits neuronal loss in HD (4). One of the important mechanisms for neurodegeneration in HD is the inflammation caused by mutant HTT, which is the focus of this review for discussion. Generally, mutant HTT causes the inflammatory response in both of the central nervous system (CNS) and peripheral tissues directly. Immunomodulatory messengers can cross the blood-brain barrier (BBB) in both directions, with inflammation spreading from

the periphery to the CNS or vice versa (5, 6). The degree of microgliosis and astrogliosis correlates with disease severity directly, and result in an increased production of inflammatory mediators. The increase in microglial activation seemed to be a very early event that occurs before the neuropathological changes. Pathological processes underlying HD, such as excitotoxicity or oxidative stress, induce the morphological change of microglia by a constant expression of inflammatory mediators (7). The secreted pro-inflammatory cytokines can damage nerve cells, leading to their death (8). Astrocytes are also susceptible to inflammatory processes in the brain, becoming activated locally by dead neurons or pathogens. For example, the mutant HTT expressed in glial cells, primarily astrocytes in HD mouse models, could reduce their neuroprotective function (9, 10). However, in some conditions, the neuroinflammation can benefit neuronal tissue by promoting cell debris clearance (11). This review will summarize the growing evidence on inflammatory changes, including the pros and cons of pathogenic mutant HTT, from HD animal models to patients' clinical phenotypes. The better understanding of the impact of neuroinflammation cytokines on HD will open new venues for the development of novel therapeutic targets.

2 Neuroinflammation in different animal models of Huntington's disease

2.1 Drosophila models

Drosophila melanogaster has been used as an early HD animal model to identify metabolic biomarkers at pre-symptomatic and symptomatic stages of the disease. *In vivo* experiments have revealed that the ectopic overexpression of mutant human HTT (exon 1 with expanded CAG repeats) in the neural tissue of transgenic flies causes neurodegeneration (12, 13). However, there was limited work on neuroinflammation in Drosophila model of HD. Lin et al. observed that the expression of mutant HTT (Q93) in hemocytes did not directly affect the animal survival, but the numbers of circulating hemocytes were significantly decreased, leading to an impaired immune response against pathogenic invasion. Mutant HTT caused the altered production of cytokines, such as *upd3*, *dome*, *tep1, totA, totB, and totC*, and consequently the immune dysregulation in Drosophila (14).

2.2 Mouse models

Identification of the genetic mutation for HD has accelerated the establishment of various transgenic or knock-in HD mouse models, in which human mutant HD gene is inserted randomly into the mouse genome or precisely into the endogenous mouse *HTT* gene. In HD transgenic mice, the animals expressed a full-length or a fragment of the mutant *HTT* gene in addition to the two normal copies of the endogenous mouse *HTT* gene (15). Common transgenic models of HD are divided as follow. Firstly, N-terminal HTT fragment models with expanded CAG-repeats, such as R6/2, R6/1 or N171-82Q, present an earlier onset of motor and cognitive abnormalities (16). The R6/1 and R6/2 transgenic mice were the first transgenic model developed to study HD, which express exon 1 of the human HD gene with around 115 and 150 CAG repeats respectively (16). Secondly, full-length transgenic mouse models carry mutant HTT in a yeast artificial chromosome (YAC) or bacterial artificial chromosome (BAC), which present a slow progression of the disease with comparable neuropathology and motor-related behavior changes (17, 18). Despite the wealth of different transgenic HD mice available currently, the R6/2 and YAC128 mouse strains are widely used to study many pathological aspects of HD (19). Symptoms of motor impairment in R6/2 mice appear around 3-6 weeks of age. A continuous loss of weight results in death between 11-14 weeks of age (20–22). In YAC128 mice with its full length mutant HTT spanning about 120 CAG repeats (18), hypoactivity is first seen until the age of 8 months. Additionally, progressive gait abnormalities, ataxia, hind limb clasping, and a progressive decline in the forced motor function occur over time (23). In HD knock-in mice, the pathological CAG-repeat is integrated into the mouse *HTT* gene in a homozygous or heterozygous manner. Because knock-in mice carry the mutation in its appropriate genetic and protein context, they are the most faithful genetic model of human disease. The widely used HdhQ150 and zQ175 mouse models exemplarily belong to this group (24–26). Knock-in mice exhibit more slow progression and age-dependent development of behavioral, pathological, cellular, and molecular abnormalities, which makes these animals valuable for studying age-dependent neuropathology and symptoms.

Based on the various transgenic or knock-in HD mouse models, many researchers reported the important role of the immune system in HD. Träger et al. investigated the myeloid cells from different HD mouse models of R6/2, HdhQ150 and YAC128, to assess whether they are similarly hyperresponsive as HD patient cells. They found that blood CD11b+ cells isolated from 12-week-old R6/2 and 22-month-old HdhQ150 are hyper-reactive upon LPS stimulation *in vitro*, replicating the phenotype observed in human cells. Analyzing the cytokine profile in 3-month-old YAC128 mice, peritoneal macrophages were found to produce high levels of IL-6 upon the standard endotoxin stimulation. These mouse models recapitulate altered cytokine profiles identified in HD patients, and therefore they could be used to model the immune phenotype (27). Pido-Lopez et al. revealed that the increased TNF-α and IL-12 levels in the striatum of 14-week-old R6/2 mice while IL-12 and IL-1β decreased in the cortex (28). In contrast, Godavarthi et al. found no differences in cytokine expression, including MCP-1,

IL-6, IL-10, TNF-α, interferon-γ and IL-12, in the brain samples of R6/2 mice compared with the age-matched control. However, the Iba-1 immunostaining did not reveal any significant increase in the numbers of microglia in the cortex and striatum of R6/2 mice at 12 weeks, an age when these mice show severe symptoms (29). Recently, the fractalkine signaling axis has been explored in the 8-20 weeks old R6/1 HD mouse, and the expression levels of cx3cl1 have been significantly decreased (30), which may lead to an increase in synaptic pruning mediated by microglia, as the total density of PSD-95 puncta decreased and the density of PSD-95 puncta in Iba1 stained microglia increased significantly (30). Furthermore, administering CX3CL1 protein was able to rescue R6/1 mice from long-term depression. It appears that striatal synaptic plasticity dysfunction in pre-symptomatic R6/1 mice is caused by a reduction in CX3CL1 (30), which is consistent with the previous research that CX3CL1 regulates microglia engulfment of synapse (31). Additionally, quinolinic acid (QUIN) has been associated with neuroinflammation in various neurological diseases (32). It is possible that the activation of microglia and astroglia may result in a production of the excitotoxic metabolites, such as the kynurenine pathway, before morphological markers of glial activation are apparent (33–35). Thus, abnormalities in the kynurenic acid pathway are an interesting aspect of HD pathology. QUIN and 3-hydroxykynurenine (3-HK) are neurotoxic metabolites that are elevated in the cortex of HD patients at the early stages of disease progression (36). Especially the 3-HK levels were elevated in the striatum, cortex and cerebellum in the R6/2 starting at 4 weeks of age significantly and selectively, and both 3-HK and QUIN levels were increased in the striatum and cortex in Hdh^{Q92} and Hdh^{Q111} at 15 months and YAC128 mouse at 8 months (35).

The altered immune system function, not only in the CNS but also peripherally, has been implicated in HD pathogenesis (6, 37). To elucidate peripheral inflammatory activation in HD, Björkqvist et al. measured the levels of multiple cytokines in the serum of R6/2, Hdh150Q/150Q and YAC128 mice. In 12-week-old R6/2 mice, IL-6, IL-10, IL-1β, and IL-12p70 were significantly increased as compared to control animals. However, the IL-6, IL-10, and IL-12p70 were significantly elevated in 22-month-old Hdh150Q/150Q mice. In the YAC128 mouse model of HD at 12 months of age, the similar elevations in serum IL-6 and IL-8 were observed (6). Similary, Chang et al. detected the levels of inflammatory markers in the plasma of R6/2 mice. IL-6 levels in R6/2 mice at different disease stages (9, 11 and 13 weeks) were higher than those in the age-matched wild-type (WT) littermates, with higher levels of MMP-9 and TGF-β1 compared with their WT littermates from 11 weeks. In contrast, the plasma level of IL-18 (11-13 weeks old) was lower than control mice (38). Furthermore, Disatnik et al. also measured the levels of inflammatory cytokines such as TNF and IL-6, the levels of both these cytokines were two times higher in the plasma of 13 weeks old R6/2 mice than WT mice (39). Interestingly, treatment with P110 (a selective peptide inhibitor, of excessive mitochondrial fission) for 8 weeks was efficient in reducing the levels of these inflammatory levels to the level in WT mice (39, 40). A study including 9-12 months old YAC128 mice serum samples revealed the significant increases in IL-8 and IL-10 levels. However, the elevation of IL-6 in serum of YAC128 mice occurred at 12 months of age whereas the level of IFN-γ increased in serum samples from YAC128 mice were at 6-9 months old (41). More recently, Podlacha et al. also reported the significant elevation of levels of inflammatory markers, such as IL-6, TNF-α, IL-1β and IL-12, in R6/1 mouse peripheral blood. This allows for a more objective assessment of particular biomarkers in course of a slower progression of symptoms in HD mice. At early stages of disease, the anti-inflammatory defense mechanism was significantly impaired, with a marked decrease in IL-10 levels (42). Valadão et al. also investigated the immune changes in many peripheral organs of the 12-month-old BAC HD model. They found significant changes in cytokine levels in all organs analyzed, including heart, liver, spleen and kidney. Levels of IL-6 and IL-12p70 were increased in the heart of BAC HD mice as compared with WT animals. In the liver samples of BAC HD mice, enhanced IL-12p70 and TNF-α levels were observed. In the spleen, there was an increase in the levels of the cytokine IL-4, but a decrease in the levels of IL-5 and IL-6 in BAC HD mice. However the increased level of IL-6 was exhibited in kidney (43).

Considering that pro-inflammatory cytokine IL-6 may actively influence the disease course in HD, an excessive IL-6 release was also detected in YAC128 mice at 12 months and R6/2 mice at 9, 11 and 13 weeks of age (6, 38). Bouchard et al., administered an IL-6 neutralizing antibody to R6/2 mice to test whether IL-6 influences the disease course. In their work, treatment with the specific antibody in R6/2 mice reduced weight loss at late stages and partially rescued motor deficits on the rotarod performance as compared with the control IgG treatment (44). Following this line of reasoning, Wertz et al. tested the hypothesis that IL-6 deficiency would be protective against the effects of mutant huntingtin and therefore generated the R6/2 mice model lacking IL-6. Contrary to previous studies, the lack of IL-6 exacerbated R6/2 associated behavioral phenotypes. Based on a single nuclear RNA sequencing of striatal cell types, it was evident that IL-6 deficiency affected normal regulation of various genes associated with synaptic function, as well as the BDNF receptor Ntrk2 (45).

Although relatively shorter experimental period and lower housing cost of using rodents are the advantages of studying mouse models of HD, the data on activation of the inflammatory system in HD mouse models are sparse and contradictory, possibly due to the considerable pathological differences between different transgenic strains and at different disease stages. The immunocytochemical staining of GFAP or Iba-1 has been widely used to identify gliosis, an early CNS damage that is associated with neuroinflammation in HD (46–49).

Different levels of gliosis activation were observed in mouse models with full-length or a fragment HTT at different ages. Yu et al. systematically compared that the N171-82Q mouse striatum and found a GFAP increase, at 3 months of age and became prominent at 4-5 months of age, indicated by intense labeling throughout astroglial cell bodies and their fibrous processes. Although the striatum of R6/2 mice at 12 weeks of age displayed some increased GFAP staining, the overall number of GFAP-labeled glial cells was not increased in R6/2 mice compared with that of their littermates or other HD mice. In R6/1 mice even at 9 months of age, there was still little GFAP staining in the striatum (50). Reiner et al. also compared the cortex (22 and 30 weeks) and striatum (30 weeks) of R6/2 chimeras, which displayed some glial cells with increasingly upregulated GFAP staining, much later than pure R6/2 mice that died at 12-15 weeks of age (51). Gatto et al. found that the neuroinflammatory processes with activated astrocyte GFAP occurred in the 11- and 30-week-old R6/1 mice (52). In 17-week-old R6/2 mouse model, reactive astrocytes with processes enveloping degenerating neurons was seen, though no accompanying inflammatory response or increased macrophages and microglia was observed (53). Simmons et al. also reported that the Iba-1 positive cells did not obviously differ in both R6/2 and WT mice at the age of <7 weeks, but increased in R6/2 mice older than 8 weeks (54). While Hdh150Q knock-in mice at 27-30 weeks of age also did not show intense GFAP immunoreactivity, a significant increase in GFAP immunoreactivity was found in the striatum of Hdh^{150Q} mice at 14 months of age, as reported previously (26, 50). In HD KI mice expressing shorter CAG repeats (HdhQ92 or HTTQ111), no gliosis was found up to 17-18 months of age (55, 56). However, in 18-20 months old Hdh175/175 mouse, the expression of full-length mHTT in microglia was able to promote pro-inflammatory transcription (57). Different extents of gliosis seen in HD mice are likely associated with genetic background, CAG repeats in the HD gene, housing and experimental conditions. However, the data in HD patients show that the inflammatory response is activated at the very early disease stage (6). It is also necessary to point out that most of rodent models fail to mimic the overt and typic neurodegeneration seen in HD patients (58–60). The lack of overt neuronal loss and robust gliosis in HD mouse models prevents the rigorous evaluation of the therapeutic effects on neurodegeneration. The summarized evidence on gliosis activation, including the pros and cons observations, was listed in Table 1.

2.3 Large animal models

The biological differences between humans and mice may account for the failure of some mouse models to replicate pathology seen in humans. Thus, it is possible that larger transgenic animal models may be able to mimic important neurodegenerative features. However, an HD transgenic sheep model (OVT73) was created, which does not exhibit many of the overt phenotypes observed in HD patients and was thought to be a model of prodromal or early-stage HD (65). By using CRISPR/Cas9, Yan et al. established the 140Q KI pig that endogenously expresses full-length mutant HTT (66). Importantly, the increased immunohistochemical staining of GFAP was firstly observed in the dorsal caudate nucleus and putamen of the 4-5-month-old F0 KI and F1 KI pigs. Also, immunostaining with the antibody to Iba-1 revealed a marked increase of the microglial cells in KI pig brain, which is more abundant in the striatum than in the cortex. Quantification of the number of different types of cells revealed that the KI striatum had the most severe loss of NeuN-positive cells and the highest increase in glial cell numbers, which was not observed in the age-matched HD KI mouse (66, 67). Additionally, Valekova et al. used transgenic HD minipig, which was generated by injecting lentiviral vectors carrying truncated mutant huntingtin genes that encode 124 glutamine repeats integrated into chromosome 1q24-q25 and transmitted through successive three generations (68), for investigating multiple cytokines (69). At the same time, various cytokines were analyzed in the secretomes of microglia and blood monocytes, as well as in the cerebrospinal fluid (CSF) and serum collected from pre-symptomatic HD minipigs. A decline in IFN-α was observed in CSF collected from an early time at 9 months of age and lasted at least up to 36 months. The transgenic minipigs at 36 months of age had lower levels of IL-10 in the CSF. IFN-α and IL-10 levels were also decreased in secretome of microglia, whilst elevated IL-8 and IL-1β levels were secreted by primary microglia that were isolated from the HD minipigs at 36 months of age. In serum samples collected from 36-month-old HD minipigs had significantly higher levels of IL-8 than WT ones (69).

Besides, transgenic non-human primate models expressing the disease genes were established, transgenic HD rhesus monkeys, which express exon 1 mutant HTT with 84Q under the control of the human ubiquitin promoter, were generated by injecting lentiviruses into fertilized oocytes to express mutant HTT (70). HD transgenic monkeys with 84Q die postnatally, and this early death was associated with the overexpression of N-terminal mutant HTT. Despite their early death, some transgenic monkeys developed key clinical HD features including dystonia, chorea, and seizure (70). Interestingly, the 5-year-old transgenic HD monkeys expressing N-terminal HTT 509 amino acids with approximately 67-72Q under the human *HTT* promoter (71) exhibited increased pro-inflammatory cytokines and higher induction of immune pathway genes, such as inflammatory response factor IL-6, TNF-α, and C-Reactive Protein (CRP) (72) (Figure 1).

TABLE 1 Changes in glial cells in different HD mouse models at multiple ages.

Model	Age	Glial status	Reference
R6/2	2-16 weeks	Iba-1 positive cells did not obviously differ in both R6/2 and wild-type mice at 2-4 and 5-7 weeks, but increased at 8-10 and 11-13 weeks old in R6/2 mice.	(54)
R6/2	4 and 13 weeks	Microglia increased significantly in the 4 and 13 weeks old R6/2 mice compared to the age-matched WT.	(61)
R6/2	8-12 weeks	R6/2 striatal revealed increased Iba-1$^+$ microglia and GFAP$^+$ astroglia at a symptomatic stage of 12 weeks old.	(62)
R6/2	12 weeks	Reactive astrocytes were rarely observed in the cortex and striatum of HD and microglia did not change in number at 12 weeks old R6/2.	(29)
R6/2	22 and 30 weeks	GFAP was increasingly upregulated in the cortex (22 and 30 weeks), and striatum (30 weeks) of R6/2 chimeras much later than pure R6/2 mice that died at 12-15 weeks of age.	(51)
R6/1	11 and 30 weeks	GFAP, an astrocyte activated markers, was found in the early stage of the disease in R6/1 mice.	(52)
R6/1	9 months	Little GFAP staining in the striatum in R6/1 mice at 9 months of age.	(50)
N171-82Q	3-5 months	GFAP staining increased in the striatum of N171-82Q mice at 3 months of age and became prominent at 4-5 months of age.	(50)
YAC128	3, 6 and 12 months	Microglia increase in both size and number in YAC128 mice at 12 months old.	(63)
KI 140Q	4,12 and 23 months	GFAP immunostaining showed no change in KI mice at 4 months but increased at >12 months.	(64)
Hdh150Q	27-30 weeks and 42-52 weeks	Hdh150Q mice at 27-30 weeks of age did not show intense GFAP immunoreactivity but a marked increase in GFAP immunoreactivity in the striatum of 42-52-week-old Hdh150Q.	(26)
Hdh150Q	14 months	14-month-old HdhCAG150 mice had a greater number of GFAP-positive cells.	(50)
HttQ111	2-18 months	HttQ111/+ mice at 18 months of age did not show increased gliosis.	(56)
HdhQ175	18-20 months	Expression of full-length mHTT in microglia was able to promote pro-inflammatory transcription at 18-20 months of age.	(57)

3 Neuroinflammation in Huntington's disease patients

Robust evidence regarding neuroinflammation in HD comes from postmortem studies of brain tissues from patients with HD. Evidence for involvement of inflammation in the pathogenesis of HD includes upregulation of inflammatory cytokines or chemokines and activation of the complement system. Björkqvist et al. have reported the increase of multiple cytokines in the plasma (IL-1β, IL-4, IL-6, IL-8, TNF-α, IL-10) and striatum (IL-6, IL-8 and TNF-α) of HD patients (6). Interestingly, a significant increase in IL-6 plasma levels was found in pre-symptomatic HD mutation carriers 16 years prior to the predicted onset of the disease, suggesting that inflammatory changes occurs very early in the disease process (6). According to Dalrymple et al., the level of IL-6 in HD patient plasma was increased significantly (37). Likewise, HD patients also exhibit increased expression of IL-6, IL-8, and matrix metalloproteinase-9 (MMP-9) in their cortex and cerebellum (73). Remarkably, MCP1/CCL2 and IL-10 mRNA levels were significantly higher in the striatum of HD patients than controls (73). Gang et al. also quantified the plasma concentrations of IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNF-α from HD patients. Patients with HD have significantly lower plasma concentrations of IL-4, a marker of responses from T-helper-2 cells, than healthy controls. In contrast, no significant differences was observed in the plasma concentrations of IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, IL-13 or TNF-α in HD patients (74). Recently, von Essen MR et al. reported the immune abnormalities before motor onset of disease (75). Proinflammatory cytokines, including IL-17, were detected in the CSF of HTT mutation carriers, as well as the increased IL-7 consumption before motor onset of HD. Moreover, they reported an increased prevalence of IL-17 producing Th17.1 cells in the CSF of HTT mutation carriers, predominantly in pre-motor manifest individuals. There was a negative correlation between intrathecal Th17.1 cell frequency and the progression of HD, suggesting that Th17.1 cells play an important role at the early stages of the disease. Moreover, the author found that the balance of pro-inflammatory and regulatory T cells was skewed. This skewing further favors a pro-inflammatory environment in the CSF of HTT mutation carriers (75).

In particular, a significant elevation of chemokines (C-C motif) ligand (CCL)-2, CCL4, CCL11, CCL13, CCL26 has been detected in the plasma from HD patients (76). Wild et al. have also reported that CCL11 and related chemokines may directly contribute to the neurodegenerative processes in CNS. As a result of analyzing plasma levels of cytokines in two separate cohorts of HD patients, the elevated levels of CCL11 and CCL26 were found in HD group from their first cohort of 65 HD patients (76). There were also significant differences in CCL11 and CCL26 levels across all HD clinical stages. In their second cohort of 68 HD patients and 26 healthy controls, plasma CCL11 and CCL26 levels were significantly increased with more advanced HD cases. In addition, CCL11 levels were positively correlated with standardized assessments of motor impairment while negatively correlated with functional scores (76, 77). Thus, it is worth performing future studies of both CCL11 and CCL26

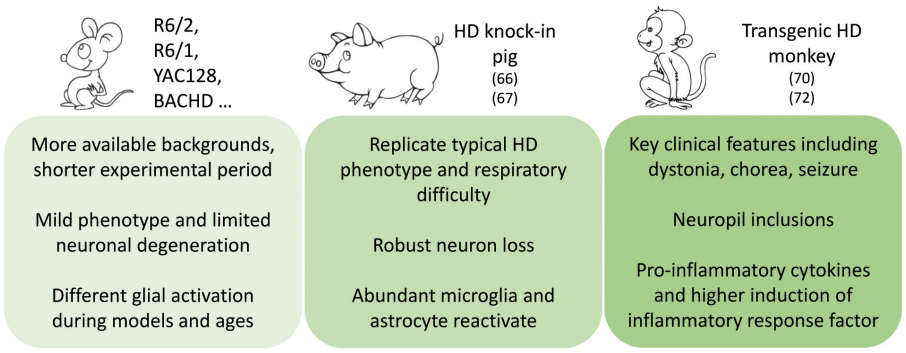

FIGURE 1
Pathogenic insights from and phenotype differences between animal models expressing mutant HTT.

as potential biomarkers in HD. It has been shown that the complement factors C1QC, C2, and C3 are also increased in CSF samples taken from living HD patients in comparison with controls (78). Singhrao et al. also found the complement activation, and increased mRNA levels of complement proteins in HD brains (79). Increased protein expression of complement components C7 and C9, complement inhibitor cluster proteins, and acute phase protein α-2-macroglobulin were shown in HD patient plasma and CSF (37).

Neuroinflammation can be modulated by neuron-glial signaling through various soluble factors, such as cluster of differentiation CD22 (80), CD47 (81), CD200 (82, 83), the family of CD300 receptors (84) and CX3CL1 (85). CX3CL1 produced in neurons is the sole member of the CX3C family of chemokines (86), and it is known that CX3CL1 is the only ligand binding to CX3CR1, a 7 transmembrane domain class A G-protein coupled receptor that is expressed in microglia, monocytes, natural killer cells (NK), T cells and smooth muscle cells (87). Interestingly, CX3CL1 was also found to be an important novel factor in HD pathogenesis and survival following a network analysis of microarray data from human post-mortem tissue (88). An investigation of the fractalkine signaling axis in HD patients revealed that cx3cl1 gene expression was significantly reduced in their putamen (30) (Figure 2).

4 Immune therapies

The accumulating evidences supports that inflammation plays a key role in neurodegenerative diseases and has stimulated the use of immunotherapeutic strategies to modulate neuroinflammatory diseases. Several preclinical and clinical trials of potential immunomodulatory drugs have been investigated in HD, such as minocycline and cannabinoids, laquinimod, TNF-α inhibitors, anti-SEMA4D monoclonal antibody, gangliosides and so on.

4.1 Minocycline and cannabinoids

It has also been demonstrated that minocycline and cannabinoids have anti-inflammatory properties, even though they are not a member of the classical definition of anti-inflammatory drugs. R6/2 mice were administered with the anti-inflammatory tetracycline minocycline showed improvements in behavioral and neuropathological deficits (89, 90), which is also supported by an excitotoxic rat model of HD (91). A clinical study revealed that minocycline at 100 and 200 mg/day was well tolerated for 8 weeks. The study involved 60 patients who were randomly assigned to receive placebo (n = 23), minocycline 100 mg/day (n = 18), or minocycline 200 mg/day (n = 19). However, there was no effect on the UHDRS (Unified HD Rating Scale) scores, with no efficacy observed (92). Cannabidiol was considered safe and well tolerated during a 6 weeks clinical trial conducted on 15 patients affected by HD. However, clinical outcomes did not show the significant improvement (93).

4.2 Laquinimod

The immunomodulator laquinimod (LAQ) could downregulate both the production of pro-inflammatory cytokines in peripheral blood mononuclear cells and neuroglial activation in the brain, which was initially known as an immunomodulatory agent and was used to treat multiple sclerosis. For example, the neuroprotective effect of LAQ is well supported by its therapeutic effects on animal models of neuroinflammatory diseases, such as the experimental

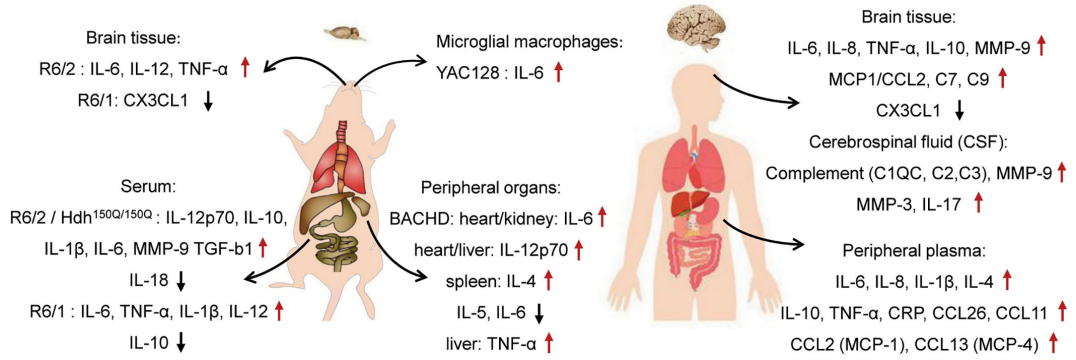

FIGURE 2
Schematic summary of brain and peripheral inflammatory changes in HD mice and patients.

autoimmune encephalomyelitis (EAE) (94, 95), and by alleviation of demyelination in different diseases (96–99). LAQ has also been shown to improve behavioral phenotypes and white matter integrity in many HD mouse models (100–104). Especially, LAQ rescues evidence of cortico-striatal neurodegeneration, demyelination of white matter, and behavioral deficits in YAC128 HD mice (105). A mild ameliorative effect of LAQ was also observed in R6/2 mice with motor function deficits and striatal neuropathology (106). Thus, the therapeutic effect of LAQ is therefore thought to be due to an anti-inflammation effect (98, 103). Although the precise mechanism of action of LAQ is unclear, Dobson et al. demonstrated that LAQ significantly dampened the release of hyper-reactive cytokines from stimulated premanifest and manifest HD patient monocytes, and may exert its neuroprotective effects by promoting BDNF production (94). However, LAQ had no efficacy observed in the Phase 2 clinical trials of HD patients unfortunately. (https://clinicaltrials.gov/ct2/show/NCT02215616). Thus, whether LAQ can be used to treat HD patients remains to be verified.

4.3 TNF-α inhibitor

TNF-α is a multifunctional cytokine associated with cellular proliferation, differentiation, inflammation, immune responses and apoptosis (107). Based on the evidence of increased levels of TNF-α in HD, one study investigated the therapeutic potential of DN-TNF-α (XPro1595), which demonstrated that intracerebroventricular (ICV) injection of DN-TNF-α modulates neuroinflammation, caspase activation, mHTT aggregate burden, and motor function deficit in R6/2 HD transgenic mice (108). However, the clinical efficacy of this molecule in human HD warrants further investigation based on the fact that DN-TNF-α's systemic injection rather than an ICV injection shows lesser efficacy on motor function in R6/2 mice (109). However, in a study of R6/2 mice carried out by Pido-Lopez et al., the systemic injection of etanercept, a drug that inhibits TNF-α, dampened the levels of TNF-α in plasma and other peripheral proinflammatory molecules such as IL-1β and IL-6. However, the level of TNF-α and IL-6 expression in the striatum is not affected by etanercept treatment. According to the follow-up study, etanercept partially reduced brain atrophy, but failed to improve HD related functional and cognitive deficits in R6/2 mice (110).

4.4 SEMA4D antibody

Semaphorin 4D (SEMA4D), also called CD100, is chemorepulsive axonal guidance and immunoregulatory transmembrane signaling molecule. It signals *via* three receptor subtypes of Plexin-B1 (PLXNB1), Plexin-B2 (PLXNB2) and CD72. In the CNS, SEMA4D interacts with PLXNB1 in neuronal cells *via* Rho-GTPases-RhoA and R-Ras GTPase-activating protein activities, inducing axonal growth cone collapse (111, 112). In previous studies, the treatment on YAC128 HD transgenic mice with neutralizing SEMA4D antibody has been shown to ameliorate the neuropathological deficits and behavioral symptoms (111). However, it is still unclear whether SEMA4D inhibition will be beneficial in human HD.

4.5 Gangliosides and others

Besides, therapeutic administration of the brain gangliosides, GM1, has also been used to provide neuroprotection in models of neuronal injury and neurodegeneration of Alzheimer's Disease (AD) (113), Parkinson's Disease (PD) (114, 115) and HD (116). Furthermore, the intraventricular administration of GM1 showed profound disease-modifying effects across HD mouse models exhibiting varying genetic backgrounds, and there is a reduction

in mutant HTT levels after GM1 administration (116). The treatment of R6/2 mice with GM1 slows down the white matter atrophy and body weight loss, while the motor functions have been significantly improved. The administration of GM1 also ameliorated psychiatric-like and cognitive dysfunctions and gait abnormalities. It was also shown that GM1 administration improves psychiatric-like and cognitive dysfunctions in YAC128 mice (116). Prados et al. reported an efficacy of the compound betulinic acid hydroxamate (BAH), a hypoximimetic derivative of betulinic acid, against the striatal HD neurodegeneration. In striatal STHdhQ111/Q111 cells, BAH stabilized HIF-1α protein and protected against mitochondrial toxin-induced cytotoxicity. Pharmacokinetic analyses showed that BAH had a good brain penetrability in 3-nitropropionic acid-treated mouse model with striatal neurodegeneration, improved the clinical symptoms, prevented neuronal loss, decreased reactive astrogliosis and microgliosis, and inhibited the upregulation of proinfammatory markers in the brain (117). Purushothaman et al. also reported the neuropharmacological protective effect of Baicalein (BC) against the Quinolinic Acid (QA)-induced HD-like rat models that displayed the psychological and behavioural changes. This study proved that BC is efficient to revive the level of enzymatic and non-enzymatic antioxidants and mitochondrial complexes by decreasing a number of inflammatory mediators such as Malondialdehyde (MDA), protein carbonyls and Nitric Oxide. It also restores the amount of BDNF and GDNF, thereby preventing the neurophysiological changes (118). Taking together, these findings show that targeting cytokines might assist in resolving neuroinflammation, but evidence suggests that merely suppressing the neuroinflammatory processes would be insufficient to restore functional capacity in HD.

5 Concluding remarks

The contribution of neuroinflammation to neurodegeneration has previously been defined. However, the role of CNS and peripheral inflammatory changes in HD remains poorly understood. This is because neuroinflammatory and neuroimmune reactions can be beneficial or detrimental, and there are various interactions between diverse brain cell types and the signaling cascades triggered in HD. Various families of cytokines and cytokine receptors, growth factors, and chemokines influence the apoptotic or survival pathways of neurons and the degree of inflammatory processes in the CNS. It is unclear whether inflammatory changes are caused by neurodegeneration or represent an independent pathological mechanism. Thus, it is important to refine our understanding of these more specific immune and inflammatory mechanisms involved in HD.

Although HD mouse models have been widely used to investigate HD pathogenesis and neuropathology, there are various differences in neurodegenerative pathophysiology between rodent models and clinical patients. In particular, neuronal inflammatory responses were found in HD patients at the early disease stage but were not consistently seen in many mouse models of HD at young ages. Given the lack of obvious neurodegeneration phenotypes in most genetic mouse models, the demand for establishing large animal models to study neurodegenerative diseases is well-appreciated. Investigation of large animal models would be highly valuable for understanding the novel pathogenic mechanisms and identifying neuroinflammation alterations, which may not be uncovered in small animals, though there are challenges and limitations that are largely stemmed from the costly and time-consuming investigation.

In addition, it remains to be investigated whether peripheral immune response and inflammatory alterations mirror the changes and the putative pathways in the CNS in HD. Yet, whether the inflammatory response is an active or a reactive (or both) mechanism in HD pathophysiology remains controversial. Further mechanistic studies are need and would require use of multiple animal models including those large animal models that can more closely mimic the pathological changes in HD patients. Advancing our understanding of the involvement of the immune system in HD pathophysiology would help identify a valid target for new therapeutic interventions to halt the progression of HD.

Author contributions

QJ and PY wrote the manuscript. X-JL and SL edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. MacDonald ME, Ambrose CM, Duyao MP, Myers RH, Lin C, Srinidhi L, et al. A novel gene containing a trinucleotide repeat that is expanded and unstable on huntington's disease chromosomes. *Cell* (1993) 72(6):971–83. doi: 10.1016/0092-8674(93)90585-E

2. Li S-H, Li X-J. Huntingtin–protein interactions and the pathogenesis of huntington's disease. *Trends Genet* (2004) 20(3):146–54. doi: 10.1016/j.tig.2004.01.008

3. van der Burg JM, Björkqvist M, Brundin P. Beyond the brain: Widespread pathology in huntington's disease. *Lancet Neurol* (2009) 8(8):765–74. doi: 10.1016/s1474-4422(09)70178-4

4. Zoghbi HY, Orr HT. Glutamine repeats and neurodegeneration. *Annu Rev Neurosci* (2000) 23:217. doi: 10.1146/annurev.neuro.23.1.217

5. Ellrichmann G, Reick C, Saft C, Linker RA. The role of the immune system in huntington's disease. *Clin Dev Immunol* (2013) 2013:541259. doi: 10.1155/2013/541259

6. Björkqvist M, Wild EJ, Thiele J, Silvestroni A, Andre R, Lahiri N, et al. A novel pathogenic pathway of immune activation detectable before clinical onset in huntington's disease. *J Exp Med* (2008) 205(8):1869–77. doi: 10.1084/jem.20080178

7. Sapp E, Kegel K, Aronin N, Hashikawa T, Uchiyama Y, Tohyama K, et al. Early and progressive accumulation of reactive microglia in the huntington disease brain. *J Neuropathology Exp Neurol* (2001) 60(2):161–72. doi: 10.1093/jnen/60.2.161

8. Pavese N, Gerhard A, Tai Y, Ho A, Turkheimer F, Barker R, et al. Microglial activation correlates with severity in huntington disease: A clinical and pet study. *Neurology* (2006) 66(11):1638–43. doi: 10.1212/01.wnl.0000222734.56412.17

9. Shin J-Y, Fang Z-H, Yu Z-X, Wang C-E, Li S-H, Li X-J. Expression of mutant huntingtin in glial cells contributes to neuronal excitotoxicity. *J Cell Biol* (2005) 171(6):1001–12. doi: 10.1083/jcb.200508072

10. Bradford J, Shin J-Y, Roberts M, Wang C-E, Sheng G, Li S, et al. Mutant huntingtin in glial cells exacerbates neurological symptoms of huntington disease mice. *J Biol Chem* (2010) 285(14):10653–61. doi: 10.1074/jbc.M109.083287

11. Kaushik DK, Basu A. A friend in need may not be a friend indeed: Role of microglia in neurodegenerative diseases. *CNS Neurol Disord Drug Targets* (2013) 12(6):726–40. doi: 10.2174/18715273113126660170

12. Steffan JS, Bodai L, Pallos J, Poelman M, McCampbell A, Apostol BL, et al. Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in drosophila. *Nature* (2001) 413(6857):739–43. doi: 10.1038/35099568

13. Song W, Smith MR, Syed A, Lukacsovich T, Barbaro BA, Purcell J, et al. Morphometric analysis of huntington's disease neurodegeneration in drosophila. *Tandem Repeats Genes Proteins Disease. Springer* (2013) 1017:41–57. doi: 10.1007/978-1-62703-438-8_3

14. Lin Y-H, Maaroufi HO, Ibrahim E, Kucerova L, Zurovec M. Expression of human mutant huntingtin protein in drosophila hemocytes impairs immune responses. *Front Immunol* (2019) 10:2405. doi: 10.3389/fimmu.2019.02405

15. Menalled LB. Knock-in mouse models of huntington's disease. *NeuroRx* (2005) 2(3):465–70. doi: 10.1602/neurorx.2.3.465

16. Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, et al. Exon 1 of the hd gene with an expanded cag repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell* (1996) 87(3):493–506. doi: 10.1016/S0092-8674(00)81369-0

17. Gray M, Shirasaki DI, Cepeda C, André VM, Wilburn B, Lu X-H, et al. Full-length human mutant huntingtin with a stable polyglutamine repeat can elicit progressive and selective neuropathogenesis in bachd mice. *J Neurosci* (2008) 28(24):6182–95. doi: 10.1523/JNEUROSCI.0857-08.2008

18. Slow EJ, Van Raamsdonk J, Rogers D, Coleman SH, Graham RK, Deng Y, et al. Selective striatal neuronal loss in a Yac128 mouse model of huntington disease. *Hum Mol Genet* (2003) 12(13):1555–67. doi: 10.1093/hmg/ddg169

19. Stack EC, Kubilus JK, Smith K, Cormier K, Del Signore SJ, Guelin E, et al. Chronology of behavioral symptoms and neuropathological sequela in R6/2 huntington's disease transgenic mice. *J Comp Neurol* (2005) 490(4):354–70. doi: 10.1002/cne.20680

20. Lüesse H-G, Schiefer J, Spruenken A, Puls C, Block F, Kosinski CM. Evaluation of R6/2 hd transgenic mice for therapeutic studies in huntington's disease: Behavioral testing and impact of diabetes mellitus. *Behav Brain Res* (2001) 126(1-2):185–95. doi: 10.1016/S0166-4328(01)00261-3

21. Murphy KP, Carter RJ, Lione LA, Mangiarini L, Mahal A, Bates GP, et al. Abnormal synaptic plasticity and impaired spatial cognition in mice transgenic for exon 1 of the human huntington's disease mutation. *J Neurosci* (2000) 20(13):5115–23. doi: 10.1523/JNEUROSCI.20-13-05115.2000

22. Carter RJ, Lione LA, Humby T, Mangiarini L, Mahal A, Bates GP, et al. Characterization of progressive motor deficits in mice transgenic for the human huntington's disease mutation. *J Neurosci* (1999) 19(8):3248–57. doi: 10.1523/JNEUROSCI.19-08-03248.1999

23. Van Raamsdonk JM, Murphy Z, Slow EJ, Leavitt BR, Hayden MR. Selective degeneration and nuclear localization of mutant huntingtin in the Yac128 mouse model of huntington disease. *Hum Mol Genet* (2005) 14(24):3823–35. doi: 10.1093/hmg/ddi407

24. Menalled LB, Sison JD, Dragatsis I, Zeitlin S, Chesselet MF. Time course of early motor and neuropathological anomalies in a knock-in mouse model of huntington's disease with 140 cag repeats. *J Comp Neurol* (2003) 465(1):11–26. doi: 10.1002/cne.10776

25. Menalled LB, Kudwa AE, Miller S, Fitzpatrick J, Watson-Johnson J, Keating N, et al. Comprehensive behavioral and molecular characterization of a new knock-in mouse model of huntington's disease: Zq175. *PloS One* (2012) 7(12):e49838. doi: 10.1371/journal.pone.0049838

26. Lin C-H, Tallaksen-Greene S, Chien W-M, Cearley JA, Jackson WS, Crouse AB, et al. Neurological abnormalities in a knock-in mouse model of huntington's disease. *Hum Mol Genet* (2001) 10(2):137–44. doi: 10.1093/hmg/10.2.137

27. Träger U, Andre R, Magnusson-Lind A, Miller JR, Connolly C, Weiss A, et al. Characterisation of immune cell function in fragment and full-length huntington's disease mouse models. *Neurobiol Dis* (2015) 73:388–98. doi: 10.1016/j.nbd.2014.10.012

28. Pido-Lopez J, Andre R, Benjamin AC, Ali N, Farag S, Tabrizi SJ, et al. *In vivo* neutralization of the protagonist role of macrophages during the chronic inflammatory stage of huntington's disease. *Sci Rep* (2018) 8(1):1–14. doi: 10.1038/s41598-018-29792-x

29. Godavarthi SK, Narender D, Mishra A, Goswami A, Rao SN, Nukina N, et al. Induction of chemokines, mcp-1, and kc in the mutant huntingtin expressing neuronal cells because of proteasomal dysfunction. *J neurochemistry* (2009) 108(3):787–95. doi: 10.1111/j.1471-4159.2008.05823.x

30. Kim A, García-García E, Straccia M, Comella-Bolla A, Miguez A, Masana M, et al. Reduced fractalkine levels lead to striatal synaptic plasticity deficits in huntington's disease. *Front Cell Neurosci* (2020) 14:163. doi: 10.3389/fncel.2020.00163

31. Gunner G, Cheadle L, Johnson KM, Ayata P, Badimon A, Mondo E, et al. Sensory lesioning induces microglial synapse elimination *Via* Adam10 and fractalkine signaling. *Nat Neurosci* (2019) 22(7):1075–88. doi: 10.1038/s41593-019-0419-y

32. Maddison DC, Giorgini F eds. The kynurenine pathway and neurodegenerative disease. *Seminars in cell & developmental biology*. (2015) 40:134–41. doi: 10.1016/j.semcdb.2015.03.002

33. Stoy N, Mackay G, Forrest C, Christofides J, Egerton M, Stone T, et al. Tryptophan metabolism and oxidative stress in patients with huntington's disease. *J neurochemistry* (2005) 93(3):611–23. doi: 10.1111/j.1471-4159.2005.03070.x

34. Guidetti P, Reddy PH, Tagle DA, Schwarcz R. Early kynurenergic impairment in huntington's disease and in a transgenic animal model. *Neurosci Lett* (2000) 283(3):233–5. doi: 10.1016/S0304-3940(00)00956-3

35. Guidetti P, Bates GP, Graham RK, Hayden MR, Leavitt BR, MacDonald ME, et al. Elevated brain 3-hydroxykynurenine and quinolinate levels in huntington disease mice. *Neurobiol Dis* (2006) 23(1):190–7. doi: 10.1016/j.nbd.2006.02.011

36. Guidetti P, Luthi-Carter RE, Augood SJ, Schwarcz R. Neostriatal and cortical quinolinate levels are increased in early grade huntington's disease. *Neurobiol Dis* (2004) 17(3):455–61. doi: 10.1016/j.nbd.2004.07.006

37. Dalrymple A, Wild EJ, Joubert R, Sathasivam K, Björkqvist M, Petersén Å, et al. Proteomic profiling of plasma in huntington's disease reveals neuroinflammatory activation and biomarker candidates. *J Proteome Res* (2007) 6(7):2833–40. doi: 10.1021/pr0700753

38. Chang K-H, Wu Y-R, Chen Y-C, Chen C-M. Plasma inflammatory biomarkers for huntington's disease patients and mouse model. *Brain behavior Immun* (2015) 44:121–7. doi: 10.1016/j.bbi.2014.09.011

39. Disatnik M-H, Joshi AU, Saw NL, Shamloo M, Leavitt BR, Qi X, et al. Potential biomarkers to follow the progression and treatment response of huntington's disease. *J Exp Med* (2016) 213(12):2655–69. doi: 10.1084/jem.20160776

40. Qi X, Qvit N, Su Y-C, Mochly-Rosen D. A novel Drp1 inhibitor diminishes aberrant mitochondrial fission and neurotoxicity. *J Cell Sci* (2013) 126(3):789–802. doi: 10.1242/jcs.114439

41. Kwan W, Magnusson A, Chou A, Adame A, Carson MJ, Kohsaka S, et al. Bone marrow transplantation confers modest benefits in mouse models of huntington's disease. *J Neurosci* (2012) 32(1):133–42. doi: 10.1523/JNEUROSCI.4846-11.2012

42. Podlacha M, Pierzynowska K, Gaffke L, Jerzemowska G, Piotrowska E, Węgrzyn G. Behavioral-and blood-based biomarkers for huntington's disease: Studies on the R6/1 mouse model with prospects for early diagnosis and monitoring of the disease. *Brain behavior immunity-health* (2022) 23:100482. doi: 10.1016/j.bbih.2022.100482

43. Valadão PAC, da Silva Oliveira B, Joviano-Santos JV, Vieira ÉLM, Rocha NP, Teixeira AL, et al. Inflammatory changes in peripheral organs in the bachd murine model of huntington's disease. *Life Sci* (2019) 232:116653. doi: 10.1016/j.lfs.2019.116653

44. Bouchard J, Truong J, Bouchard K, Dunkelberger D, Desrayaud S, Moussaoui S, et al. Cannabinoid receptor 2 signaling in peripheral immune cells modulates disease onset and severity in mouse models of huntington's disease. *J Neurosci* (2012) 32(50):18259–68. doi: 10.1523/JNEUROSCI.4008-12.2012

45. Wertz MH, Pineda SS, Lee H, Kulicke R, Kellis M, Heiman M. Interleukin-6 deficiency exacerbates huntington's disease model phenotypes. *Mol neurodegeneration* (2020) 15(1):1–8. doi: 10.1186/s13024-020-00379-3

46. Hedreen JC, Folstein SE. Early loss of neostriatal striosome neurons in huntington's disease. *J Neuropathology Exp Neurol* (1995) 54(1):105–20. doi: 10.1097/00005072-199501000-00013

47. Sharp AH, Ross CA. Neurobiology of huntington's disease. *Neurobiol Dis* (1996) 3(1):3–15. doi: 10.1006/nbdi.1996.0002

48. Etxeberria-Rekalde E, Alzola-Aldamizetxebarria S, Flunkert S, Hable I, Daurer M, Neddens J, et al. Quantification of huntington's disease related markers in the R6/2 mouse model. *Front Mol Neurosci* (2021) 13:617229. doi: 10.3389/fnmol.2020.617229

49. Palpagama TH, Waldvogel HJ, Faull RL, Kwakowsky A. The role of microglia and astrocytes in huntington's disease. *Front Mol Neurosci* (2019) 12:258. doi: 10.3389/fnmol.2019.00258

50. Yu Z-X, Li S-H, Evans J, Pillarisetti A, Li H, Li X-J. Mutant huntingtin causes context-dependent neurodegeneration in mice with huntington's disease. *J Neurosci* (2003) 23(6):2193–202. doi: 10.1523/JNEUROSCI.23-06-02193.2003

51. Reiner A, Del Mar N, Deng YP, Meade CA, Sun Z, Goldowitz D. R6/2 neurons with intranuclear inclusions survive for prolonged periods in the brains of chimeric mice. *J Comp Neurol* (2007) 505(6):603–29. doi: 10.1002/cne.21515

52. Gatto RG, Weissmann C, Amin M, Angeles-López QD, García-Lara L, Castellanos LCS, et al. Evaluation of early microstructural changes in the R6/1 mouse model of huntington's disease by ultra-high field diffusion Mr imaging. *Neurobiol Aging* (2021) 102:32–49. doi: 10.1016/j.neurobiolaging.2021.02.006

53. Turmaine M, Raza A, Mahal A, Mangiarini L, Bates GP, Davies SW. Nonapoptotic neurodegeneration in a transgenic mouse model of huntington's disease. *Proc Natl Acad Sci* (2000) 97(14):8093–7. doi: 10.1073/pnas.110078997

54. Simmons DA, Casale M, Alcon B, Pham N, Narayan N, Lynch G. Ferritin accumulation in dystrophic microglia is an early event in the development of huntington's disease. *Glia* (2007) 55(10):1074–84. doi: 10.1002/glia.20526

55. Wheeler VC, White JK, Gutekunst C-A, Vrbanac V, Weaver M, Li X-J, et al. Long glutamine tracts cause nuclear localization of a novel form of huntingtin in medium spiny striatal neurons in hdh Q92 and hdh Q111 knock-in mice. *Hum Mol Genet* (2000) 9(4):503–13. doi: 10.1093/hmg/9.4.503

56. Kovalenko M, Milnerwood A, Giordano J, St Claire J, Guide JR, Stromberg M, et al. HTT Q111/+ huntington's disease knock-in mice exhibit brain region-specific morphological changes and synaptic dysfunction. *J Huntington's Dis* (2018) 7(1):17–33. doi: 10.3233/JHD-170282

57. Crotti A, Benner C, Kerman BE, Gosselin D, Lagier-Tourenne C, Zuccato C, et al. Mutant huntingtin promotes autonomous microglia activation *Via* myeloid lineage-determining factors. *Nat Neurosci* (2014) 17(4):513–21. doi: 10.1038/nn.3668

58. Deng H-X, Siddique T. Transgenic mouse models and human neurodegenerative disorders. *Arch Neurol* (2000) 57(12):1695–702. doi: 10.1001/archneur.57.12.1695

59. Crook ZR, Housman D. Huntington's disease: Can mice lead the way to treatment? *Neuron* (2011) 69(3):423–35. doi: 10.1016/j.neuron.2010.12.035

60. Dawson TM, Golde TE, Lagier-Tourenne C. Animal models of neurodegenerative diseases. *Nat Neurosci* (2018) 21(10):1370–9. doi: 10.1038/s41593-018-0236-8

61. Paldino E, D'Angelo V, Sancesario G, Fusco FR. Pyroptotic cell death in the R6/2 mouse model of huntington's disease: New insight on the inflammasome. *Cell Death Discovery* (2020) 6(1):1–12. doi: 10.1038/s41420-020-00293-z

62. Palazuelos J, Aguado T, Pazos MR, Julien B, Carrasco C, Resel E, et al. Microglial Cb2 cannabinoid receptors are neuroprotective in huntington's disease excitotoxicity. *Brain* (2009) 132(11):3152–64. doi: 10.1093/brain/awp239

63. Franciosi S, Ryu JK, Shim Y, Hill A, Connolly C, Hayden MR, et al. Age-dependent neurovascular abnormalities and altered microglial morphology in the Yac128 mouse model of huntington disease. *Neurobiol Dis* (2012) 45(1):438–49. doi: 10.1016/j.nbd.2011.09.003

64. Hickey MA, Kosmalska A, Enayati J, Cohen R, Zeitlin S, Levine MS, et al. Extensive early motor and non-motor behavioral deficits are followed by striatal neuronal loss in knock-in huntington's disease mice. *Neuroscience* (2008) 157 (1):280–95. doi: 10.1016/j.neuroscience.2008.08.041

65. Jacobsen JC, Bawden CS, Rudiger SR, McLaughlan CJ, Reid SJ, Waldvogel HJ, et al. An ovine transgenic huntington's disease model. *Hum Mol Genet* (2010) 19(10):1873–82. doi: 10.1093/hmg/ddq063

66. Yan S, Tu Z, Liu Z, Fan N, Yang H, Yang S, et al. A huntingtin knockin pig model recapitulates features of selective neurodegeneration in huntington's disease. *Cell* (2018) 173(4):989–1002.e13. doi: 10.1016/j.cell.2018.03.005

67. Bai D, Yin P, Zhang Y, Sun F, Chen L, Lin L, et al. Lack of association of somatic cag repeat expansion with striatal neurodegeneration in hd knock-in animal models. *Hum Mol Genet* (2021) 30(16):1497–508. doi: 10.1093/hmg/ddab129

68. Baxa M, Hruska-Plochan M, Juhas S, Vodicka P, Pavlok A, Juhasova J, et al. A transgenic minipig model of huntington's disease. *J Huntingtons Dis* (2013) 2 (1):47–68. doi: 10.3233/jhd-130001

69. Valekova I, Jarkovska K, Kotrcova E, Bucci J, Elledérova Z, Juhas S, et al. Revelation of the ifnα, il-10, il-8 and il-1β as promising biomarkers reflecting immuno-pathological mechanisms in porcine huntington's disease model. *J Neuroimmunol* (2016) 293:71–81. doi: 10.1016/j.jneuroim.2016.02.012

70. Yang S-H, Cheng P-H, Banta H, Piotrowska-Nitsche K, Yang J-J, Cheng EC, et al. Towards a transgenic model of huntington's disease in a non-human primate. *Nature* (2008) 453(7197):921–4. doi: 10.1038/nature06975

71. Chan AW, Jiang J, Chen Y, Li C, Prucha MS, Hu Y, et al. Progressive cognitive deficit, motor impairment and striatal pathology in a transgenic huntington disease monkey model from infancy to adulthood. *PloS One* (2015) 10(5):e0122335. doi: 10.1371/journal.pone.0122335

72. Raper J, Bosinger S, Johnson Z, Tharp G, Moran SP, Chan AWS. Increased irritability, anxiety, and immune reactivity in transgenic huntington's disease monkeys. *Brain Behav Immun* (2016) 58:181–90. doi: 10.1016/j.bbi.2016.07.004

73. Silvestroni A, Faull RL, Strand AD, Möller T. Distinct neuroinflammatory profile in post-mortem human huntington's disease. *Neuroreport* (2009) 20 (12):1098–103. doi: 10.1097/WNR.0b013e32832e34ee

74. Du G, Dong W, Yang Q, Yu X, Ma J, Gu W, et al. Altered gut microbiota related to inflammatory responses in patients with huntington's disease. *Front Immunol* (2021) 11:603594. doi: 10.3389/fimmu.2020.603594

75. von Essen MR, Hellem MN, Vinther-Jensen T, Ammitzbøll C, Hansen RH, Hjermind LE, et al. Early intrathecal T helper 17.1 cell activity in huntington disease. *Ann Neurol* (2020) 87(2):246–55. doi: 10.1002/ana.25647

76. Wild E, Magnusson A, Lahiri N, Krus U, Orth M, Tabrizi SJ, et al. Abnormal peripheral chemokine profile in huntington's disease. *PloS currents* (2011) 3:1–9. doi: 10.1371/currents.RRN1231

77. Huber AK, Giles DA, Segal BM, Irani DN. An emerging role for eotaxins in neurodegenerative disease. *Clin Immunol* (2018) 189:29–33. doi: 10.1016/j.clim.2016.09.010

78. Fang Q, Strand A, Law W, Faca VM, Fitzgibbon MP, Hamel N, et al. Brain-specific proteins decline in the cerebrospinal fluid of humans with huntington disease. *Mol Cell Proteomics* (2009) 8(3):451–66. doi: 10.1074/mcp.M800231-MCP200

79. Singhrao S, Neal J, Morgan B, Gasque P. Increased complement biosynthesis by microglia and complement activation on neurons in huntington's disease. *Exp Neurol* (1999) 159(2):362–76. doi: 10.1006/exnr.1999.7170

80. Mott RT, Ait-Ghezala G, Town T, Mori T, Vendrame M, Zeng J, et al. Neuronal expression of Cd22: Novel mechanism for inhibiting microglial

proinflammatory cytokine production. *Glia* (2004) 46(4):369–79. doi: 10.1002/glia.20009

81. Junker A, Krumbholz M, Eisele S, Mohan H, Augstein F, Bittner R, et al. Microrna profiling of multiple sclerosis lesions identifies modulators of the regulatory protein Cd47. *Brain* (2009) 132(12):3342–52. doi: 10.1093/brain/awp300

82. Lago N, Pannunzio B, Amo-Aparicio J, López-Vales R, Peluffo H. Cd200 modulates spinal cord injury neuroinflammation and outcome through Cd200r1. *Brain Behavior Immun* (2018) 73:416–26. doi: 10.1016/j.bbi.2018.06.002

83. Hoek RM, Ruuls SR, Murphy CA, Wright GJ, Goddard R, Zurawski SM, et al. Down-regulation of the macrophage lineage through interaction with Ox2 (Cd200). *Science* (2000) 290(5497):1768–71. doi: 10.1126/science.290.5497.1768

84. Borrego F. The Cd300 molecules: An emerging family of regulators of the immune system. *Blood J Am Soc Hematol* (2013) 121(11):1951–60. doi: 10.1182/blood-2012-09-435057

85. Harrison JK, Jiang Y, Chen S, Xia Y, Maciejewski D, McNamara RK, et al. Role for neuronally derived fractalkine in mediating interactions between neurons and Cx3cr1-expressing microglia. *Proc Natl Acad Sci* (1998) 95(18):10896–901. doi: 10.1073/pnas.95.18.10896

86. Nomiyama H, Osada N, Yoshie O. Systematic classification of vertebrate chemokines based on conserved synteny and evolutionary history. *Genes to Cells* (2013) 18(1):1–16. doi: 10.1111/gtc.12013

87. Sheridan GK, Murphy KJ. Neuron–glia crosstalk in health and disease: Fractalkine and Cx3cr1 take centre stage. *Open Biol* (2013) 3(12):130181. doi: 10.1098/rsob.130181

88. Chandrasekaran S, Bonchev D. Network analysis of human post-mortem microarrays reveals novel genes, micrornas, and mechanistic scenarios of potential importance in fighting huntington's disease. *Comput Struct Biotechnol J* (2016) 14:117–30. doi: 10.1016/j.csbj.2016.02.001

89. Stack EC, Smith KM, Ryu H, Cormier K, Chen M, Hagerty SW, et al. Combination therapy using minocycline and coenzyme Q10 in R6/2 transgenic huntington's disease mice. *Biochim Biophys Acta (BBA)-Molecular Basis Dis* (2006) 1762(3):373–80. doi: 10.1016/j.bbadis.2005.11.002

90. Wang X, Zhu S, Drozda M, Zhang W, Stavrovskaya IG, Cattaneo E, et al. Minocycline inhibits caspase-independent and-dependent mitochondrial cell death pathways in models of huntington's disease. *Proc Natl Acad Sci* (2003) 100 (18):10483–7. doi: 10.1073/pnas.1832501100

91. Ryu J, Choi H, McLarnon J. Combined minocycline plus pyruvate treatment enhances effects of each agent to inhibit inflammation, oxidative damage, and neuronal loss in an excitotoxic animal model of huntington's disease. *Neuroscience* (2006) 141(4):1835–48. doi: 10.1016/j.neuroscience.2006.05.043

92. Huntington Study Group. Minocycline safety and tolerability in huntington disease. *Neurology* (2004) 63(3):547–9. doi: 10.1212/01.wnl.0000133403.30559.ff

93. Consroe P, Laguna J, Allender J, Snider S, Stern L, Sandyk R, et al. Controlled clinical trial of cannabidiol in huntington's disease. *Pharmacol Biochem Behav* (1991) 40(3):701–8. doi: 10.1016/0091-3057(91)90386-g

94. Aharoni R, Saada R, Eilam R, Hayardeny L, Sela M, Arnon R. Oral treatment with laquinimod augments regulatory T-cells and brain-derived neurotrophic factor expression and reduces injury in the cns of mice with experimental autoimmune encephalomyelitis. *J Neuroimmunol* (2012) 251(1-2):14–24. doi: 10.1016/j.jneuroim.2012.06.005

95. Kaye J, Piryatinsky V, Birnberg T, Hingaly T, Raymond E, Kashi R, et al. Laquinimod arrests experimental autoimmune encephalomyelitis by activating the aryl hydrocarbon receptor. *Proc Natl Acad Sci U.S.A.* (2016) 113(41):E6145–e52. doi: 10.1073/pnas.1607843113

96. Coskun M, Vermeire S, Nielsen OH. Novel targeted therapies for inflammatory bowel disease. *Trends Pharmacol Sci* (2017) 38(2):127–42. doi: 10.1016/j.tips.2016.10.014

97. Gentile A, Musella A, De Vito F, Fresegna D, Bullitta S, Rizzo FR, et al. Laquinimod ameliorates excitotoxic damage by regulating glutamate re-uptake. *J Neuroinflamm* (2018) 15(1):5. doi: 10.1186/s12974-017-1048-6

98. Katsumoto A, Miranda AS, Butovsky O, Teixeira AL, Ransohoff RM, Lamb BT. Laquinimod attenuates inflammation by modulating macrophage functions in traumatic brain injury mouse model. *J Neuroinflamm* (2018) 15(1):26. doi: 10.1186/s12974-018-1075-y

99. Wilmes AT, Reinehr S, Kühn S, Pedreiturria X, Petrikowski L, Faissner S, et al. Laquinimod protects the optic nerve and retina in an experimental autoimmune encephalomyelitis model. *J Neuroinflamm* (2018) 15(1):183. doi: 10.1186/s12974-018-1208-3

100. Ellrichmann G, Blusch A, Fatoba O, Brunner J, Reick C, Hayardeny L, et al. Laquinimod treatment in the R6/2 mouse model. *Sci Rep* (2017) 7(1):4947. doi: 10.1038/s41598-017-04990-1

101. Garcia-Miralles M, Hong X, Tan LJ, Caron NS, Huang Y, To XV, et al. Laquinimod rescues striatal, cortical and white matter pathology and results in modest behavioural improvements in the Yac128 model of huntington disease. *Sci Rep* (2016) 6:31652. doi: 10.1038/srep31652

102. Garcia-Miralles M, Yusof N, Tan JY, Radulescu CI, Sidik H, Tan LJ, et al. Laquinimod treatment improves myelination deficits at the transcriptional and ultrastructural levels in the Yac128 mouse model of huntington disease. *Mol Neurobiol* (2019) 56(6):4464–78. doi: 10.1007/s12035-018-1393-1

103. Varrin-Doyer M, Zamvil SS, Schulze-Topphoff U. Laquinimod, an up-and-Coming immunomodulatory agent for treatment of multiple sclerosis. *Exp Neurol* (2014) 262 Pt A:66–71. doi: 10.1016/j.expneurol.2014.04.002

104. Yin P, Liu Q, Pan Y, Yang W, Yang S, Wei W, et al. Phosphorylation of myelin regulatory factor by Prkg2 mediates demyelination in huntington's disease. *EMBO Rep* (2020) 21(6):e49783. doi: 10.15252/embr.201949783

105. Ehrnhoefer DE, Caron NS, Deng Y, Qiu X, Tsang M, Hayden MR. Laquinimod decreases bax expression and reduces caspase-6 activation in neurons. *Exp Neurol* (2016) 283(Pt A):121–8. doi: 10.1016/j.expneurol.2016.06.008

106. Ellrichmann G, Blusch A, Fatoba O, Brunner J, Reick C, Hayardeny L, et al. Author correction: Laquinimod treatment in the R6/2 mouse model. *Sci Rep* (2019) 9(1):4960. doi: 10.1038/s41598-018-37926-4

107. Locksley RM, Killeen N, Lenardo MJ. The tnf and tnf receptor superfamilies: Integrating mammalian biology. *Cell* (2001) 104(4):487–501. doi: 10.1016/s0092-8674(01)00237-9

108. Hsiao HY, Chiu FL, Chen CM, Wu YR, Chen HM, Chen YC, et al. Inhibition of soluble tumor necrosis factor is therapeutic in huntington's disease. *Hum Mol Genet* (2014) 23(16):4328–44. doi: 10.1093/hmg/ddu151

109. Fatoba O, Ohtake Y, Itokazu T, Yamashita T. Immunotherapies in huntington's disease and A-synucleinopathies. *Front Immunol* (2020) 11:337. doi: 10.3389/fimmu.2020.00337

110. Pido-Lopez J, Tanudjojo B, Farag S, Bondulich MK, Andre R, Tabrizi SJ, et al. Inhibition of tumour necrosis factor alpha in the R6/2 mouse model of huntington's disease by etanercept treatment. *Sci Rep* (2019) 9(1):7202. doi: 10.1038/s41598-019-43627-3

111. Southwell AL, Franciosi S, Villanueva EB, Xie Y, Winter LA, Veeraraghavan J, et al. Anti-semaphorin 4d immunotherapy ameliorates neuropathology and some cognitive impairment in the Yac128 mouse model of huntington disease. *Neurobiol Dis* (2015) 76:46–56. doi: 10.1016/j.nbd.2015.01.002

112. Ito Y, Oinuma I, Katoh H, Kaibuchi K, Negishi M. Sema4d/Plexin-B1 activates gsk-3beta through r-ras gap activity, inducing growth cone collapse. *EMBO Rep* (2006) 7(7):704–9. doi: 10.1038/sj.embor.7400737

113. Blennow K, Davidsson P, Wallin A, Frcdman P, Gottfries C, Månsson J, et al. Differences in cerebrospinal fluid gangliosides between "Probable alzheimer's disease" and normal aging. *Aging Clin Exp Res* (1992) 4(4):301–6. doi: 10.1007/BF03324111

114. Schneider J. Gm1 ganglioside in the treatment of parkinson's disease a. *Ann New York Acad Sci* (1998) 845(1):363–73. doi: 10.1111/j.1749-6632.1998.tb09688.x

115. Schneider JS, Aras R, Williams CK, Koprich JB, Brotchie JM, Singh V. Gm1 ganglioside modifies A-synuclein toxicity and is neuroprotective in a rat A-synuclein model of parkinson's disease. *Sci Rep* (2019) 9(1):1–12. doi: 10.1038/s41598-019-42847-x

116. Alpaugh M, Galleguillos D, Forero J, Morales LC, Lackey SW, Kar P, et al. Disease-modifying effects of ganglioside Gm1 in huntington's disease models. *EMBO Mol Med* (2017) 9(11):1537–57. doi: 10.15252/emmm.201707763

117. Prados ME, Correa-Sáez A, Unciti-Broceta JD, Garrido-Rodríguez M, Jimenez-Jimenez C, Mazzone M, et al. Betulinic acid hydroxamate is neuroprotective and induces protein phosphatase 2a-dependent hif-1α stabilization and post-transcriptional dephosphorylation of prolyl hydrolase 2. *Neurotherapeutics* (2021) 18(3):1849–61. doi: 10.1007/s13311-021-01089-4

118. Purushothaman B, Sumathi T. 5, 6, 7 trihydroxy flavone armoured neurodegeneration caused by quinolinic acid induced huntington's like disease in rat striatum-reinstating the level of brain neurotrophins with special reference to cognitive-socio behaviour, biochemical and histopathological aspects. *Neurosci Res* (2022) 174:25–35. doi: 10.1016/j.neures.2021.08.003

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Glossary

HTT	Huntingtin
HD	Huntington's disease
polyQ	polyglutamine
CNS	Central nervous system
BBB	Blood-brain barrier
YAC	Yeast artificial chromosome
BAC	Bacterial artificial chromosome
LPS	Lipopolysaccharides
IL	Interleukin
TNF	Tumor necrosis factor
MCP-1	Monocyte chemoattractant protein-1
PSD-95	Postsynaptic density protein 95
IBA1	Ionized calcium-binding adapter molecule 1
CX3CL1	C-X3-C motif chemokine ligand 1
QUIN	Quinolinic acid
3-HK	3-hydroxykynurenine
WT	Wild-type
IFN-γ	Interferon gamma
BDNF	Brain-derived neurotrophic factor
NTRK2	Neurotrophic receptor tyrosine kinase 2
GFAP	Glial fibrillary acidic protein
CRISPR	Clustered regularly interspaced short palindromic repeats
KI	Knock-in
CSF	Cerebrospinal fluid
CRP	C-reactive protein
MMP-9	Matrix metalloproteinase-9
CCL	Chemokines (C-C motif) ligand
C1QC	Complement C1q subcomponent subunit C
NK	Natural killer
SEMA4D	Semaphorin-4D
UHDRS	Unified HD rating Scale
LAQ	Laquinimod
EAE	Experimental autoimmune encephalomyelitis
ICV	Intracerebroventricular
PLXNB1	Plexin-B1
PLXNB2	Plexin-B2
AD	Alzheimer's Disease
PD	Parkinson's Disease
BAH	Betulinic acid hydroxamate
HIF-1α	Hypoxia-inducible factor 1-alpha
BC	Baicalein
QA	Quinolinic acid
GDNF	Glial cell derived neurotrophic factor
MDA	Malondialdehyde

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Cytosolic DNA sensors and glial responses to endogenous DNA

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Genomic instability is a key driving force for the development and progression of many neurodegenerative diseases and central nervous system (CNS) cancers. The initiation of DNA damage responses is a critical step in maintaining genomic integrity and preventing such diseases. However, the absence of these responses or their inability to repair genomic or mitochondrial DNA damage resulting from insults, including ionizing radiation or oxidative stress, can lead to an accumulation of self-DNA in the cytoplasm. Resident CNS cells, such as astrocytes and microglia, are known to produce critical immune mediators following CNS infection due to the recognition of pathogen and damage-associated molecular patterns by specialized pattern recognition receptors (PRRs). Recently, multiple intracellular PRRs, including cyclic GMP-AMP synthase, interferon gamma-inducible 16, absent in melanoma 2, and Z-DNA binding protein, have been identified as cytosolic DNA sensors and to play critical roles in glial immune responses to infectious agents. Intriguingly, these nucleic acid sensors have recently been shown to recognize endogenous DNA and trigger immune responses in peripheral cell types. In the present review, we discuss the available evidence that cytosolic DNA sensors are expressed by resident CNS cells and can mediate their responses to the presence of self-DNA. Furthermore, we discuss the potential for glial DNA sensor-mediated responses to provide protection against tumorigenesis versus the initiation of potentially detrimental neuroinflammation that could initiate or foster the development of neurodegenerative disorders. Determining the mechanisms that underlie the detection of cytosolic DNA by glia and the relative role of each pathway in the context of specific CNS disorders and their stages may prove pivotal in our understanding of the pathogenesis of such conditions and might be leveraged to develop new treatment modalities.

KEYWORDS

genomic integrity, DNA sensors, neuroinflammation, neurodegeneration, glia

1 Introduction

In 2022, there were an estimated 1.9 million new cancer cases and over 600,000 deaths due to these diseases in the United States (1). Of these, approximately 25,000 were associated with the brain and the central nervous system (CNS), and these have a poor prognosis with only a 5-year survival rate of 32.5% (2). Additionally, lethal cases of

neurodegenerative diseases including Alzheimer's disease (AD) and Parkinson's disease (PD) are becoming increasingly prevalent, and this is a serious concern as an estimated 6.5 million Americans were reported to live with AD in 2022 and 1 million having PD in 2019 (3, 4). While multiple mechanisms, including cellular senescence and dysfunctional mitophagy, have been associated with cancer development and age-related diseases (5–8), genomic instability arising from excessive DNA damage and/or DNA repair mechanism dysfunction is recognized as a key driving force for such diseases in general (9), and the development of CNS cancers and neurodegenerative diseases more specifically (10, 11).

High fidelity DNA repair is required to maintain genomic integrity. Breaks in DNA are a relatively common occurrence and can occur naturally up to tens of thousands of times per day, per cell. However, the number of such breaks can be drastically increased by exogenous insults such as ionizing radiation (IR) and oxidative stress. Exposure to high levels of IR can be tremendously detrimental and affects nearly every macromolecule in a cell. These effects can either occur directly or indirectly. For example, IR alone directly disrupts the structure of DNA, creating breaks in its molecular backbone (12). Alternatively, IR can elicit radiolysis of cellular water whereby the chemical bonds of water molecules are broken down resulting in the generation of reactive oxygen species (ROS) including hydroxyl radicals, ionized water, superoxide anions, and hydrogen peroxide (12). While ROS play essential roles in many cellular processes, excessive levels of ROS disrupt redox homeostasis and can induce DNA lesions (13). DNA lesions/breaks initiate DNA damage responses (DDR), a collection of mechanisms that ensure efficient DNA repair and maintain genomic integrity (14, 15). However, DNA repair is not infallible. If cells are permitted to replicate with unrepaired or incompletely repaired DNA, an accumulation of mutations and DNA damage may lead to cancer development, neurodegenerative disorders, and other age-related diseases. Furthermore, DNA damage has recently been implicated in the generation of detrimental inflammation, and this effect is often associated with the presence of self-DNA in the cytoplasm (16–19).

DNA damage due to exposure to IR, oxidative stress, or even chemotherapy, results in cytosolic DNA accumulation, often in the form of micronuclei (20). Micronuclei are small nuclei-like structures containing lagging or damaged chromosome fragments that continue into the interphase following completion of mitosis or meiosis (20) The nuclear envelopes surrounding micronuclei are typically defective and prone to rupture, after which, their DNA cargo is liberated into the cytosol. Additionally, micronuclei have been described as a source of complex genome rearrangements, including one-off catastrophic rearrangement events known as chromothripsis (21). Furthermore, the presence of micronuclei is associated with many autoimmune diseases (22–26), neurodegenerative diseases (27–32), and aggressive cancers (33) in affected tissues. Indeed, the presence of micronuclei has historically been used as a means to assess the genotoxicity of chemicals and mutagens *via* the cytokinesis-block micronucleus assay, and their contribution to the initiation of innate immune responses is now becoming apparent (34).

While micronuclei are likely to be a major source of cytoplasmic genomic self-DNA, mitochondria may also serve as a source of DNA in the cytosol (35). Mitochondria primarily function to produce the ATP necessary for normal cell activity *via* oxidative phosphorylation. Outside of oxidative phosphorylation, mitochondria also perform many other metabolic and non-metabolic roles ranging from the regulation of apoptosis to the generation of ROS that are necessary for maintaining redox homeostasis (36, 37). What makes mitochondria truly unique, however, is that they contain their own circular genome due to their endosymbiotic origin (38). The various means by which mitochondrial contents, including mitochondrial DNA (mtDNA), are released have been extensively reviewed elsewhere (39–44), but generally involves passive/accidental release due to mitochondrial stress and dysfunction, and cell death pathways including necrosis and apoptosis (40).

Since DNA is normally sequestered in the nucleus and mitochondria, its presence in the cytosol can function as a damage associated molecular pattern (DAMP) and serve to trigger inflammatory innate immune responses. In the CNS, such responses to both endogenous and exogenous insults must be tightly regulated to avoid damaging, or even lethal, inflammation. While the recruitment of peripheral leukocytes to the CNS and their subsequent activation are important in the development of immune responses in disease states, it is now recognized that resident glial cells, such as microglia and astrocytes, play a critical role in the initiation of detrimental neuroinflammation.

2 Glial cells play a critical role in the initiation and progression of immune responses in the CNS

In the healthy brain, microglia and astrocytes are essential for homeostasis. Microglia perform critical housekeeping functions such as synaptic remodeling and pruning (45, 46), and removal of cellular debris and dead or dying cells (47), which is necessary for creating a regenerative environment (48). Astrocytes are the most abundant glial cell in the brain and have a crucial role in synaptogenesis, synaptic transmission, and neurotransmitter recycling, and for the maintenance of the blood brain barrier (BBB) (49–53). Importantly, it is now apparent that microglia and astrocytes both serve as sentinel cells that initiate and exacerbate immune responses associated with CNS pathology (54, 55). Given their wide distribution throughout the CNS, they are ideally positioned to confront and respond to trauma or invading pathogens. In disease states, microglia and astrocytes are activated and produce a wide array of potent proinflammatory mediators, such as IL-6, IL-1β, and TNF, as well as chemokines that promote the recruitment of peripheral leukocytes across the BBB that can further contribute to potentially damaging neuroinflammation (56–59). The initiation of microglial and astrocytic responses is now recognized to be mediated by multiple families of cell surface, endosomal, and cytosolic PRRs that are triggered by DAMPs and pathogen-associated molecular patterns (PAMPs). This subsequently results in the activation of transcription factors that precipitate the production of cell surface and secreted immune mediators.

Of these PRRs, perhaps the best studied are the cell surface and endosomal Toll-like receptors (TLRs) and the cytosolic nucleotide-binding and oligomerization domain-containing (NOD)-like receptors (NLRs), and these sensors have been exhaustively studied for their roles in antimicrobial and antiviral responses (as reviewed in 60–69). The TLR and NLR families consist of at least 10 and 22 members in mammals, respectively, and these receptors are widely expressed throughout the body on/in peripheral leukocytes and non-leukocytic cell types (70–72). Importantly, we, and others, have demonstrated the constitutive and/or inducible expression of TLRs (54, 55, 58, 73–75) and the NLRs, NOD1 and NOD2 (76–78), on/in both microglia and astrocytes.

These TLRs and NLRs can detect a variety of bacterial or viral extra- and intracellular PAMPs and DAMPs to activate downstream signaling cascades and initiate proinflammatory and/or antiviral activity by glial cells. However, NOD1/2 appears to be limited to the detection of bacterial cell wall components (78). Furthermore, while several TLRs are able to detect and respond to nucleic acids in endosomal compartments (72, 79, 80), they are not well suited to detect compromised cytosolic sterility or damage. Rather, it now appears that cells utilize discrete cytosolic PRR families that are capable of responding to the presence of foreign and/or self-nucleic acids in the cytoplasm.

3 Detection of cytosolic nucleic acids by glial cells

PRR families, in addition to TLRs and NLRs, have recently been identified that serve as cytosolic sensors for foreign or altered self-nucleic acids (81). These include the retinoic acid-inducible gene I (RIG-I)-like family of receptors (RLRs) that can detect bacterial and viral nucleic acids and, more specifically, dsRNA (82–84). There are currently three known RLRs: RIG-I, melanoma differentiation-associated gene 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2). All three RLRs share a common central helicase domain and carboxy-terminal domain, although only RIG-I and MDA5 possess two caspase activation and recruitment domains (CARDs) that are required for downstream signal transduction (85). In contrast, LGP2 lacks a CARD domain and, instead, negatively regulates RIG-I and MDA5 activity (85, 86). Upon RNA binding, RIG-I and MDA5 interact with the CARD domain found on mitochondrial antiviral-signaling (MAVS), a mitochondria-localized adaptor protein, which subsequently activates TRAF family member-associated nuclear factor-kappa-B activator (TANK)-binding kinase 1 (TBK1) and inhibitor of nuclear factor kappa-B kinase subunit epsilon (IKKε). TBK1 and IKKε, in turn, activate the transcription factors interferon regulatory factors 3 and 7 (IRF3 and IRF7), which finally induce the transcription of type-I interferons (IFNs) among other antiviral mediators. Importantly, we have shown that RIG-I and MDA5 are constitutively expressed in microglia and astrocytes and that such expression is upregulated following viral infection (87). Furthermore, we have recently demonstrated that RIG-I not only recognizes viral dsRNA in glia, but is also able to respond to bacterial dsRNA (88).

Surprisingly, RIG-I may elicit immune responses to cytosolic DNA in addition to RNA, albeit in an indirect manner mediated by the actions of RNA polymerase III (RP3) (88–93), wherein RP3 reverse transcribes cytosolic dsDNA into a 5' triphosphate-containing dsRNA ligand that then can subsequently be detected by RIG-I. Such a mechanism might explain the earlier, and perhaps erroneous, description of RP3 as a DNA sensor (92, 93).

However, it is now apparent that cells, including glia, possess a number of molecules that specifically serve as cytosolic DNA sensing molecules to initiate responses to foreign or self-DNA. The first of such molecules to be discovered was DNA sensor Z-DNA binding protein (ZBP1; previously known as DNA-dependent activator of IRFs (DAI)) in 2007 (94). Since then, our knowledge of cytosolic DNA sensing PRRs has expanded to include proteins such as cyclic GMP-AMP synthase (cGAS) (95), interferon gamma-inducible 16 (IFI16) (96), and absent in melanoma 2 (AIM2) (97–99). Importantly, we, and others, have described the expression and activity of cGAS (100, 101), IFI16 (102–104), ZBP1 (105–108), and AIM2 (101, 109, 110), in resident CNS cells including human and murine microglia and/or astrocytes.

As shown in Figure 1, cGAS and IFI16 use the endoplasmic reticulum-localized protein stimulator of interferon genes (STING) as an adaptor molecule to exert their responses, while ZBP1 and AIM2 recruit their own adaptor molecules, receptor interacting serine/threonine-protein (RIP) kinases 1 and 3 (RIPK1/3), and apoptosis-associated speck-like protein containing a CARD (ASC), respectively (99, 111). Upon activation, STING facilitates the activation of transcription factors TBK1, IRF3, and nuclear factor kappa-B (NF-κB) that then initiate the transcription of type-I IFNs, proinflammatory cytokines, and chemokines (112). ZBP1 recruits RIPK1 and 3 *via* interactions with its RIP homotypic interaction motif (RHIM) domains, which subsequently activates NF-κB (113). Both IFI16 and AIM2 can, like other receptors upstream of inflammasome formation, recruit ASC following DNA detection (99, 114). AIM2 and IFI16 can both form an inflammasome complex with ASC to recruit the effector protein, caspase-1, that subsequently cleaves pro-IL-1β and pro-IL18 to their mature forms for release (Figure 1) (99, 114). Additionally, AIM2-ASC inflammasome complex formation can lead to pyroptotic cell death *via* cleavage of gasdermin-D (GSDMD) (97, 98).

Finally, several other putative DNA sensors such as DNA-dependent protein kinase (DNA-PK) (115), DEAD-box helicase 41 (DDX41) (116), and meiotic recombination 11 homolog A (MRE11) (117) have been described in peripheral cell types, but their functions as PRRs for DNA in the CNS have not been investigated to date.

Peripheral and resident CNS cells exhibit inflammatory phenotypes following DNA damage (118–120), although the mechanisms underlying the initiation of these responses have remained elusive. Interestingly, several cytosolic DNA sensors appear to be capable of detecting mitochondrial and genomic self-DNA resulting from insults such as oxidative stress and IR (41, 121–128). As such, cytosolic DNA sensors may be the link between DNA damage and subsequent innate immune responses.

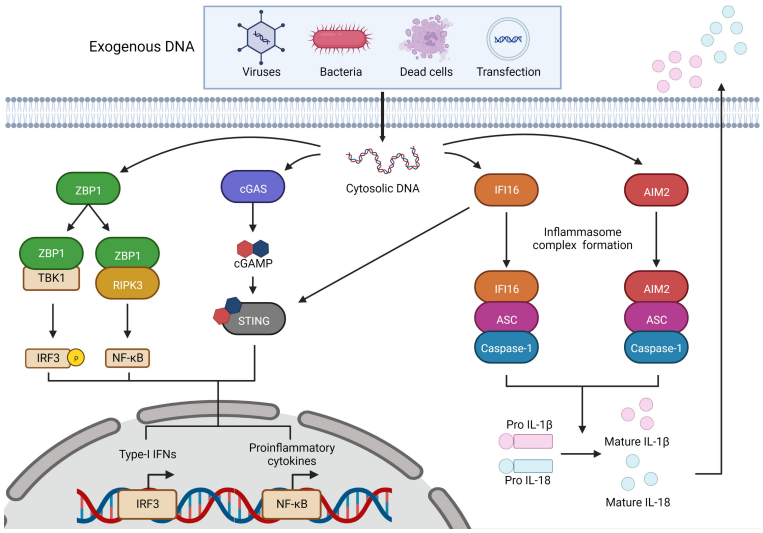

FIGURE 1
Cytosolic DNA sensors and their signaling pathways. Sensing of dsDNA by ZBP1 induces its association with RIPK3 and subsequent activation of NF-κB to elicit pro-inflammatory cytokine production, and/or its interaction with TBK1 to induce IRF3 activation and type-I IFN expression. Sensing of dsDNA by cGAS catalyzes the production of cGAMP that activates STING and leads to TBK1 activation that induces IRF3 activation and type-1 IFN expression. IFI16 can directly interact with STING following DNA sensing resulting in NF-κB and IRF3 activation, or can associate with ASC to form an inflammasome complex resulting in caspase-1-mediated IL-1β and IL-18 release. AIM2 sensing of dsDNA also leads to inflammasome complex formation with ASC and mature IL-1β and IL-18 release. This figure was created with Biorender.com.

4 Cytosolic DNA sensors lie at the intersection of DNA damage and innate immunity

The presence of DNA in the cytoplasm is indicative of cell compromise as it is normally confined to the nucleus or mitochondria in healthy cells. While cytosolic DNA sensors have been extensively studied with regard to bacterial and viral infections, it has only been recently recognized that they may also play a critical role in the generation of immune responses to cytosolic mtDNA and genomic self-DNA (42, 121, 129–131). In contrast to endosomal nucleic acid sensors such as TLR9 that detect prokaryotic DNA based on their distinct methylation patterns (132), cytosolic DNA sensors seem to be unable to discriminate between foreign and self-DNA, and so can detect and respond to either (133, 134). However, it remains unclear whether such responses to self-DNA are protective or detrimental, especially in the context of the CNS where there is strong potential for damaging neuroinflammation. Regardless, it is now apparent that glial cells express multiple sensors capable of initiating their immune functions in response to the presence of cytosolic DNA as described below.

4.1 cGAS

Of all the DNA sensors, cGAS has risen to the forefront of nucleic acid sensor research since it's discovery in 2013 (95). Over the past decade, our understanding of its role has expanded from the triggering of antiviral immunity to include the inhibition of homologous recombination-mediated DNA repair (135, 136), control of DNA replication dynamics (137), cellular senescence (35, 138–141), cell death (142–147), and tumorigenesis (136).

As shown in Figure 2A, following DNA binding, cGAS catalyzes the production of the secondary messenger molecule 2'3'cyclic guanosine monophosphate-adenosine monophosphate (cGAMP), which subsequently binds to STING that is located on the surface of the endoplasmic reticulum (95, 148, 149). Following interaction with cGAMP, STING undergoes a conformational change that initiates the recruitment of TBK1, which then phosphorylates IRF3 and liberates NF-κB. Activation of this signaling cascade results in the production of potent proinflammatory cytokines and chemokines including IL-6, TNF, and IL-8, and the type-I IFNs such as IFN-β (112, 150–152).

The discrimination, or lack thereof, between foreign and self-DNA has been recognized as a significant issue regarding cytosolic DNA sensors since indiscriminate DNA binding could lead to the development of autoimmune responses. Indeed, several autoimmune diseases, including Aicardi-Goutières syndrome (AGS), are associated with increased levels of cytoplasmic DNA and inflammatory mediator production (153). While DNA-mediated activation of cGAS occurs in a length dependent manner, with robust activity occurring only with DNA longer than 45 bp or shorter DNA fragments with flayed ends (154, 155), its ability to discriminate between DNA fragments seems to end here.

DNA damage and genomic instability has long been known to induce inflammatory responses (123, 156, 157), but it has only been recently that this phenomenon has been shown to be connected to cytosolic nucleic acid sensors including cGAS. In 2017, Mackenzie et al. (121) discovered that a primary culprit in the induction of

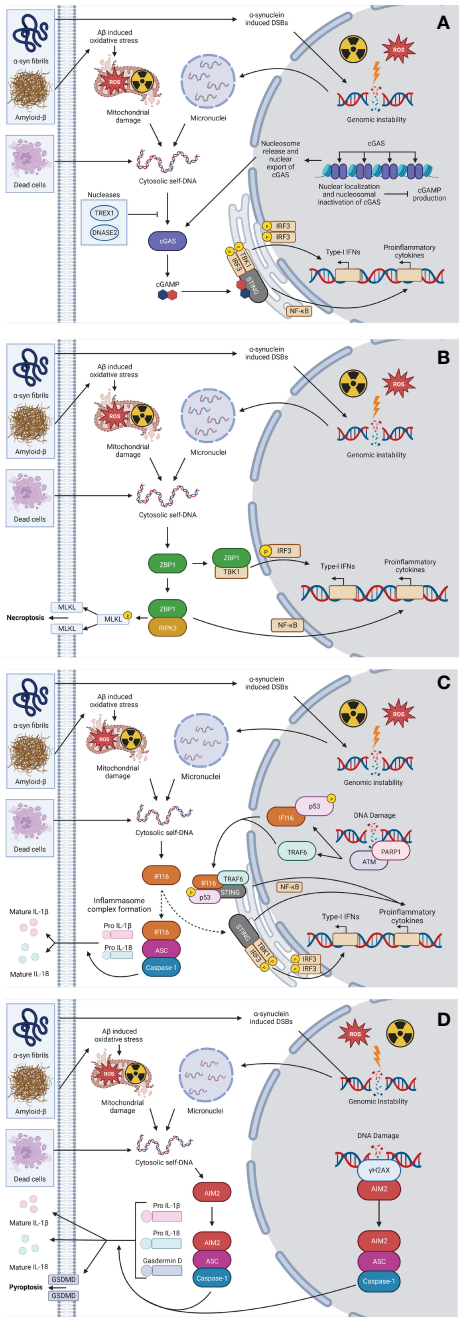

FIGURE 2
Cytosolic self-DNA sensing pathways. In the CNS, the uptake of α-Syn fibrils, amyloid-β, and/or cellular debris by resident CNS cells, or exposure to exogenous oxidative stress or ionizing radiation (IR), can lead to liberation of self-DNA to the cytosol *via* mitochondrial damage or genomic instability leading to micronuclei formation. The presence of self-DNA in the cytoplasm can then be perceived *via* DNA sensing PRRs including cGAS **(A)**, ZBP1 **(B)**, IFI16 **(C)**, and/or AIM2 **(D)**, leading to sensor-specific signaling pathways that precipitate the production of inflammatory cytokines, type-I IFNs and/or immunogenic necroptotic and pyroptotic cell death pathways. This figure was created with Biorender.com.

inflammatory responses following IR-induced DNA damage was the formation of micronuclei. The breakdown of the micronuclear envelope and subsequent exposure of self-DNA was associated with rapid cGAS translocation to the micronuclei and the onset of proinflammatory immune responses (121), a result that has since been corroborated in other studies (122, 158–163). Additionally, DNA damaging events often precipitate the release of mtDNA to the cytoplasm and this can similarly be recognized by cGAS (19, 164–169).

Importantly, mammalian cells appear to possess mechanisms that serve to limit cGAS activation to prevent excessive or prolonged activation that could lead to devastating autoimmunity. First, recent studies have determined that cGAS may primarily be localized to the nucleus in cells at rest (170, 171), rather than being cytosolic as was initially thought (95). In the nucleus, cGAS is tightly bound to nucleosomes where its DNA binding sites are prevented from interacting with nucleosomal DNA (172–177). Furthermore, while nuclear cGAS activation can occur, cGAMP production resulting from such activation is approximately 500-fold less than that generated when DNA is administered to the cytoplasm (171).

Second, cytoplasmic nucleases, such as three prime repair exonuclease 1 (TREX1) and deoxyribonuclease II (DNASE2) restrict cytoplasmic DNA accumulation thereby limiting cGAS activation (162). The impact of these nucleases is particularly apparent in inflammatory autoimmune disorders that occur due to mutations that result in their loss of function (141, 178, 179). Together, these studies reinforce the importance of the regulation of DNA sensing proteins, especially cGAS.

4.2 ZBP1

ZBP1 was the first cytosolic DNA sensor discovered (94) and its importance in host responses to viral infection have been extensively described (105, 180). ZBP1 was initially found to be a critical component in the detection of the dsDNA of HSV-1 (94) and other viruses in both peripheral and CNS cell types (105, 107, 108, 111, 181). More recently, it appears that this sensor may also recognize RNA motifs in addition to DNA (182). Furthermore, this cytosolic sensor has been implicated in the initiation of the necroptotic pathway of immunogenic cell death (105–108, 111, 182–188).

As shown in Figure 2B, upon binding to DNA, ZBP1 recruits RIPK3 that subsequently activates NF-κB to induce proinflammatory cytokine production (113). Additionally, ZBP1 has been shown to associate with TBK1 and IRF3 to regulate the activation of IRF3 and, thus, drive type-I IFN production (94). However, it is important to note that this receptor may act in a ligand and cell type specific manner, as ZBP1 knockdown was found to have no effect on exogenous DNA-induced IFN production in mouse embryonic fibroblasts (189) or a lung epithelial cell line (190), while similar knockdown significantly reduced B-DNA-induced IFN-β production in an immortalized murine fibroblast cell line (190).

Importantly, our current knowledge of the role of ZBP1 in responses to self-DNA remains limited, especially since the precise identity of the ligand(s) for ZBP1 remains controversial (181, 182, 187, 189, 191). However, there is data to support the notion that mtDNA serves as a ligand for ZBP1 (192, 193). A recent study has

indicated that mtDNA release induced by low level oxidative stress, in the absence of detectable damage to nuclear DNA, elicits a type-I IFN response by pulmonary epithelial cells (192). Interestingly, in this study, fragments of mtDNA were shown to be released in exosomes that were capable of initiating further inflammatory responses in naïve epithelial cells (192). Furthermore, a second study reported the ability of glucose deprivation to induce mtDNA release *via* the actions of NOXA in FVB/NJ mice following implantation of the MVT-1 mammary cancer cell line (193), which subsequently initiated necroptosis in a ZBP1-dependent manner (193).

4.3 IFI16

IFI16 is a DNA sensor that is a member of the pyrin and HIN domain (PYHIN) family. While it was first identified in 1992 (194), it wasn't until 2010 that it was characterized as a cytosolic DNA sensor capable of inducing IFN-β production in response to transfected exogenous DNA (96), and such responses have been reported to occur following direct interaction with STING (195) (Figure 2C). Interestingly, IFI16 exhibits both nuclear and cytosolic localization (196). In the nucleus, IFI16 has been shown to respond to viral DNA, which leads to the formation of an inflammasome that then translocates to the cytoplasm where it participates in the cleavage of pro IL-1β and IL-18 into their mature forms for release (114, 197, 198).

With regard to self-DNA detection, the murine ortholog of IFI16, IFI204, was found to mediate the detection of DNA released into the cytoplasm following DNA damage resulting from ataxia-telangiectasia mutated (ATM) deficiency in a murine model of ataxia telangiectasia (A-T) (123). Since the work of Mackenzie et. al (121), has indicated that cytosolic self-DNA recognition occurs due to the formation of micronuclei, it is possible that a similar mechanism underlies that ability of IFI204/IFI16 to perceive the presence of self-DNA, although further study will be necessary to confirm such a hypothesis.

While IFI16 is capable of binding self-DNA, it displays a preference for long non-self-DNA due to its ability to oligomerize into clusters, forming foci that are unable to bind nucleosomal self-DNA (199, 200). Alternatively, IFI16 may serve to detect DNA damage indirectly *via* the formation of a complex with DDR proteins that can subsequently initiate STING signaling (Figure 2C). Following etoposide-induced genotoxic stress, IFI16 has been found to combine with the DDR proteins ATM and poly (ADP-ribose) polymerase 1 (PARP1) to induce the formation of a STING signaling complex that results in the activation of NF-κB and proinflammatory cytokine production (200). This ATM-initiated nuclear mechanism results in far more rapid responses to DNA damage than those mediated by cGAS that require the formation and rupture of micronuclei (200). Furthermore, such responses predominantly result in the activation of NF-κB, rather than IRF3, resulting in a pro-inflammatory response (200). However, it is important to note that most of this work has been performed in keratinocytes and it remains to be determined whether such mechanisms exist in resident CNS cell type.

4.4 AIM2

AIM2 is another member of the PYHIN family that appears to have DNA sensing capabilities. First identified in 1997 (201), its role in dsDNA detection was not recognized until a decade later (97–99, 202). As shown in Figure 2D, AIM2 recruits the inflammasome adaptor protein ASC and procaspase-1 following binding to cytosolic DNA, forming an inflammasome complex that permits IL-1β and IL-18 maturation *via* the actions of caspase-1 (97–99, 202). While AIM2, like ZBP1, can also initiate immunogenic cell death pathways, AIM2 appears to initiate pyroptosis rather than necroptosis *via* the cleavage of gasdermin-D and subsequent pore formation (97–99, 202).

Importantly, AIM2 has been shown to detect cytosolic self-DNA and mtDNA (131, 203–205). The interaction between AIM2 and cytosolic self-DNA has primarily been studied in the context of autoimmune diseases that characteristically entail cytosolic DNA accumulation, such as arthritis, psoriasis, and systemic lupus erythematosus (131, 203, 206). Furthermore, pharmacological disruption of the nuclear envelope and the liberation of self-DNA to the cytosol has also been shown to induce AIM2 activation (205). As such, it has been inferred from these observations that AIM2 is capable of recognizing DNA and might be able to do so following micronuclei formation.

In addition, a limited number of studies suggest that AIM2 may also be localized to the nucleus in some cell types, such as macrophages (124), and can mediate responses to nuclear DNA damage (124, 207). For example, AIM2 has been reported to co-localize with the DNA damage marker gamma-H2A histone family member X (γH2AX) at sites of double strand breaks (DSBs) following IR exposure in macrophages, where it subsequently forms an AIM2-ASC-caspase-1 inflammasome complex to trigger pyroptotic cell death (124).

While the relative importance of AIM2-mediated DNA detection in the cytosol versus the nucleus remains unclear, it is noteworthy that the localization of the AIM2 inflammasome complex to sites of nuclear DNA DSBs occurs as rapidly as 8 hours following IR exposure (124). Furthermore, it is interesting that these inflammasome complexes have been observed to accumulate in the perinuclear region (124). However, it remains to be determined whether such accumulations involve additional interactions of AIM2 with cytosolic self-DNA.

5 Self-DNA detection in the CNS

To date, the mechanisms by which resident CNS cells can detect and respond to self- DNA are understudied, and it remain unclear whether the net result of such responses are beneficial or detrimental. Neuroinflammation, while crucial in protecting the brain against infection, can result in serious neurological damage if it is of inappropriate intensity or duration. Indeed, neuroinflammation that stems from DNA damage and/or deficient or defective DNA repair can underlie or exacerbate neurodegeneration in CNS disease states (11, 208–210). For example, the detection of cytosolic mtDNA and self-DNA by resident glial cells is a hallmark of CNS pathologies

including AGS, AD, PD, A-T, and Huntington's disease (HD) (44, 109, 160, 179, 211–216), as summarized in Table 1.

In some CNS disorders, such as A-T and AGS, the origins of cytosolic DNA accumulation are clear. In A-T, a critical kinase in DDRs, ATM, is defective and results in the accumulation of DSBs leading to the presence of cytosolic DNA (10, 123), while in AGS, mutations in the genes encoding products that process/degrade nucleic acids, such as TREX1 and RNAse H2, lead to cytosolic DNA accumulation and lethal autoimmunity in neonates (178, 226).

However, the origin of cytosolic DNA in other neurodegenerative diseases is either unclear or unknown. For example, in AD, amyloid-β (Aβ) plaques have been shown to induce oxidative stress that can cause mitochondrial dysfunction and subsequent mtDNA release into the cytosol (232). In contrast, the α-synuclein (α-Syn) fibrils that are commonly seen in PD can induce genomic DNA damage (223), while affected striatal neurons in HD patients and murine models of this disease show significantly higher numbers of micronuclei. However, the mechanisms underlying DNA damage in HD remain unknown (160).

We, and others, have shown that exogenous cytosolic DNA elicits reactive astrogliosis and microgliosis, and is associated with the production of proinflammatory and antiviral mediators by these cells (44, 100, 180, 212, 216, 233, 234). Importantly, the recent demonstration that glial cells express multiple cytosolic sensors has provided the means by which they perceive DNA. We showed that ZBP1 is expressed in microglia and astrocytes in an inducible manner following HSV-1 infection (106). In addition, Cox et. al (101), provided evidence that murine microglia and astrocytes express mRNA encoding cGAS, the p204 murine ortholog of IFI16, and AIM2 (101). Subsequently, we, and others, have extended these findings to demonstrate cGAS, IFI16, and AIM2, protein expression in human microglia and astrocytes, and have

TABLE 1 Expression of DNA sensors by CNS cells and links to neurogenerative disorders.

Cell Type		Species	Sensor(s)	Stimuli/Model	Disease Association	References
Microglia	Primary	Human	cGAS-STING	Ganciclovir treatment	Not applicable	(217)
		Murine	cGAS-STING	ATM deficiency	Ataxia telangiectasia	(109)
				DNA transfection	Not applicable	(101)
				Tau protein aggregation	Alzheimer's disease	(218)
			p204 (IFI16 ortholog)	DNA transfection	Not applicable	(101)
			AIM2	AIM2 deficient 5XFAD	Alzheimer's disease	(219)
				MPTP-induced PD	Parkinson's disease	(220)
				EAE	Multiple sclerosis	(221)
	Cell line	Human	cGAS-STING	DNA transfection	Not applicable	(100)
Astrocytes	Primary	Murine	p204 (IFI16 ortholog)	DNA transfection	Not applicable	(101)
			AIM2	EAE	Multiple sclerosis	(222)
Mixed Glia	Primary	Murine	cGAS-STING	α-synuclein preformed fibrils	Parkinson's disease	(223)
Neurons	Primary	Human	cGAS-STING	Micronuclei formation	Huntington's disease	(160)
			AIM2	DNA transfection	Not applicable	(224)
		Murine	cGAS-STING	Micronuclei formation	Huntington's disease	(160)
			AIM2	DNA transfection	Not applicable	(224)
	Cell line	Murine	cGAS-STING	Cytosolic mtDNA accumulation	Amyotrophic lateral sclerosis	(225)
Whole brain	N/A	Murine	cGAS-STING	TREX deficiency	Aicardi-Goutières syndrome	(226)
				Rnaseh2a$^{G37S/G37S}$ mutation	Aicardi-Goutières syndrome	(178)
				Cytosolic mtDNA accumulation	Amyotrophic lateral sclerosis	(225)
				Tau protein aggregation	Alzheimer's disease	(218)
				APP/PS1	Alzheimer's disease	(212, 227)
				EAE	Multiple sclerosis	(228)
			AIM2	APP/PS1	Alzheimer's disease	(229, 230)
				ME7	Chronic neurodegeneration	(101)
			p204 (IFI16 ortholog)	ME7	Chronic neurodegeneration	(101)
		Zebrafish	IFI16	Cytosolic mtDNA	Parkinson's disease	(231)

shown their ability to mediate glial immune responses to exogenous DNA administration (100, 102, 105, 224).

Transfection of exogenous DNA into human and murine primary glia and cell lines elicits the production of type-I IFNs and proinflammatory cytokines including CCL3, CCL5, CXCL2, TNF, IFN-β, and IL-6 (87, 88). The importance of the cytosolic DNA sensors, cGAS, IFI16 (and murine ortholog p204), and AIM2, in the generation of such responses is implied by the demonstration that dsDNA transfection into primary murine microglia and astrocytes significantly upregulates the expression of mRNA encoding these sensors (101). Importantly, we have shown that the responses of a human microglia cell line to intracellular exogenous DNA administration were significantly attenuated following CRISPR/Cas9 knockdown of cGAS expression (102). Furthermore, the secretion of mature IL-1β, downregulated dendritic growth, and enhanced axon extension of primary human and murine neurons elicited by dsDNA transfection was found to be dependent on the presence of AIM2 (224). As such, it is apparent that resident CNS cells are capable of responding to the presence of exogenous cytosolic DNA and do so *via* various sensor molecules.

With regard to the ability of resident CNS cells to perceive the presence of cytosolic self- DNA, glia have recently been shown to respond to mtDNA and self-DNA accumulation *via* sensors including cGAS and AIM2 (44, 109, 168, 214, 216, 223, 235, 236), as summarized in Table 1. For example, the addition of α-Syn preformed fibrils (PFF) to primary murine mixed glial cultures to simulate PD pathology resulted in DNA DSBs, an accumulation in cytosolic DNA, and subsequent activation of STING and TBK1 that resulted in type I IFN production (223). Importantly, these responses were attenuated by the pharmacological inhibition of STING activation (223). These findings are consistent with those in an *in vivo* mouse model where α-Syn-PFF similarly induced DNA damage, TBK1 activation, and IFN production by microglia *in situ* that preceded PD-like dopaminergic neurodegeneration, and the demonstration that substantia nigra pars compacta tissue from human PD patients show elevated STING protein levels that correlate with α-Syn-PFF accumulation (223).

The cGAS-STING axis has been implicated in the initiation of neurotoxic responses of primary microglial cultures to ATM mutations/deficiency that, again, results in the accumulation of cytosolic self-DNA and models A-T (109). This DNA sensing pathway has also been linked to striatal neuron cell death in HD (160). In primary human and murine HD-affected striatal neurons, Sharma et al. (160) reported a high incidence of micronuclei formation that coincided with cell death due to autophagy, and the increased expression of mRNA encoding CCL5 and CXCL10 that was abolished following cGAS depletion (160). Additionally, studies in mouse models of other neurodegenerative disorders have similarly indicated a role for cGAS in their progression. Most notably, models of AGS development that feature mutations of TREX1 and RNAse H2 have revealed that cGAS is essential for the initiation of the autoimmune responses associated with this disorder (178, 226).

Interestingly, AIM2-mediated responses have also been associated with neurodegenerative diseases but, in contrast to cGAS, such response appear to play a protective rather than a detrimental role, as summarized in Table 1. In primary murine microglia, AIM2 has been shown to alleviate the damaging neuroinflammation seen in the experimental autoimmune encephalitis (EAE) model of multiple sclerosis (MS), and other mouse models of AD and PD (219–221). In EAE, AIM2 was shown to negatively regulate DNA-PK activity in an inflammasome independent manner, and AIM2 deficiency was found to increase levels of microglial activation and peripheral leukocyte recruitment to the CNS (221). Furthermore, the inhibitory activity of AIM2 was found to be comparable to that of pharmacological DNA-PK inhibition (221). However, it should be noted that such findings conflict with other reports suggesting inflammasome components are required for the recruitment of peripheral T-cells to the CNS that exacerbate neuroinflammation in EAE (237, 238). As such, the role of AIM2 in inflammatory CNS diseases appears complex as it relates to both resident glia and peripheral leukocytes and further research is clearly required.

In the 5XFAD model of AD, deletion of AIM2 resulted in a decrease in Aβ deposition, but caused an elevation in the production of the key inflammatory cytokines IL-6 and IL-18, further supporting a negative regulatory role for AIM2 in neuroinflammation (219). Finally, in a neurotoxin (N-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine, MPTP)-induced PD mouse model, AIM2 activation served to limit cGAS activity *via* interference with protein kinase B (AKT)-mediated IRF3 phosphorylation, and conditional knockout of AIM2 in microglia, but not peripheral cells, exacerbated PD-like disease severity (220).

As such, while it is clear that cytosolic DNA sensors play critical roles in the initiation and/or progression of CNS pathologies in animal models (as summarized in Table 1), their specific roles can often appear contradictory and so may be sensor and/or disease condition specific. Additionally, while light has been shed on the beneficial/detrimental effects mediated by these receptors in murine CNS cells and neurodegenerative disorder models, it remains unclear whether cytosolic DNA sensors exert similar functions in human disease. A better understanding of these DNA sensors in the context of human neurons/glia and patients with CNS disorders could identify new targets for therapeutic intervention to limit neuroinflammation and/or to promote beneficial immune responses.

6 Clinical implications and concluding remarks

Taken together, it has become apparent that resident CNS cells play a critical role in the protective and detrimental immune responses associated with CNS infection and damage, and the development/progression of neurological disorders. Furthermore, it is increasingly clear that such responses are initiated *via* the detection of DAMP and PAMP motifs that are associated with cellular damage and infectious agents, respectively. The principal glial cells, microglia and astrocytes, express various cell-surface, endosomal, and cytosolic members of multiple PRR families that trigger immune mediator production to foster neuroinflammation

and recruit leukocytes to the CNS. Importantly, glia can constitutively, and/or be induced to, express PRRs that detect the presence of DNA in the cytosolic compartment. While these sensors were initially characterized as components in the detection of viral and bacterial nucleic acids by microglia and astrocytes, it is now recognized that molecules, including cGAS, IFI16, and AIM2, can play important roles in the generation of responses to the presence of self-DNA in the cytosol resulting from DNA damaging insults, such as IR or oxidative stress, or deficient/defective DNA repair. As such, these mechanisms might be targetable to either augment glia-mediated responses that serve to protect against tumorigenesis, or prevent the inflammatory responses of these cells that initiate or exacerbate damaging neuroinflammation.

To date, several small molecule inhibitors and agonists have been developed for cGAS (239) and AIM2 (240), as well as for the downstream adaptor proteins STING (241–245) and RIPK1/3 (246). These agents have shown good efficacy in both peripheral cells and isolated glia where pharmacological inhibition of these components has led to significant reductions in the production of immune mediators including IFN-β, IL-1β, and IL-6, following activation (105, 240, 241, 245, 247–250). Indeed, the STING agonists, TAK-676 (NCT04420884, NCT04879849) and E7766 (NCT04144140), are currently being tested clinically for the treatment of advanced or metastatic solid tumors, lymphomas, and leukemias, as adjunctive agents to conventional chemotherapy. As such, it might be possible in the future to target the DNA sensors or their downstream adaptor proteins associated with CNS cancers and neurodegenerative diseases.

However, considerable hurdles remain for the development and use of such agents as the available data regarding the specific role of each cytosolic DNA sensor in CNS disorders can be unclear or even contradictory. This may be due to species-specific differences, such as in the ability of STING to be activated by cGAMP (251), or it may be that the glial responses initiated by the presence of cytosolic DNA are sensor, cell type, or even disease stage, dependent. In addition, the ability of many small molecule inhibitors and agonists that target cytosolic DNA sensors to cross the BBB and their efficacy in the brain have not been determined. Indeed, marked differences in the effectiveness of such agents has been noted between studies in isolated glia and those in murine model or clinical settings, primarily due to poor BBB penetration, as reviewed elsewhere (252). While various methods have been successfully employed to circumvent this issue, including BBB disruption and intracerebral, intrathecal, or intranasal, delivery, each carries its own problems, such as neurotoxicity (BBB disruption) or a high degree of invasiveness (intrathecal delivery) (252). Finally, the potential for adverse side-effects of agents targeting DNA sensor mechanisms remains unclear, as no DNA sensor inhibitors or agonists are currently undergoing clinical trials for their efficacy in the treatment of CNS tumors or neurodegenerative disease.

Clearly, more study of these novel cytosolic DNA sensor pathways is warranted given our current lack of understanding of the role of each in glial functions in the context of specific CNS disorders and their stages. Furthermore, successfully establishing the *in situ* efficacy of agonists/antagonists of these DNA sensor pathways in the CNS, and solving the issue of delivery across the BBB, could represent an exciting new therapeutic modality that might be used alone or in conjunction with existing approaches to improve the treatment of a wide range of CNS pathologies.

Author contributions

AS and IM co-wrote this literature review article. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. *SEER cancer stat facts: Cancer of any site*. National Cancer InstituteBethesda, MD (2022). Available at: https://seer.cancer.gov/statfacts/html/all.html.

2. *SEER cancer stat facts: Brain and other nervous system cancer*. Bethesda MD: National Cancer Institute (2022). Available at: https://seer.cancer.gov/statfacts/html/brain.html.

3. Alzheimer's disease. In: *Centers for disease control and prevention*. Available at: https://www.cdc.gov/dotw/alzheimers/index.html.

4. Rong S, Xu G, Liu B, Sun Y, Snetselaar LG, Wallace RB, et al. Trends in mortality from parkinson disease in the united states, 1999–2019. *Neurology* (2021) 97(20): e1986–93.

5. Herranz N, Gil J. Mechanisms and functions of cellular senescence. *J Clin Invest [Internet]*. (2018) 128(4):1238–46. https://www.jci.org/articles/view/95148.

6. Childs BG, Durik M, Baker DJ, Van Deursen JM. Cellular senescence in aging and age-related disease: From mechanisms to therapy. *Nat Med* (2015) 21(12):1424–35.

7. Rea IM, Gibson DS, McGilligan V, McNerlan SE, Denis Alexander H, Ross OA. Age and age-related diseases: Role of inflammation triggers and cytokines. *Front Immunol* (2018) 9(APR):1–28.

8. Cai Q, Jeong YY. Mitophagy in alzheimer's disease and other age-related neurodegenerative diseases. *Cells [Internet]*. (2020) 89(1):150. https://www.mdpi.com/2073-4409/9/1/150.

9. Zhao Y, Simon M, Seluanov A, Gorbunova V, damage DNA. And repair in age-related inflammation. *Nat Rev Immunol [Internet]*. (2023) 23(2):75–89. https://www.nature.com/articles/s41577-022-00751-y.

10. Zhu L-S, Wang D-Q, Cui K, Liu D, Zhu L-Q. Emerging perspectives on dna double-strand breaks in neurodegenerative diseases. *Curr Neuropharmacol.* (2019) 17 (12):1146–57.

11. Coppedè F, Migliore L. DNA Damage in neurodegenerative diseases. *Mutat Res - Fundam Mol Mech Mutagen [Internet].* (2015) 776:84–97. doi: 10.1016/j.mrfmmm.2014.11.010

12. Reisz JA, Bansal N, Qian J, Zhao W, Furdui CM. Effects of ionizing radiation on biological molecules - mechanisms of damage and emerging methods of detection. *Antioxidants Redox Signal* (2014) 21(2):260–92.

13. Saikolappan S, Kumar B, Shishodia G, Koul S, Koul HK. Reactive oxygen species and cancer: A complex interaction. *Cancer Lett [Internet]* (2019) 452:132–43. doi: 10.1016/j.canlet.2019.03.020

14. Giglia-Mari G, Zotter A, Vermeulen W. DNA Damage response. *Cold Spring Harb Perspect Biol* (2011) 3(1):1–19.

15. Abuetabh Y, Wu HH, Chai C, Al Yousef H, Persad S, Sergi CM, et al. DNA Damage response revisited: the p53 family and its regulators provide endless cancer therapy opportunities. *Exp Mol Med [Internet].* (2022) 54(10):1658–69. doi: 10.1038/s12276-022-00863-4

16. Horn V, Triantafyllopoulou A. DNA Damage signaling and polyploid macrophages in chronic inflammation. *Curr Opin Immunol [Internet].* (2018) 50:55–63. doi: 10.1016/j.coi.2017.11.002

17. Taffoni C, Steer A, Marines J, Chamma H, Vila IK, Laguette N. Nucleic acid immunity and dna damage response: new friends and old foes. *Front Immunol* (2021) 12(April):1–10.

18. Li T, Chen ZJ. The cGAS–cGAMP–STING pathway connects DNA damage to inflammation, senescence, and cancer. *J Exp Med [Internet].* (2018) 215(5):1287–99. doi: 10.1084/jem.20180139

19. Maekawa H, Inoue T, Ouchi H, Jao TM, Inoue R, Nishi H, et al. Mitochondrial damage causes inflammation *via* cGAS-STING signaling in acute kidney injury. *Cell Rep [Internet].* (2019) 29(5):1261–1273.e6. doi: 10.1016/j.celrep.2019.09.050

20. Krupina K, Goginashvili A, Cleveland DW. Causes and consequences of micronuclei. *Curr Opin Cell Biol [Internet].* (2021) 70:91–9. doi: 10.1016/j.ceb.2021.01.004

21. Zhang CZ, Spektor A, Cornils H, Francis JM, Jackson EK, Liu S, et al. Chromothripsis from DNA damage in micronuclei. *Nature* (2015) 522(7555):179–84.

22. Ballardin M, Barsacchi R, Bodei L, Caraccio N, Cristofani R, Di Martino F, et al. Oxidative and genotoxic damage after radio-iodine therapy of graves' hyperthyroidism. *Int J Radiat Biol* (2004) 80(3):209–16.

23. Zúñiga-González GM, Batista-González CM, Gómez-Meda BC, Ramos-Ibarra ML, Zamora-Perez AL, Muñoz-Magallanes T, et al. Micronuclei in diabetes: Folate supplementation diminishes micronuclei in diabetic patients but not in an animal model. *Mutat Res - Genet Toxicol Environ Mutagen* (2007) 634(1–2):126–34.

24. Karaman A, Binici DN, Melikoğlu MA. Comet assay and analysis of micronucleus formation in patients with rheumatoid arthritis. *Mutat Res - Genet Toxicol Environ Mutagen* (2011) 721(1):1–5.

25. Donmez-Altuntas H, Sut Z, Ferahbas A, Hamurcu Z, Demirtas H. Increased micronucleus frequency in phytohaemagglutinin-stimulated blood cells of patients with vitiligo. *J Eur Acad Dermatol Venereol [Internet].* (2007) 22(2):162–7. doi: 10.1111/j.1468-3083.2007.02356.x

26. Silva FSG, Oliveira H, Moreiras A, Fernandes JC, Bronze-da-Rocha E, Figueiredo A, et al. Cytotoxic and genotoxic effects of acitretin, alone or in combination with psoralen-ultraviolet a or narrow-band ultraviolet b-therapy in psoriatic patients. *Mutat Res - Genet Toxicol Environ Mutagen [Internet].* (2013) 753 (1):42–7. doi: 10.1016/j.mrgentox.2012.12.017

27. Sprung CN, Chao M, Leong T, McKay MJ. Chromosomal radiosensitivity in two cell lineages derived from clinically radiosensitive cancer patients. *Clin Cancer Res* (2005) 11(17):6352–8.

28. Trippi F, Scarpato R, Petrozzi L, Bonuccelli U, Latorraca S, Sorbi S, et al. Spontaneous and induced chromosome damage in somatic cells of sporadic and familial alzheimer's disease patients. *Mutagenesis [Internet].* (2001) 16(4):323–7. http://mutage.oxfordjournals.org/content/16/4/323.short.

29. Sathasivam K, Woodman B, Mahal A, Bertaux F, Wanker EE, Shima DT, et al. Centrosome disorganization in fibroblast cultures derived from R6/2 huntington's disease (HD) transgenic mice and HD patients. *Hum Mol Genet* (2001) 10(21):2425–35.

30. Wang Y, Duan H, Dai Y, Bin P, Cheng J, Pan Z, et al. Antaxia-telangiectasia mutated gene polymorphisms and susceptibility to chromosomal damage among polycyclic aromatic hydrocarbons exposed workers. *Sci Total Environ [Internet].* (2009) 407(8):2615–20. doi: 10.1016/j.scitotenv.2009.01.002

31. Migliore L, Scarpato R, Coppede F, Petrozzi L, Bonuccelli U, Rodilla V. Chromosome and oxidative damage biomarkers in lymphocytes of parkinson's disease patients. *Int J Hyg Environ Health* (2001) 204(1):61–6.

32. Petrozzi L, Lucetti C, Gambaccini G, Bernardini S, Del Dotto P, Migliore L, et al. Cytogenetic analysis oxidative damage in lymphocytes of parkinson's disease patients. *Neurol Sci* (2001) 22(1):83–4.

33. Cortés-Ciriano I, Lee JJK, Xi R, Jain D, Jung YL, Yang L, et al. Comprehensive analysis of chromothripsis in 2,658 human cancers using whole-genome sequencing. *Nat Genet* (2020) 52(3):331–41.

34. Fenech M. Cytokinesis-block micronucleus cytome assay. *Nat Protoc* (2007) 2 (5):1084–104.

35. Miller KN, Victorelli SG, Salmonowicz H, Dasgupta N, Liu T, Passos JF, et al. Cytoplasmic DNA: sources, sensing, and role in aging and disease. *Cell* (2021) 184 (22):5506–26.

36. Shadel GS, Horvath TL. Mitochondrial ROS signaling in organismal homeostasis. *Cell [Internet].* (2015) 163(3):560–9. doi: 10.1016/j.cell.2015.10.001

37. Andreyev AY, Kushnareva YE. Mitochondrial metabolism of reactive oxygenSpecies. *Biochem [Internet].* (2005) 70(2):200–14. Availablr at: http://www.oxphos.org/staticfile/pubs/Mitochondrialmetabolismofreactiveoxygenspecies.pdf%0A http://protein.bio.msu.r /biokhimiya/contents/v70/pdf/bcm_0200.pdf

38. Roger AJ, Muñoz-Gómez SA, Kamikawa R. The origin and diversification of mitochondria. *Curr Biol* (2017) 27(21):R1177–92.

39. Carvalho G, Repolês BM, Mendes I, Wanrooij PH. Mitochondrial dna instability in mammalian cells. *Antioxidants Redox Signal* (2022) 36(13–15):885–905.

40. Wu Z, Sainz AG, Shadel GS. Mitochondrial DNA: cellular genotoxic stress sentinel. *Trends Biochem Sci [Internet].* (2021) 46(10):812–21. doi: 10.1016/j.tibs.2021.05.004

41. Moya GE, Rivera PD, Dittenhafer-Reed KE. Evidence for the role of mitochondrial dna release in the inflammatory response in neurological disorders. *Int J Mol Sci [Internet].* (2021) 22(13):7030. https://www.mdpi.com/1422-0067/22/13/7030.

42. De Gaetano A, Solodka K, Zanini G, Selleri V, Mattioli AV, Nasi M, et al. Molecular mechanisms of mtdna-mediated inflammation. *Vol. 10 Cells* (2021), 1–21.

43. Riley JS, Tait SW. Mitochondrial DNA in inflammation and immunity. *EMBO Rep* (2020) 21(4):1–17.

44. Zhao Y, Liu B, Xu L, Yu S, Fu J, Wang J, et al. ROS-induced mtDNA release: The emerging messenger for communication between neurons and innate immune cells during neurodegenerative disorder progression. *Antioxidants [Internet].* (2021) 10 (12):1917. https://www.mdpi.com/2076-3921/10/12/1917.

45. Kettenmann H, Kirchhoff F, Verkhratsky A. Microglia: New roles for the synaptic stripper. *Neuron [Internet].* (2013) 77(1):10–8. doi: 10.1016/j.neuron.2012.12.023

46. Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, et al. Synaptic pruning by microglia is necessary for normal brain development. *Sci (80-).* (2011) 333(6048):1456–8.

47. Tremblay M-E, Stevens B, Sierra A, Wake H, Bessis A, Nimmerjahn A. The role of microglia in the healthy brain. *J Neurosci [Internet].* (2011) 31(45):16064–9. doi: 10.1523/JNEUROSCI.4158-11.2011

48. Neumann H, Kotter MR, Franklin RJM. Debris clearance by microglia: an essential link between degeneration and regeneration. *Brain [Internet].* (2008) 132 (2):288–95. doi: 10.1093/brain/awn109

49. Tsai HH, Li H, Fuentealba LC, Molofsky AV, Taveira-Marques R, Zhuang H, et al. Regional astrocyte allocation regulates CNS synaptogenesis and repair. *Sci (80-).* (2012) 337(6092):358–62.

50. Chung WS, Clarke LE, Wang GX, Stafford BK, Sher A, Chakraborty C, et al. Astrocytes mediate synapse elimination through MEGF10 and MERTK pathways. *Nature* (2013) 504(7480):394–400.

51. Allen NJ, Bennett ML, Foo LC, Wang GX, Chakraborty C, Smith SJ, et al. Astrocyte glypicans 4 and 6 promote formation of excitatory synapses *via* GluA1 AMPA receptors. *Nature* (2012) 486(7403):410–4.

52. Molofsky AV, Kelley KW, Tsai HH, Redmond SA, Chang SM, Madireddy L, et al. Astrocyte-encoded positional cues maintain sensorimotor circuit integrity. *Nature* (2014) 509(7499):189–94.

53. Alvarez JI, Dodelet-Devillers A, Kebir H, Ifergan I, Fabre PJ, Terouz S, et al. The hedgehog pathway promotes blood-brain barrier integrity and CNS immune quiescence. *Sci (80-).* (2011) 334(6063):1727–31.

54. Carpentier PA, Duncan DS, Miller SD. Glial toll-like receptor signaling in central nervous system infection and autoimmunity. *Brain Behav Immun* (2008) 22 (2):140–7.

55. Jack CS, Arbour N, Manusow J, Montgrain V, Blain M, McCrea E, et al. TLR signaling tailors innate immune responses in human microglia and astrocytes. *J Immunol* (2005) 175(7):4320–30.

56. Cribbs DH, Berchtold NC, Perreau V, Coleman PD, Rogers J, Tenner AJ, et al. Extensive innate immune gene activation accompanies brain aging, increasing vulnerability to cognitive decline and neurodegeneration: A microarray study. *J Neuroinflammation.* (2012) 9:1–18.

57. Heneka MT, O'Banion MK. Inflammatory processes in alzheimer's disease. *J Neuroimmunol* (2007) 184(1–2):69–91.

58. Bsibsi M, Ravid R, Gveric D, Van Noort JM. Broad expression of toll-like receptors in the human central nervous system. *J Neuropathol Exp Neurol* (2002) 61 (11):1013–21.

59. Griffiths MR, Gasque P, Neal JW. The multiple roles of the innate immune system in the regulation of apoptosis and inflammation in the brain. *J Neuropathol Exp Neurol* (2009) 68(3):217–26.

60. Wicherska-Pawłowska K, Wróbel T, Rybka J. Toll-like receptors (TLRs), NOD-like receptors (NLRs), and RIG-I-Like receptors (RLRs) in innate immunity. TLRs, NLRs, and RLRs ligands as immunotherapeutic agents for hematopoietic diseases. *Int J Mol Sci [Internet].* (2021) 22(24):13397. https://www.mdpi.com/1422-0067/22/24/13397.

61. Asami J, Shimizu T. Structural and functional understanding of the toll-like receptors. *Protein Sci* (2021) 30(4):761–72.

62. Mokhtari Y, Pourbagheri-Sigaroodi A, Zafari P, Bagheri N, Ghaffari SH, Bashash D. Toll-like receptors (TLRs): An old family of immune receptors with a new face in cancer pathogenesis. *J Cell Mol Med* (2021) 25(2):639–51.

63. Lind NA, Rael VE, Pestal K, Liu B, Barton GM. Regulation of the nucleic acid-sensing toll-like receptors. *Nat Rev Immunol* (2022) 22(4):224–35.

64. Li L, Acioglu C, Heary RF, Elkabes S. Role of astroglial toll-like receptors (TLRs) in central nervous system infections, injury and neurodegenerative diseases. *Brain Behav Immun [Internet]* (2021) 91(August 2020):740–55. doi: 10.1016/j.bbi.2020.10.007

65. Johnston EL, Heras B, Kufer TA, Kaparakis-Liaskos M. Detection of bacterial membrane vesicles by NOD-like receptors. *Int J Mol Sci* (2021) 22(3):1–14.

66. Platnich JM, Muruve DA. NOD-like receptors and inflammasomes: A review of their canonical and non-canonical signaling pathways. *Arch Biochem Biophys [Internet]* (2019) 670(February):4–14. doi: 10.1016/j.abb.2019.02.008

67. Zhong Y, Kinio A, Saleh M. Functions of NOD-like receptors in human diseases. *Front Immunol* (2013) 4(October):1–18.

68. Danis J, Mellett M. Nod-like receptors in host defence and disease at the epidermal barrier. *Int J Mol Sci [Internet].* (2021) 22(9):4677. https://www.mdpi.com/1422-0067/22/9/4677.

69. Kienes I, Weidl T, Mirza N, Chamaillard M, Kufer TA. Role of nlrs in the regulation of type i interferon signaling, host defense and tolerance to inflammation. *Int J Mol Sci* (2021) 22(3):1–28.

70. Fritz JH, Ferrero RL, Philpott DJ, Girardin SE. Nod-like proteins in immunity, inflammation and disease. *Nat Immunol* (2006) 7(12):1250–7.

71. Takeda K, Takeuchi O, Akira S. Recognition of lipopeptides by toll-like receptors. *J Endotoxin Res* (2002) 8(6):459–63.

72. Ishii KJ, Akira S. Innate immune recognition of, and regulation by, DNA. *Trends Immunol* (2006) 27(11):525–32.

73. Bowman CC, Rasley A, Tranguch SL, Marriott I. Cultured astrocytes express toll-like receptors for bacterial products. *Glia* (2003) 43(3):281–91.

74. Olson JK, Miller SD. Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. *J Immunol* (2004) 173(6):3916–24.

75. Rasley A, Anguita J, Marriott I. Borrelia burgdorferi induces inflammatory mediator production by murine microglia. *J Neuroimmunol* (2002) 130(1–2):22–31.

76. Sterka D, Rati DM, Marriott I. Functional expression of NOD2, a novel pattern recognition receptor for bacterial motifs, in primary murine astrocytes. *Glia* (2006) 53 (3):322–30.

77. Chauhan VS, Sterka DG, Furr SR, Marriott I. NOD2 plays an important role in the inflammatory responses of microglia and astrocytes to bacterial CNS pathogens. *Glia* (2009) 57(4):414–23.

78. Sterka D, Marriott I. Characterization of nucleotide-binding oligomerization domain (NOD) protein expression in primary murine microglia. *J Neuroimmunol* (2006) 179(1–2):65–75.

79. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-κB by toll-like receptor 3. *Nat [Internet].* (2001) 413(6857):732–8. http://www.nature.com/articles/35099560.

80. Latz E, Schoenemeyer A, Visintin A, Fitzgerald KA, Monks BG, Knetter CF, et al. TLR9 signals after translocating from the ER to CpG DNA in the lysosome. *Nat Immunol* (2004) 5(2):190–8.

81. Roers A, Hiller B, Hornung V. Recognition of endogenous nucleic acids by the innate immune system. *Immunity* (2016) 44(4):739–54.

82. Rehwinkel J, Gack MU. RIG-i-like receptors: their regulation and roles in RNA sensing. *Nat Rev Immunol [Internet].* (2020) 20(9):537–51. doi: 10.1038/s41577-020-0288-3

83. Loo YM, Gale M. Immune signaling by RIG-i-like receptors. *Immun [Internet].* (2011) 34(5):680–92. doi: 10.1016/j.immuni.2011.05.003

84. Yoneyama M, Fujita T. Function of RIG-i-like receptors in antiviral innate immunity. *J Biol Chem [Internet].* (2007) 282(21):15315–8. doi: 10.1074/jbc.R700007200

85. Yoneyama M, Kikuchi M, Matsumoto K, Imaizumi T, Miyagishi M, Taira K, et al. Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. *J Immunol* (2005) 175(5):2851–8.

86. Rothenfusser S, Goutagny N, DiPerna G, Gong M, Monks BG, Schoenemeyer A, et al. The RNA helicase Lgp2 inhibits TLR-independent sensing of viral replication by retinoic acid-inducible gene-I. *J Immunol* (2005) 175(8):5260–8.

87. Furr SR, Chauhan V, Sterka D, Grdzelishvili V, Marriott I. Characterization of retinoic acid-inducible gene-I expression in primary murine glia following exposure to vesicular stomatitis virus. *J Neurovirol.* (2008) 14(6):503–13.

88. Johnson MB, Halman JR, Burmeister AR, Currin S, Khisamutdinov EF, Afonin KA, et al. Retinoic acid inducible gene-I mediated detection of bacterial nucleic acids in human microglial cells. *J Neuroinflammation.* (2020) 17(1):1–14.

89. Zhao Y, Karijolich J. Know thyself: RIG-I-Like receptor sensing of DNA virus infection. *J Virol [Internet]* (2019) 93(23). doi: 10.1128/JVI.01085-19

90. Zevini A, Olagnier D, Hiscott J. Crosstalk between cytoplasmic RIG-I and STING sensing pathways. *Trends Immunol [Internet].* (2017) 38(3):194–205. doi: 10.1016/j.it.2016.12.004

91. Schlee M, Hartmann G. The chase for the RIG-I ligand–recent advances. *Mol Ther [Internet].* (2010) 18(7):1254–62. https://linkinghub.elsevier.com/retrieve/pii/S1525001616310693.

92. Chiu YH, MacMillan JB, Chen ZJ. RNA Polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. *Cell [Internet].* (2009) 138 (3):576–91. doi: 10.1016/j.cell.2009.06.015

93. Ablasser A, Bauernfeind F, Hartmann G, Latz E, Fitzgerald KA, Hornung V. RIG-i-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. *Nat Immunol* (2009) 10(10):1065–72.

94. Takaoka A, Wang Z, Choi MK, Yanai H, Negishi H, Ban T, et al. DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. *Nature* (2007) 448(7152):501–5.

95. Sun L, Wu J, Du F, Chen X, Chen ZJ. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. *Sci (80-).* (2013) 339 (6121):786–91.

96. Unterholzner L, Keating SE, Baran M, Horan KA, Jensen SB, Sharma S, et al. IFI16 is an innate immune sensor for intracellular DNA. *Nat Immunol* (2010) 11 (11):997–1004.

97. Hornung V, Ablasser A, Charrel-Dennis M, Bauernfeind F, Horvath G, Caffrey DR, et al. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. *Nature* (2009) 458(7237):514–8.

98. Fernandes-Alnemri T, Yu JW, Datta P, Wu J, Alnemri ES. AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. *Nature* (2009) 458 (7237):509–13.

99. Roberts TL, Idris A, Dunn JA, Kelly GM, Burnton CM, Hodgson S, et al. HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. *Sci (80-) [Internet].* (2009) 323(5917):1057–60. doi: 10.1126/science.1169841

100. Jeffries AM, Marriott I. Human microglia and astrocytes express cGAS-STING viral sensing components. *Neurosci Lett* (2017) 658(July):53–6.

101. Cox DJ, Field RH, Williams DG, Baran M, Bowie AG, Cunningham C, et al. DNA Sensors are expressed in astrocytes and microglia *in vitro* and are upregulated during gliosis in neurodegenerative disease. *Glia* (2015) 63(5):812–25.

102. Jeffries AM, Nitika, Truman AW, Marriott I. The intracellular DNA sensors cGAS and IFI16 do not mediate effective antiviral immune responses to HSV-1 in human microglial cells. *J Neurovirol [Internet].* (2020) 26(4):544–55. doi: 10.1007/s13365-020-00852-1

103. Coulon PG, Dhanushkodi N, Prakash S, Srivastava R, Roy S, Alomari NI, et al. NLRP3, NLRP12, and IFI16 inflammasomes induction and caspase-1 activation triggered by virulent HSV-1 strains are associated with severe corneal inflammatory herpetic disease. *Front Immunol* (2019) 10(JULY):1–19.

104. Conrady CD, Zheng M, Fitzgerald KA, Liu C, Carr DJJ. Resistance to HSV-1 infection in the epithelium resides with the novel innate sensor, IFI-16. *Mucosal Immunol* (2012) 5(2):173–83.

105. Jeffries AM, Suptela AJ, Marriott I. Z-DNA binding protein 1 mediates necroptotic and apoptotic cell death pathways in murine astrocytes following herpes simplex virus-1 infection. . *J Neuroinflamm [Internet].* (2022) 19(1):1–13. doi: 10.1186/s12974-022-02469-z

106. Furr SR, Chauhan VS, Moerdyk-Schauwecker MJ, Marriott I. A role for DNA-dependent activator of interferon regulatory factor in the recognition of herpes simplex virus type 1 by glial cells. *J Neuroinflammation.* (2011) 8:1–12.

107. Daniels BP, Kofman SB, Smith JR, Norris GT, Snyder AG, Kolb JP, et al. The nucleotide sensor ZBP1 and kinase RIPK3 induce the enzyme IRG1 to promote an antiviral metabolic state in neurons. *Immun [Internet].* (2019) 50(1):64–76.e4. doi: 10.1016/j.immuni.2018.11.017

108. Rothan HA, Arora K, Natekar JP, Strate PG, Brinton MA, Kumar M. Z-DNA-Binding protein 1 is critical for controlling virus replication and survival in west nile virus encephalitis. *Front Microbiol* (2019) 10(September):1–10.

109. Song X, Ma F, Herrup K. Accumulation of cytoplasmic DNA due to ATM deficiency activates the microglial viral response system with neurotoxic consequences. *J Neurosci [Internet].* (2019) 39(32):6378–94. doi: 10.1523/JNEUROSCI.0774-19.2019

110. Yogarajah T, Ong KC, Perera D, Wong KT. AIM2 inflammasome-mediated pyroptosis in enterovirus a71-infected neuronal cells restricts viral replication. *Sci Rep [Internet].* (2017) 7(1):1–16. doi: 10.1038/s41598-017-05589-2

111. Upton JW, Kaiser WJ, Mocarski ES. DAI/ZBP1/DLM-1 complexes with RIP3 to mediate virus-induced programmed necrosis that is targeted by murine cytomegalovirus vIRA. *Cell Host Microbe [Internet].* (2012) 11(3):290–7. doi: 10.1016/j.chom.2012.01.016

112. Ishikawa H, Barber GN. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. *Nature* (2008) 455(7213):674–8.

113. Rebsamen M, Heinz LX, Meylan E, Michallet MC, Schroder K, Hofmann K, et al. DAI/ZBP1 recruits RIP1 and RIP3 through RIP homotypic interaction motifs to activate NF-κB. *EMBO Rep* (2009) 10(8):916–22.

114. Kerur N, Veettil MV, Sharma-Walia N, Bottero V, Sadagopan S, Otageri P, et al. IFI16 acts as a nuclear pathogen sensor to induce the inflammasome in response to kaposi sarcoma-associated herpesvirus infection. *Cell Host Microbe [Internet].* (2011) 9(5):363–75. doi: 10.1016/j.chom.2011.04.008

115. Ferguson BJ, Mansur DS, Peters NE, Ren H, Smith GL. DNA-PK is a DNA sensor for IRF-3-dependent innate immunity. *Elife* (2012) 2012(1):1–17.

116. Zhang Z, Yuan B, Bao M, Lu N, Kim T, Liu YJ. The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. *Nat Immunol* (2011) 12(10):959–65.

117. Kondo T, Kobayashi J, Saitoh T, Maruyama K, Ishii KJ, Barber GN, et al. DNA Damage sensor MRE11 recognizes cytosolic double-stranded DNA and induces type i interferon by regulating STING trafficking. *Proc Natl Acad Sci U S A*. (2013) 110 (8):2969–74.

118. Murata M, Kawanishi S. Oxidative DNA damage induced by nitrotyrosine, a biomarker of inflammation. *Biochem Biophys Res Commun* (2004) 316(1):123–8.

119. Bartsch H, Nair J. Chronic inflammation and oxidative stress in the genesis and perpetuation of cancer: Role of lipid peroxidation, DNA damage, and repair. *Langenbeck's Arch Surg* (2006) 391(5):499–510.

120. Kiernan EA, Smith SMC, Mitchell GS, Watters JJ. Mechanisms of microglial activation in models of inflammation and hypoxia: Implications for chronic intermittent hypoxia. *J Physiol* (2016) 594(6):1563–77.

121. Mackenzie KJ, Carroll P, Martin C-AA, Murina O, Fluteau A, Simpson DJ, et al. cGAS surveillance of micronuclei links genome instability to innate immunity. *Nat [Internet]*. (2017) 548(7668):461–5. doi: 10.1038/nature23449

122. Harding SM, Benci JL, Irianto J, Discher DE, Minn AJ, Greenberg RA. Mitotic progression following DNA damage enables pattern recognition within micronuclei. *Nat [Internet]*. (2017) 548(7668):466–70. doi: 10.1038/nature23470

123. Härtlova A, Erttmann SF, Raffi FAM, Schmalz AM, Resch U, Anugula S, et al. DNA Damage primes the type i interferon system *via* the cytosolic dna sensor sting to promote anti-microbial innate immunity. *Immunity* (2015) 42(2):332–43.

124. Hu B, Jin C, Li HB, Tong J, Ouyang X, Cetinbas NM, et al. The DNA-sensing AIM2 inflammasome controls radiation-induced cell death and tissue injury. *Sci (80-).* (2016) 354(6313):765–8.

125. Hou Y, Liang H, Rao E, Zheng W, Huang X, Deng L, et al. Non-canonical NF-κB antagonizes STING sensor-mediated DNA sensing in radiotherapy. *Immun [Internet]*. (2018) 49(3):490–503.e4. doi: 10.1016/j.immuni.2018.07.008

126. Chatterjee J, Nairy RK, Langhnoja J, Tripathi A, Patil RK, Pillai PP, et al. ER stress and genomic instability induced by gamma radiation in mice primary cultured glial cells. *Metab Brain Dis* (2018) 33(3):1–14.

127. Banoth B, Cassel SL. Mitochondria in innate immune signaling. *Transl Res [Internet]*. (2018) 202:52–68. doi: 10.1016/j.trsl.2018.07.014

128. Forrester SJ, Kikuchi DS, Hernandes MS, Xu Q, Griendling KK. Reactive oxygen species in metabolic and inflammatory signaling. *Circ Res* (2018) 122(6):877–902.

129. Gao D, Li T, Li X-D, Chen X, Li Q-Z, Wight-Carter M, et al. Activation of cyclic GMP-AMP synthase by self-DNA causes autoimmune diseases. *Proc Natl Acad Sci [Internet]*. (2015) 112(42):E5699–705. doi: 10.1073/pnas.1516465112

130. Kumar V. The trinity of cGAS, TLR9, and ALRs guardians of the cellular galaxy against host-derived self-DNA. *Front Immunol* (2021) 11(February):1–31.

131. Jakobs C, Perner S, Hornung V. AIM2 drives joint inflammation in a self-DNA triggered model of chronic polyarthritis. *PloS One* (2015) 10(6).

132. Hiroaki H, Osamu, Taro K, Tsuneyasu K, Shintaro S, Hideki S, et al. A toll-like receptor recognizes bacterial DNA. *Nat [Internet]* (2000) 408(DECEMBER):740–5. www.nature.com.

133. Zhang F, Yuan Y, Ma F. Function and regulation of nuclear dna sensors during viral infection and tumorigenesis. *Front Immunol* (2021) 11(January):1–11.

134. Briard B, Place DE, Kanneganti T-D. DNA Sensing in the innate immune response. *Physiol [Internet]*. (2020) 35(2):112–24. doi: 10.1152/physiol.00022.2019

135. Jiang H, Xue X, Panda S, Kawale A, Hooy RM, Liang F, et al. Chromatin-bound cGAS is an inhibitor of DNA repair and hence accelerates genome destabilization and cell death. *EMBO J [Internet]*. (2019) 38(21):1–17. doi: 10.15252/embj.2019102718

136. Liu H, Zhang H, Wu X, Ma D, Wu J, Wang L, et al. Nuclear cGAS suppresses DNA repair and promotes tumorigenesis. *Nat [Internet]*. (2018) 563(7729):131–6. doi: 10.1038/s41586-018-0629-6

137. Chen H, Chen H, Zhang J, Wang Y, Simoneau A, Yang H, et al. cGAS suppresses genomic instability as a decelerator of replication forks. *Sci Adv [Internet]* (2020) 6(42). doi: 10.1126/sciadv.abb8941

138. Yang H, Wang H, Ren J, Chen Q, Chen ZJ. cGAS is essential for cellular senescence. *Proc Natl Acad Sci [Internet]*. (2017) 114(23):E4612–20. doi: 10.1073/pnas.1705499114

139. Glück S, Guey B, Gulen MF, Wolter K, Kang TW, Schmacke NA, et al. Innate immune sensing of cytosolic chromatin fragments through cGAS promotes senescence. *Nat Cell Biol* (2017) 19(9):1061–70.

140. Dou Z, Ghosh K, Vizioli MG, Zhu J, Sen P, Wangensteen KJ, et al. Cytoplasmic chromatin triggers inflammation in senescence and cancer. *Nat [Internet]*. (2017) 550 (7676):402–6. doi: 10.1038/nature24050

141. Takahashi A, Loo TM, Okada R, Kamachi F, Watanabe Y, Wakita M, et al. Downregulation of cytoplasmic DNases is implicated in cytoplasmic DNA accumulation and SASP in senescent cells. *Nat Commun [Internet]*. (2018) 9(1):1–12. doi: 10.1038/s41467-018-03555-8

142. Sarhan J, Liu BC, Muendlein HI, Weindel CG, Smirnova I, Tang AY, et al. Constitutive interferon signaling maintains critical threshold of MLKL expression to license necroptosis. *Cell Death Differ [Internet]*. (2019) 26(2):332–47. doi: 10.1038/s41418-018-0122-7

143. Liang Q, Seo GJ, Choi YJ, Kwak MJ, Ge J, Rodgers MA, et al. Crosstalk between the cGAS DNA sensor and beclin-1 autophagy protein shapes innate antimicrobial immune responses. *Cell Host Microbe [Internet]*. (2014) 15(2):228–38. doi: 10.1016/j.chom.2014.01.009

144. Liu D, Wu H, Wang C, Li Y, Tian H, Siraj S, et al. STING directly activates autophagy to tune the innate immune response. *Cell Death Differ [Internet]*. (2019) 26 (9):1735–49. doi: 10.1038/s41418-018-0251-z

145. Gui X, Yang H, Li T, Tan X, Shi P, Li M, et al. Autophagy induction *via* STING trafficking is a primordial function of the cGAS pathway. *Nat [Internet]*. (2019) 567 (7747):262–6. doi: 10.1038/s41586-019-1006-9

146. Brault M, Olsen TM, Martinez J, Stetson DB, Oberst A. Intracellular nucleic acid sensing triggers necroptosis through synergistic type I IFN and TNF signaling. *J Immunol* (2018) 200(8):2748–56.

147. Chen D, Tong J, Yang L, Wei L, Stolz DB, Yu J, et al. PUMA amplifies necroptosis signaling by activating cytosolic DNA sensors. *Proc Natl Acad Sci U S A*. (2018) 115(15):3930–5.

148. Ablasser A, Goldeck M, Cavlar T, Deimling T, Witte G, Röhl I, et al. CGAS produces a 2′-5′-linked cyclic dinucleotide second messenger that activates STING. *Nature* (2013) 498(7454):380–4.

149. Gao P, Ascano M, Wu Y, Barchet W, Gaffney BL, Zillinger T, et al. Cyclic [G (2′,5′)pA(3′,5′)p] is the metazoan second messenger produced by DNA-activated cyclic GMP-AMP synthase. *Cell* (2013) 153(5):1094–107.

150. Sun W, Li Y, Chen L, Chen H, You F, Zhou X, et al. ERIS, an endoplasmic reticulum IFN stimulator, activates innate immune signaling through dimerization. *Proc Natl Acad Sci U S A*. (2009) 106(21):8653–8.

151. Wu J, Sun L, Chen X, Du F, Shi H, Chen C, et al. Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. *Sci (80-).* (2013) 339(6121):826–30.

152. Zhong B, Yang Y, Li S, Wang YY, Li Y, Diao F, et al. The adaptor protein MITA links virus-sensing receptors to IRF3 transcription factor activation. *Immun [Internet]*. (2008) 29(4):538–50. doi: 10.1016/j.immuni.2008.09.003

153. Crow YJ, Rehwinkel J. Aicardi-goutie'res syndrome and related phenotypes: Linking nucleic acid metabolism with autoimmunity. *Hum Mol Genet* (2009) 18 (R2):130–6.

154. Herzner AM, Hagmann CA, Goldeck M, Wolter S, Kübler K, Wittmann S, et al. Sequence-specific activation of the DNA sensor cGAS by y-form DNA structures as found in primary HIV-1 cDNA. *Nat Immunol* (2015) 16(10):1025–33.

155. Andreeva L, Hiller B, Kostrewa D, Lässig C, De Oliveira Mann CC, Jan Drexler D, et al. CGAS senses long and HMGB/TFAM-bound U-turn DNA by forming protein-DNA ladders. *Nature* (2017) 549(7672):394–8.

156. Paludan SR. Activation and regulation of DNA-driven immune responses. *Microbiol Mol Biol Rev* (2015) 79(2):225–41.

157. Ahn J, Xia T, Konno H, Konno K, Ruiz P, Barber GN. Inflammation-driven carcinogenesis is mediated through STING. *Nat Commun [Internet]*. (2014) 5(1):5166.

158. Motwani M, Fitzgerald KA. cGAS micro-manages genotoxic stress. *Immun [Internet]*. (2017) 47(4):616–7. http://linkinghub.elsevier.com/retrieve/pii/S1074761317304314.

159. Bakhoum SF, Ngo B, Laughney AM, Cavallo JA, Murphy CJ, Ly P, et al. Chromosomal instability drives metastasis through a cytosolic DNA response. *Nature* (2018) 553(7689):467–72.

160. Sharma M, Rajendrarao S, Shahani N, Ramírez-Jarquín UN, Subramaniam S. Cyclic GMP-AMP synthase promotes the inflammatory and autophagy responses in huntington disease. *Proc Natl Acad Sci U S A*. (2020) 117(27):15989–99.

161. de Oliveira Mann CC, Kranzusch PJ. cGAS conducts micronuclei DNA surveillance. *Trends Cell Biol [Internet]*. (2017) 27(10):697–8. doi: 10.1016/j.tcb.2017.08.007

162. Mohr L, Toufektchan E, von Morgen P, Chu K, Kapoor A, Maciejowski J. ER-directed TREX1 limits cGAS activation at micronuclei. *Mol Cell* (2021) 81(4):724–738.e9.

163. Zhao M, Wang F, Wu J, Cheng Y, Cao Y, Wu X, et al. CGAS is a micronucleophagy receptor for the clearance of micronuclei. *Autophagy [Internet]*. (2021) 17(12):3976–91. doi: 10.1080/15548627.2021.1899440

164. Guo Y, Gu R, Gan D, Hu F, Li G, Xu G. Mitochondrial DNA drives noncanonical inflammation activation *via* cGAS–STING signaling pathway in retinal microvascular endothelial cells. *Cell Commun Signal* (2020) 18(1):1–12.

165. Nadalutti CA, Ayala-Peña S, Santos JH. Mitochondrial DNA damage as driver of cellular outcomes. *Am J Physiol - Cell Physiol* (2022) 322(2):C136–50.

166. Liu S, Feng M, Guan W. Mitochondrial DNA sensing by STING signaling participates in inflammation, cancer and beyond. *Int J Cancer*. (2016) 139(4):736–41.

167. West AP, Khoury-Hanold W, Staron M, Tal MC, Pineda CM, Lang SM, et al. Mitochondrial DNA stress primes the antiviral innate immune response. *Nature* (2015) 520(7548):553–7.

168. Zhang W, Li G, Luo R, Lei J, Song Y, Wang B, et al. Cytosolic escape of mitochondrial DNA triggers cGAS-STING-NLRP3 axis-dependent nucleus pulposus cell pyroptosis. *Exp Mol Med* (2022) 54(2):129–42.

169. Huang LS, Hong Z, Wu W, Xiong S, Zhong M, Gao X, et al. mtDNA activates cGAS signaling and suppresses the YAP-mediated endothelial cell proliferation

program to promote inflammatory injury. *Immun [Internet].* (2020) 52(3):475–486.e5. doi: 10.1016/j.immuni.2020.02.002

170. Volkman HE, Cambier S, Gray EE, Stetson DB. Tight nuclear tethering of cGAS is essential for preventing autoreactivity. *Elife* (2019) 8:1–21.

171. Gentili M, Lahaye X, Nadalin F, Nader GPF, Puig Lombardi E, Herve S, et al. The n-terminal domain of cGAS determines preferential association with centromeric DNA and innate immune activation in the nucleus. *Cell Rep [Internet].* (2019) 26 (9):2377–2393.e13. doi: 10.1016/j.celrep.2019.01.105

172. Boyer JA, Spangler CJ, Strauss JD, Cesmat AP, Liu P, McGinty RK, et al. Structural basis of nucleosome-dependent cGAS inhibition. *Sci (80-).* (2020) 370 (6515):450–4.

173. Cao D, Han X, Fan X, Xu RM, Zhang X. Structural basis for nucleosome-mediated inhibition of cGAS activity. *Cell Res [Internet].* (2020) 30(12):1088–97. doi: 10.1038/s41422-020-00422-4

174. Kujirai T, Zierhut C, Takizawa Y, Kim R, Negishi L, Uruma N, et al. Structural basis for the inhibition of cGAS by nucleosomes. *Sci (80-).* (2020) 370(6515):455–8.

175. Michalski S, de Oliveira Mann CC, Stafford CA, Witte G, Bartho J, Lammens K, et al. Structural basis for sequestration and autoinhibition of cGAS by chromatin. *Nat [Internet].* (2020) 587(7835):678–82. doi: 10.1038/s41586-020-2748-0

176. Pathare GR, Decout A, Glück S, Cavadini S, Makasheva K, Hovius R, et al. Structural mechanism of cGAS inhibition by the nucleosome. *Nat [Internet].* (2020) 587(7835):668–72. doi: 10.1038/s41586-020-2750-6

177. Zhao B, Xu P, Rowlett CM, Jing T, Shinde O, Lei Y, et al. The molecular basis of tight nuclear tethering and inactivation of cGAS. *Nat [Internet].* (2020) 587(7835):673–7. doi: 10.1038/s41586-020-2749-z

178. Pokatayev V, Hasin N, Chon H, Cerritelli SM, Sakhuja K, Ward JM, et al. RNase H2 catalytic core aicardi-goutières syndrome-related mutant invokes cGAS-STING innate immunesensing pathway in mice. *J Exp Med* (2016) 213(3):329–36.

179. Bartsch K, Knittler K, Borowski C, Rudnik S, Damme M, Aden K, et al. Absence of RNase H2 triggers generation of immunogenic micronuclei removed by autophagy. *Hum Mol Genet* (2017) 26(20):3960–72.

180. Jeffries AM, Marriott I. Cytosolic dna sensors and cns responses to viral pathogens. *Front Cell Infect Microbiol* (2020) 10(September):1–17.

181. Thapa RJ, Ingram JP, Ragan KB, Nogusa S, Boyd DF, Benitez AA, et al. DAI senses influenza a virus genomic RNA and activates RIPK3-dependent cell death. *Cell Host Microbe [Internet].* (2016) 20(5):674–81. doi: 10.1016/j.chom.2016.09.014

182. Maelfait J, Liverpool L, Bridgeman A, Ragan KB, Upton JW, Rehwinkel J. Sensing of viral and endogenous RNA by ZBP 1/ DAI induces necroptosis. *EMBO J* (2017) 36(17):2529–43.

183. Pham TH, Kwon KM, Kim Y-E, Kim KK, Ahn J-H. DNA Sensing-independent inhibition of herpes simplex virus 1 replication by DAI/ZBP1. *J Virol* (2013) 87 (6):3076–86.

184. Hao Y, Yang B, Yang J, Shi X, Yang X, Zhang D, et al. ZBP1: A powerful innate immune sensor and double-edged sword in host immunity. *Int J Mol Sci* (2022) 23 (18):1–23.

185. Ingram JP, Thapa RJ, Fisher A, Tummers B, Zhang T, Yin C, et al. ZBP1/DAI drives RIPK3-mediated cell death induced by IFNs in the absence of RIPK1. *J Immunol* (2019) 203(5):1348–55.

186. Lin J, Kumari S, Kim C, Van TM, Wachsmuth L, Polykratis A, et al. RIPK1 counteracts ZBP1-mediated necroptosis to inhibit inflammation. *Nat [Internet].* (2016) 540(7631):124–8. doi: 10.1038/nature20558

187. Kuriakose T, Man SM, Subbarao Malireddi RK, Karki R, Kesavardhana S, Place DE, et al. ZBP1/DAI is an innate sensor of influenza virus triggering the NLRP3 inflammasome and programmed cell death pathways. *Sci Immunol [Internet].* (2016) 1 (2):665–76. doi: 10.1126/sciimmunol.aag2045

188. Guo H, Gilley RP, Fisher A, Lane R, Landsteiner VJ, Ragan KB, et al. Species-independent contribution of ZBP1/DAI/DLM-1-triggered necroptosis in host defense against HSV1. *Cell Death Dis [Internet].* (2018) 9(8):816. doi: 10.1038/s41419-018-0868-3

189. Wang ZC, Choi MK, Ban T, Yanai H, Negishi H, Lu Y, et al. Regulation of innate immune responses by DAI (DLM-1/ZBP1) and other DNA-sensing molecules. *Proc Natl Acad Sci U S A.* (2008) 105(14):5477–82.

190. Lippmann J, Rothenburg S, Deigendesch N, Eitel J, Meixenberger K, Van Laak V, et al. IFNβ responses induced by intracellular bacteria or cytosolic DNA in different human cells do not require ZBP1 (DLM-1/DAI). *Cell Microbiol* (2008) 10(12):2579–88.

191. Semenova N, Bosnjak M, Markelc B, Znidar K, Cemazar M, Heller L. Multiple cytosolic DNA sensors bind plasmid DNA after transfection. *Nucleic Acids Res* (2019) 47(19):10235–46.

192. Szczesny B, Marcatti M, Ahmad A, Montalbano M, Brunyánszki A, Bibli SI, et al. Mitochondrial DNA damage and subsequent activation of z-DNA binding protein 1 links oxidative stress to inflammation in epithelial cells. *Sci Rep* (2018) 8(1):1–11.

193. Baik JY, Liu Z, Jiao D, Kwon HJ, Yan J, Kadigamuwa C, et al. ZBP1 not RIPK1 mediates tumor necroptosis in breast cancer. *Nat Commun [Internet].* (2021) 12(1):1–14. doi: 10.1038/s41467-021-23004-3

194. Trapani JA, Browne KA, Dawson MJ, Ramsay RG, Eddy RL, Shows TB, et al. A novel gene constitutively expressed in human lymphoid cells is inducible with interferon-γ in myeloid cells. *Immunogenetics* (1992) 36(6):369–76.

195. Cridland JA, Curley EZ, Wykes MN, Schroder K, Sweet MJ, Roberts TL, et al. The mammalian PYHIN gene family: Phylogeny, evolution and expression. *BMC Evol Biol [Internet].* (2012) 12(1):140. doi: 10.1186/1471-2148-12-140

196. Costa S, Borgogna C, Mondini M, De Andrea M, Meroni PL, Berti E, et al. Redistribution of the nuclear protein IFI16 into the cytoplasm of ultraviolet b-exposed keratinocytes as a mechanism of autoantigen processing. *Br J Dermatol* (2011) 164 (2):282–90.

197. Monroe KM, Yang Z, Johnson JR, Geng X, Doitsh G, Krogan NJ, et al. IFI16 DNA sensor is required for death of lymphoid CD4 T cells abortively infected with HIV. *Sci (80-).* (2014) 343(6169):428–32.

198. Doitsh G, Galloway NLK, Geng X, Yang Z, Monroe KM, Zepeda O, et al. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. *Nat [Internet].* (2014) 505(7484):509–14. https://www.nature.com/articles/nature12940.

199. Morrone SR, Wang T, Constantoulakis LM, Hooy RM, Delannoy MJ, Sohn J. Cooperative assembly of IFI16 filaments on dsDNA provides insights into host defense strategy. *Proc Natl Acad Sci [Internet]* (2014) 111(1). doi: 10.1073/pnas.1313577111

200. Dunphy G, Flannery SM, Almine JF, Connolly DJ, Paulus C, Jønsson KL, et al. Non-canonical activation of the dna sensing adaptor sting by atm and ifi16 mediates nf-κb signaling after nuclear dna damage. *Mol Cell [Internet].* (2018) 71(5):745–60. doi: 10.1016/j.molcel.2018.07.034

201. DeYoung KL, Ray ME, Su YA, Anzick SL, Johnstone RW, Trapani JA, et al. Cloning a novel member of the human interferon-inducible gene family associated with control of tumorigenicity in a model of human melanoma. *Oncogene [Internet].* (1997) 15(4):453–7. doi: 10.1038/sj.onc.1201206

202. Bürckstümmer T, Baumann C, Blüml S, Dixit E, Dürnberger G, Jahn H, et al. An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. *Nat Immunol [Internet].* (2009) 10(3):266–72. doi: 10.1038/ni.1702

203. Baum R, Sharma S, Carpenter S, Li Q-Z, Busto P, Fitzgerald KA, et al. Cutting edge: AIM2 and endosomal TLRs differentially regulate arthritis and autoantibody production in DNase II–deficient mice. *J Immunol [Internet].* (2015) 194(3):873–7. doi: 10.4049/jimmunol.1402573

204. Dang EV, McDonald JG, Russell DW, Cyster JG. Oxysterol restraint of cholesterol synthesis prevents AIM2 inflammasome activation. *Cell [Internet].* (2017) 171(5):1057–1071.e11. doi: 10.1016/j.cell.2017.09.029

205. Micco A, Frera G, Lugrin J, Jamilloux Y, Hsu ET, Tardivel A, et al. AIM2 inflammasome is activated by pharmacological disruption of nuclear envelope integrity. *Proc Natl Acad Sci U S A.* (2016) 113(32):E4671–80.

206. Kimkong I, Avihingsanon Y, Hirankarn N. Expression profile of HIN200 in leukocytes and renal biopsy of SLE patients by real-time RT-PCR. *Lupus* (2009) 18 (12):1066–72.

207. Diner BA, Li T, Greco TM, Crow MS, Fuesler JA, Wang J, et al. The functional interactome of PYHIN immune regulators reveals IFIX is a sensor of viral DNA. *Mol Syst Biol [Internet].* (2015) 11(2):787. doi: 10.15252/msb.20145808

208. Subhramanyam CS, Wang C, Hu Q, Dheen ST. Microglia-mediated neuroinflammation in neurodegenerative diseases. *Semin Cell Dev Biol* (2019) 94 (May):112–20.

209. Ransohoff RM. How neuroinflammation contributes to neurodegeneration. *Sci (80-).* (2016) 353(6301):777–83.

210. Shiwaku H, Okazawa H. Impaired DNA damage repair as a common feature of neurodegenerative diseases and psychiatric disorders. *Curr Mol Med* (2015) 15(2):119–28.

211. Xu Q, Xu W, Cheng H, Yuan H, Tan X. Efficacy and mechanism of cGAMP to suppress alzheimer's disease by elevating TREM2. *Brain Behav Immun [Internet]* (2019) 81(July):495–508. doi: 10.1016/j.bbi.2019.07.004

212. Hou Y, Wei Y, Lautrup S, Yang B, Wang Y, Cordonnier S, et al. NAD + supplementation reduces neuroinflammation and cell senescence in a transgenic mouse model of alzheimer's disease *via* cGAS–STING. *Proc Natl Acad Sci [Internet]* (2021) 118(37). doi: 10.1073/pnas.2011226118

213. Paul BD, Snyder SH, Bohr VA. Signaling by cGAS–STING in neurodegeneration, neuroinflammation, and aging. *Trends Neurosci* (2021) 44(2):83–96.

214. Healy LM, Stratton JA, Kuhlmann T, Antel J. The role of glial cells in multiple sclerosis disease progression. *Nat Rev Neurol* (2022) 18(4):237–48.

215. Zia S, Rawji KS, Michaels NJ, Burr M, Kerr BJ, Healy LM, et al. Microglia diversity in health and multiple sclerosis. *Front Immunol* 202011(November):1–14.

216. Nissanka N, Moraes CT. Mitochondrial DNA damage and reactive oxygen species in neurodegenerative disease. *FEBS Lett* (2018) 592(5):728–42.

217. Mathur V, Burai R, Vest RT, Bonanno LN, Lehallier B, Zardeneta ME, et al. Activation of the STING-dependent type I interferon response reduces microglial reactivity and neuroinflammation. *Neuron [Internet].* (2017) 96(6):1290–1302.e6. doi: 10.1016/j.neuron.2017.11.032

218. Jin M, Shiwaku H, Tanaka H, Obita T, Ohuchi S, Yoshioka Y, et al. Tau activates microglia *via* the PQBP1-cGAS-STING pathway to promote brain inflammation. *Nat Commun [Internet].* (2021) 12(1):6565. doi: 10.1038/s41467-021-26851-2

219. Wu PJ, Hung YF, Liu HY, Hsueh YP. Deletion of the inflammasome sensor Aim2 mitigates aβ deposition and microglial activation but increases inflammatory cytokine expression in an Alzheimer disease mouse model. *Neuroimmunomodulation* (2017) 24(1):29–39.

220. Rui W, Li S, Yang L, Liu Y, Fan Y, Hu Y, et al. Microglial AIM2 alleviates antiviral-related neuro-inflammation in mouse models of parkinson's disease. *Glia [Internet].* (2022) 70(12):2409–25. doi: 10.1002/glia.24260

221. Ma C, Li S, Hu Y, Ma Y, Wu Y, Wu C, et al. AIM2 controls microglial inflammation to prevent experimental autoimmune encephalomyelitis. *J Exp Med [Internet]* (2021) 218(5). doi: 10.1084/jem.20201796/211880/AIM2-controls-microglial-inflammation-to-prevent

222. Barclay WE, Aggarwal N, Deerhake ME, Inoue M, Nonaka T, Nozaki K, et al. The AIM2 inflammasome is activated in astrocytes during the late phase of EAE. *JCI Insight [Internet].* (2022) 7(8):27710. doi: 10.1172/jci.insight.155563

223. Hinkle JT, Patel J, Panicker N, Karuppagounder SS, Biswas D, Belingon B, et al. STING mediates neurodegeneration and neuroinflammation in nigrostriatal α-synucleinopathy. *Proc Natl Acad Sci U S A.* (2022) 119(15):1–8.

224. Wu PJ, Liu HY, Huang TN, Hsueh YP. AIM 2 inflammasomes regulate neuronal morphology and influence anxiety and memory in mice. *Sci Rep2016* 6(August):1–12.

225. Yu CH, Davidson S, Harapas CR, Hilton JB, Mlodzianoski MJ, Laohamonthonkul P, et al. TDP-43 triggers mitochondrial DNA release *via* mPTP to activate cGAS/STING in ALS. *Cell [Internet].* (2020) 183(3):636–649.e18. doi: 10.1016/j.cell.2020.09.020

226. Gray EE, Treuting PM, Woodward JJ, Stetson DB. Cutting edge: cGAS is required for lethal autoimmune disease in the Trex1-deficient mouse model of aicardi–goutières syndrome. *J Immunol* (2015) 195(5):1939–43.

227. Larrick JW, Mendelsohn AR. Modulation of cGAS-STING pathway by nicotinamide riboside in alzheimer's disease. *Rejuvenation Res [Internet].* (2021) 24 (5):397–402. doi: 10.1089/rej.2021.0062

228. Lemos H, Huang L, Chandler PR, Mohamed E, Souza GR, Li L, et al. Activation of the STING adaptor attenuates experimental autoimmune encephalitis. *J Immunol [Internet].* (2014) 192(12):5571–8. doi: 10.4049/jimmunol.1303258

229. Cao L-L, Guan P-P, Zhang S-Q, Yang Y, Huang X-S, Wang P. Downregulating expression of OPTN elevates neuroinflammation *via* AIM2 inflammasome- and RIPK1-activating mechanisms in APP/PS1 transgenic mice. *J Neuroinflamm [Internet].* (2021) 18(1):281. doi: 10.1186/s12974-021-02327-4

230. Chen J, Shu S, Chen Y, Liu Z, Yu L, Yang L, et al. AIM2 deletion promotes neuroplasticity and spatial memory of mice. *Brain Res Bull [Internet].* (2019) 152 (8):85–94. doi: 10.1016/j.brainresbull.2019.07.011

231. Matsui H, Ito J, Matsui N, Uechi T, Onodera O, Kakita A. Cytosolic dsDNA of mitochondrial origin induces cytotoxicity and neurodegeneration in cellular and zebrafish models of parkinson's disease. *Nat Commun [Internet].* (2021) 12(1):3101. doi: 10.1038/s41467-021-23452-x

232. Yoo SM, Park J, Kim SH, Jung YK. Emerging perspectives on mitochondrial dysfunction and inflammation in alzheimer's disease. *BMB Rep* (2020) 53(1):35–46.

233. Gleichman AJ, Carmichael ST. Glia in neurodegeneration: Drivers of disease or along for the ride? *Neurobiol Dis [Internet]* (2020) 142(May):104957. doi: 10.1016/j.nbd.2020.104957

234. Booth HDE, Hirst WD, Wade-Martins R. The role of astrocyte dysfunction in parkinson's disease pathogenesis. *Trends Neurosci [Internet].* (2017) 40(6):358–70. doi: 10.1016/j.tins.2017.04.001

235. Soni C, Reizis B. Self-DNA at the epicenter of SLE: Immunogenic forms, regulation, and effects. *Front Immunol* (2019) 10(JULY):1–18.

236. Sliter DA, Martinez J, Hao L, Chen X, Sun N, Fischer TD, et al. Parkin and PINK1 mitigate STING-induced inflammation. *Nature* (2018) 561(7722):258–62.

237. Martin BN, Wang C, Zhang C, Kang Z, Gulen MF, Zepp JA, et al. T Cell–intrinsic ASC critically promotes TH17-mediated experimental autoimmune encephalomyelitis. *Nat Immunol [Internet].* (2016) 17(5):583–92. https://www.nature.com/articles/ni.3389.

238. Inoue M, Williams KL, Gunn MD, Shinohara ML. NLRP3 inflammasome induces chemotactic immune cell migration to the CNS in experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A.* (2012) 109(26):10480–5.

239. Decout A, Katz JD, Venkatraman S, Ablasser A. The cGAS–STING pathway as a therapeutic target in inflammatory diseases. *Nat Rev Immunol [Internet].* (2021) 21 (9):548–69. doi: 10.1038/s41577-021-00524-z

240. Jiao Y, Nan J, Mu B, Zhang Y, Zhou N, Yang S, et al. Discovery of a novel and potent inhibitor with differential species-specific effects against NLRP3 and AIM2 inflammasome-dependent pyroptosis. *Eur J Med Chem [Internet].* (2022) 232:114194. https://linkinghub.elsevier.com/retrieve/pii/S0223523422000964.

241. Haag SM, Gulen MF, Reymond L, Gibelin A, Abrami L, Decout A, et al. Targeting STING with covalent small-molecule inhibitors. *Nat [Internet].* (2018) 559 (7713):269–73. doi: 10.1038/s41586-018-0287-8

242. Tian X, Xu F, Zhu Q, Feng Z, Dai W, Zhou Y, et al. Medicinal chemistry perspective on cGAS-STING signaling pathway with small molecule inhibitors. *Eur J Med Chem [Internet]* (2022) 244(September):114791. doi: 10.1016/j.ejmech.2022.114791

243. Hong Z, Mei J, Li C, Bai G, Maimaiti M, Hu H, et al. STING inhibitors target the cyclic dinucleotide binding pocket. *Proc Natl Acad Sci [Internet]* (2021) 118(24). doi: 10.1073/pnas.2105465118

244. Wu JJ, Zhao L, Hu HG, Li WH, Li YM. Agonists and inhibitors of the STING pathway: Potential agents for immunotherapy. *Med Res Rev* (2020) 40(3):1117–41.

245. Liu K, Lan Y, Li X, Li M, Cui L, Luo H, et al. Development of small molecule inhibitors/agonists targeting STING for disease. *BioMed Pharmacother [Internet].* (2020) 132:110945. doi: 10.1016/j.biopha.2020.110945

246. Speir M, Djajawi TM, Conos SA, Tye H, Lawlor KE. Targeting rip kinases in chronic inflammatory disease. *Biomolecules* (2021) 11(5):1–22.

247. Vincent J, Adura C, Gao P, Luz A, Lama L, Asano Y, et al. Small molecule inhibition of cGAS reduces interferon expression in primary macrophages from autoimmune mice. *Nat Commun [Internet].* (2017) 8(1):1–12. doi: 10.1038/s41467-017-00833-9

248. Wiser C, Kim B, Vincent J, Ascano M. Small molecule inhibition of human cGAS reduces total cGAMP output and cytokine expression in cells. *Sci Rep [Internet].* (2020) 10(1):1–11. doi: 10.1038/s41598-020-64348-y

249. Zhang H, You Q-D, Xu X-L. Targeting stimulator of interferon genes (STING): A medicinal chemistry perspective. *J Med Chem [Internet].* (2020) 63(8):3785–816. doi: 10.1021/acs.jmedchem.9b01039.

250. Zorman J, Sušjan P, Hafner-Bratkovič I. Shikonin suppresses NLRP3 and AIM2 inflammasomes by direct inhibition of caspase-1. *PloS One [Internet].* (2016) 11 (7):e0159826. doi: 10.1371/journal.pone.0163887.

251. Chin K-H, Tu Z-L, Su Y-C, Yu Y-J, Chen H-C, Lo Y-C, et al. Novel c-di-GMP recognition modes of the mouse innate immune adaptor protein STING. *Biol Crystallogr [Internet].* (2013) 69(3):352–66. https://scripts.iucr.org/cgi-bin/paper?S0907444912047269.

252. Akhtar A, Andleeb A, Waris TS, Bazzar M, Moradi AR, Awan NR, et al. Neurodegenerative diseases and effective drug delivery: A review of challenges and novel therapeutics. *J Control Release [Internet]* (2021) 330(June 2020):1152–67. doi: 10.1016/j.jconrel.2020.11.021

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Effect and mechanism of chlorogenic acid on cognitive dysfunction in mice by lipopolysaccharide-induced neuroinflammation

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Background: Neuroinflammation is an important factor causing numerous neurodegenerative pathologies. Inflammation can lead to abnormal neuronal structure and function and even death, followed by cognitive dysfunction. There is growing evidence that chlorogenic acid has anti-inflammatory effects and immunomodulatory activity.

Purpose: The aim of this study was to elucidate the potential targets and molecular mechanisms of chlorogenic acid in the treatment of neuroinflammation.

Methods: We used the lipopolysaccharide-induced neuroinflammation mouse model and the lipopolysaccharide-stimulated BV-2 cells *in vitro* model. Behavioral scores and experiments were used to assess cognitive dysfunction in mice. HE staining and immunohistochemistry were used to assess neuronal damage in the mouse brain. Immunofluorescence detected microglia polarization in mouse brain. Western blot and flow cytometry detected the polarization of BV-2 cells. The migration of BV-2 cells was detected by wound healing assay and transwell assay. Potential targets for chlorogenic acid to exert protective effects were predicted by network pharmacology. These targets were then validated using molecular docking and experiments.

Results: The results of *in vivo* experiments showed that chlorogenic acid had an obvious ameliorating effect on neuroinflammation-induced cognitive dysfunction. We found that chlorogenic acid was able to inhibit BV-2 cells M1 polarization and promote BV-2 cells M2 polarization *in vitro* while also inhibiting the abnormal migration of BV-2 cells. Based on the network pharmacology results, we identified the TNF signaling pathway as a key signaling pathway in which chlorogenic acid exerts anti-neuroinflammatory effects. Among them,

Akt1, TNF, MMP9, PTGS2, MAPK1, MAPK14, and RELA are the core targets for chlorogenic acid to function.

Conclusion: Chlorogenic acid can inhibit microglial polarization toward the M1 phenotype and improve neuroinflammation-induced cognitive dysfunction in mice by modulating these key targets in the TNF signaling pathway.

KEYWORDS

chlorogenic acid, neuroinflammation, cognitive dysfunction, microglia, polarization, TNF signaling pathway

1 Introduction

Neuroinflammation, as an important pathological change in the development of neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis), affects neuronal structure and function and can even lead to neuronal death (1, 2). Lipopolysaccharide (LPS) is a component of the outer wall of gram-negative bacterial cell walls and is expressed on the membrane surface of immune cells such as macrophages and microglia (3). Activation of TLR-4, a specific receptor for LPS, the results in the production of proinflammatory cytokines, oxidative stress factors and chemokines, all of which are key mediators in the development of neuroinflammation (4, 5). In previous studies, we found that LPS can cause cognitive dysfunction and a range of complex behaviors, including decreased learning memory, reduced motor complexity, increased anxiety, and the emergence of depressive behaviors (6). A previous study demonstrated that intraperitoneal injection of LPS can be used as a model to study neuroinflammation-induced cognitive dysfunction in mice (7). Microglia, as intrinsic immune cells of the mammalian central nervous system (CNS), play a key role in maintaining brain homeostasis by monitoring their surroundings under physiological conditions (8). Under pathological conditions, resting microglia are activated and polarized into two cellular phenotypes, M1 and M2, *via* the classical activation pathway and the alternative activation pathway, respectively; the M1 phenotype is proinflammatory, and the M2 phenotype is anti-inflammatory (9, 10). M1 microglia mainly produce proinflammatory cytokines such as TNF-α, interleukin-1 (IL-1β), interleukin-6 (IL-6), and oxidative stress factors such as nitric oxide (NO) and reactive oxygen species (ROS), causing damage to neurons in the surrounding environment (11). M2 microglia produce interleukin-4 (IL-4), interleukin-10 (IL-10) and other anti-inflammatory factors that antagonize the M1 pro-inflammatory response and exert a protective effect on neurons (11). M2 microglia play a crucial role in suppressing inflammation, scavenging toxic factors and protecting the brain (12). Therefore, altering the M1/M2 phenotype can influence the progression of inflammation, and promoting microglial polarization toward the M2 phenotype is a key target for the treatment of neuroinflammation-based diseases.

In recent years, Chinese herbal medicines have received wide attention for their safety and efficacy. We found that chlorogenic acid (CGA) is often present as an active ingredient in plants such as Eucommiae (Eucommia ulmoides Oliv.), Honeysuckle (Lonicera japonica Thunb.), and green coffee bean. There is growing evidence that CGA has multiple pharmacological effects, including antioxidant, antibacterial, antiviral, antitumor, and immunomodulatory effects (13). Based on the strong antioxidant and anti-inflammatory effects of CGA, many scholars have found that it has a good neuroprotective effect (14). In addition, it has been shown that CGA is able to cross the blood-brain barrier (BBB) and can treat certain neurological disorders (15). Several clinical and preclinical studies have shown that coffee extract (CGA, the main component) exhibits good therapeutic effects in Alzheimer's disease and Parkinson's disease (16, 17). Hermawati found that CGA improved memory loss and hippocampal cell death after transient total cerebral ischemia and prevented CA1 pyramidal cell death after bilateral common carotid artery occlusion (18). Therefore, CGA may have a potential therapeutic effect on cognitive dysfunction caused by neuroinflammation. However, it is unclear whether CGA can affect microglial polarization and thus exert a therapeutic effect. In the present study, we investigated the effect of CGA on microglial polarization in mice with cognitive dysfunction caused by neuroinflammation and explored the protective mechanisms of CGA. Our study found that chlorogenic acid was able to ameliorate neuroinflammation and associated cognitive dysfunction by inhibiting microglia activation. Furthermore, through network pharmacological analysis, we found that chlorogenic acid may act on the TNF signaling pathway. This study may help determine the translational application of chlorogenic acid in clinical treatment.

2 Materials and methods

2.1 Chemicals and reagents

BV-2 cells were purchased from the Cell Bank of the Chinese Academy of Sciences. Lipopolysaccharide (LPS) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Chlorogenic acid was purchased from Shanghai Yuanye Biotechnology Co. Ltd. (Shanghai, China). Anti-iNOS (1:1000, ab178945) and anti-MMP9 (1:1000, ab228402) were purchased from Abcam. Anti-CD86 (1:1000, BM4121), anti-Arg-1 (1:1000, M01106-4), anti-IL-10 (1:1000, RP1015), anti-CD206 (1:1000, A02285-2), anti-CXCL12 (1:1000, BA1389), anti-CXCR4

(1:1000, A00031-4), anti-PTGS2 (1:1000, A00084), and anti-TNF (1:1000, BA0131) were purchased from Boster Biological Technology Co. Ltd. (Shanghai, China). Cleaved caspase-3 (1:500, GB11532), IBA-1 (1:500, GB12105), and CD206 (1:400, GB113497) were purchased from Wuhan Servicebio Biotechnology Co. Ltd. (Wuhan, China). p-Akt1 (1:1000, 9018S), Akt1 (1:1000, 75692S), p-NF-κB (1:1000, 3033S), NF-κB (1:1000, 8242S), p-ERK1/2 (1:1000, 4370T), ERK1/2 (1:1000, 4696S), p-P38 (1:1000, 4511T), and P38 (1:1000, 8690T) antibodies were purchased from CST.

2.2 Animals and experimental protocols

Male C57BL/6 mice (18-22 g, 8-10 w) were obtained from Henan Speckles Biotechnology Co. Ltd. Housing conditions for all mice included a room temperature of 23°C, 50% humidity, and a 12-hour light-dark cycle. As shown in Figure 1A, 48 mice were randomly numbered and then randomly divided into four groups of 12 mice each: Sham group (DMSO-treated group), LPS group (lipopolysaccharide-treated group), LPS+CGA group (chlorogenic acid pretreatment + lipopolysaccharide-treated group), and CGA group (chlorogenic acid-treated group). The grouping of mice and the sample size of each group were determined based on previous studies(19, 20). The LPS+CGA group and the CGA group were injected intraperitoneally with CGA (40 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$) daily for 11 d. The dose of chlorogenic acid is based on previous investigations and our pre-experiments (21). From the 5th day, LPS (750 $\mu g{\cdot}kg^{-1}{\cdot}d^{-1}$ (22)) was given intraperitoneally for another 7 days in the LPS and LPS+CGA groups, and the daily LPS injection time was second to the CGA injection for 3 h. In addition, DMSO (5%) was injected intraperitoneally daily in the Sham and LPS groups as a solvent control. Behavioral tests were performed on days 6-10, and mice were euthanized after 11 days by administering an overdose of sodium pentobarbital. The study protocol was approved by the Experimental Animal Ethics Committee of the First Affiliated Hospital of Shihezi University School of Medicine (licence number A2018-052-01).

2.3 Neurobehavioral score

The neurobehavioral score assesses four components: auricular reflex, flip-right reflex, corneal reflex, caudal flexion and escape response (23). A score of "0" means the mouse has no reflex, "1" means the mouse's reflex time is more than 1 s, and "2" means the mouse's reflex time is 1 s. The lower the score obtained by the mice, the more severe the damage to the nervous system of the mouse brain. Neurobehavioral scores were recorded and calculated by two independent and single-blind researchers.

2.4 Open field experiment

Three hours before the experiment, all mice were placed in the room for behavioral testing to adapt to the environment. A white experimental chamber with a size of 50 cm×50 cm×40 cm was placed for the mice to move freely in it. After setting the corresponding parameters in the software, the experimenter records the date, the number of animals and other information. Remove the mice from the cage, put them into the central area of the experimental box and leave them immediately. The trajectory of the mice in the experimental box for 10 min was monitored by the animal behavior analysis software, and the total distance the mice moved, the number of times they entered the central area, the percentage of time they were in the central area, and the percentage of time the mice moved at different speeds were recorded. The experimental chamber was cleaned with alcohol, and the next mouse was tested after an interval of 5-10 min.

2.5 Morris water maze test

Place a round platform in a round basin with a diameter of 1 m, and then add tap water of approximately 25°C to the basin, with the water surface 2-3 centimeters above the platform (22). Prior to LPS injection, we trained four groups of mice twice over a four-day period. Groups of mice were trained to find a hidden platform in the water maze within 60 s. If the mouse could not find the platform within 60 s, it was guided to the platform and allowed to stay on the platform for 10 s. The locus navigation experiment was performed on the fifth day after LPS injection, and the escape latency, i.e., the time from when the experimenter let go of the mouse to 3 s after the mouse remained on the platform, was recorded. On the next day, the platform was removed from the water maze, and the mice were subjected to spatial exploration experiments. The mice swam freely in the maze for 60 s, the trajectory of the mice was monitored, and data such as the average swimming speed, the distance swum and the percentage of total time spent in the target quadrant were recorded. The data were analyzed using Tracking Master V3.0 software (Shanghai Vanbee Intelligent Technology Co., Ltd.).

2.6 Tissue preparation and H&E staining

Mice were euthanized, perfused with saline *via* the heart, and then perfused with 4% paraformaldehyde solution. The brain tissue was carefully peeled out and fixed in 4% paraformaldehyde solution overnight. The tissue was dehydrated in ethanol and then embedded in paraffin to make 4 μm thick coronal sections of the brain. The paraffin sections were dewaxed in water, stained sequentially with hematoxylin and eosin, dehydrated and sealed, and neuronal damage in the hippocampal region of the mouse brain was observed by microscopy.

2.7 Immunohistochemistry

Prepared paraffin sections were dewaxed in water for antigen repair. The sections were covered uniformly with 3% BSA and blocked at room temperature for 30 min. Cleaved- caspase-3 primary antibody was added dropwise to the sections, which were

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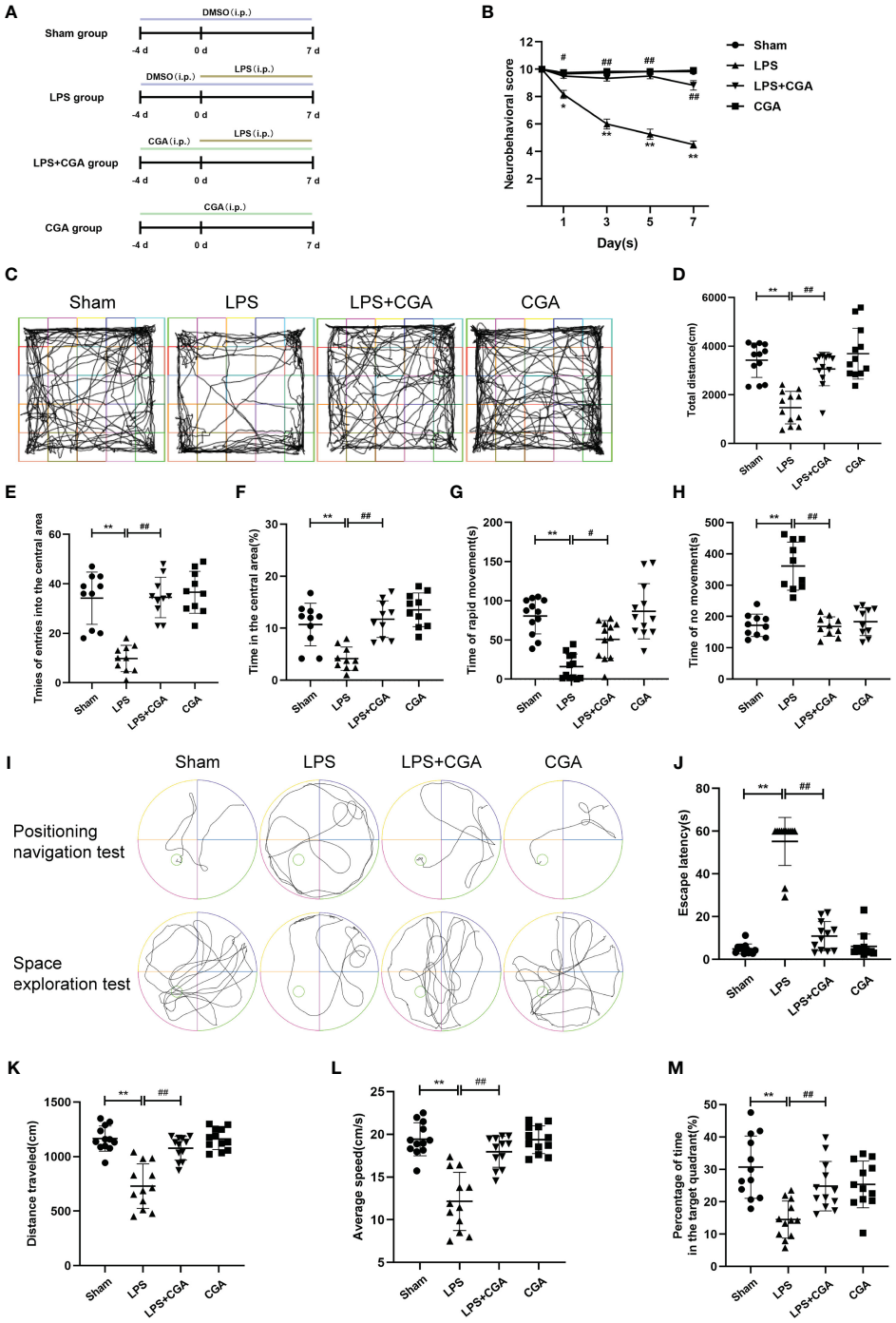

FIGURE 1
Chlorogenic acid improves cognitive dysfunction in a mouse model of LPS-induced neuroinflammation. **(A)** Timeline of *in vivo* experimental interventions. **(B)** Neurobehavioral scores at different time points. **(C-H)** Autonomous movement trajectory, total distance, number of times entering the central area, percentage of time in the central area and time of rapid movement and immobility of mice within ten minutes recorded in the open field experiment. **(I-M)** The Morris water maze test was used to record the autonomous movement trajectory map and escape latency of mice. The space exploration test recorded the autonomous movement trajectory, swimming distance, mean swimming speed and percentage of time in the target quadrant of mice. $^{**}P < 0.01$ vs. the sham group, $^{\#\#}P < 0.01$ vs. the LPS group. (n=10).

incubated flat in a wet box at 4°C overnight. Then, the corresponding secondary antibody was incubated at room temperature. DAB color development solution was added dropwise, the color development time was controlled under the microscope, and the positive color was brownish yellow. Then, hematoxylin was restained for approximately 3 min, washed, and then hematoxylin differentiation solution was used to differentiate for a few seconds, washed, hematoxylin return blue solution returned to blue, and rinsed with running water. After dehydration and sealing, the sections were placed under a light microscope to observe neuronal damage in the hippocampal region of the mouse brain.

2.8 Immunohistofluorescence

Paraffin sections were dewaxed in water and then subjected to antigen repair. The sections were blocked with 3% BSA at room temperature. A mixed primary antibody of IBA-1 and CD86 or CD206 was added dropwise to the section and incubated overnight at 4°C, with the corresponding secondary antibody incubated at room temperature. DAPI was used to restain the cell nucleus. After washing, the slices were sealed. Sections were imaged with a fluorescence microscope, and pictures were collected to observe the polarization of microglia in the hippocampal region of the mouse brain.

2.9 Cell culture

BV-2 cells were cultured in MEM containing 10% serum, 0.5% penicillin and 0.5% streptomycin. The cell culture conditions were 37°C and 5% CO_2. BV-2 cells were divided into the control group, the LPS group, and the LPS+CGA (50, 100, and 200 μM) group. After preintervention with CGA (50, 100, 200 μM) for 24 h, the cells were stimulated with LPS (1 $\mu g \cdot ml^{-1}$) for 24 h (24).

2.10 Western blotting analysis

Protein samples were electrophoresed on 10% or 12% SDS polyacrylamide gels (35-50 μg) and later transferred to PVDF membranes, which were blocked with 5% skim milk or 5% bovine serum albumin at room temperature for 2 h. Primary antibodies were added dropwise and incubated overnight at 4°C. The membranes were then incubated with the corresponding secondary antibodies at room temperature for 2 h. The PVDF membranes were incubated with enhanced chemiluminescence (ECL) reagents and exposed to a fully automated chemiluminescence image analysis system. The relative expression levels of target proteins were analyzed using ImageJ.

2.11 Flow cytometry assay

The treated BV-2 cells were transferred to centrifuge tubes, washed with PBS, counted, and blocked with 3% BSA for 30 min. The corresponding primary antibody was added and incubated at 37°C for 2 h. The corresponding fluorescent secondary antibody was added and incubated at 37°C for 2 h in the dark. After washing with PBS, the percentage of positive cells was detected by flow cytometry (BD, USA).

2.12 Wound healing assay

BV-2 cells were inoculated in 6-well plates and cultured until the cell density was approximately 90%. The cell layer was scratched with the tip of a 1 ml sterile pipette to form a straight line. After washing away floating cells and cell debris with PBS, interventions were performed in the above manner for different groupings. At 0 and 48 h after scratching, cell migration was observed by light microscopy, and images were collected. The number of migrating cells or the area of the trabeculae was counted using ImageJ.

2.13 Transwell assay

BV-2 cells (4×10^5 $cells \cdot ml^{-1}$) were inoculated in the upper chamber of Transwell culture plates. The upper chamber was filled with MEM containing 2% serum, and the lower chamber was filled with MEM containing 10% serum. After intervention as described above, the plates were washed with PBS. After gently wiping off the unperforated cells in the upper chamber with a cotton swab, the perforated cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet for 30 min. Four to five fields of view were randomly selected with a light microscope to count the number of perforated cells.

2.14 ELISA

The expression levels of TNF-α and IL-6 in mouse brain tissue homogenates were detected using ELISA kits. Within 5 min, the optical density values at 450 nm were detected by enzyme standardization, and the standard curve was plotted. The measurements were repeated three times for both standards and samples.

2.15 Network pharmacology analysis

In this study, the TCMSP database (http://tcmspw.com/tcmsp.php), SWISS target prediction database (http://swisstargetprediction.ch/), and QSAR database (Quantitative Structure Activity Relationship) were used to predict potential chlorogenic acid targets. The UniProt database was converted to UniPort ID (https://www.Unitprot.org/), and duplicate values were removed after merging the chlorogenic acid targets. The targets of neuroinflammation and cognitive dysfunction were obtained by searching "neuroinflammation" and "cognitive dysfunction", and the databases included DisGeNET (https://www.disgenet.org/), G enecards database (https://www.genecards.org/), and OMIM database (https://www.omim.org/). The targets were converted to UniProt IDs using the UniProt database. Venn diagrams of common targets for chlorogenic acid, neuroinflammation and cognitive dysfunction were created using the OmicShare tool (https://www.omicshare.com/). The common targets were transformed into corresponding gene names using the UniProt database, and then protein interaction (PPI)-related information was obtained through the String database (https://string-db.org/). Visualization was performed, and the degree values of proteins and key proteins in the PPI network were obtained by Cytoscape 3.7.0 software.

2.16 Docking analysis

In this research, molecular docking was used to evaluate the interaction between chlorogenic acid and core targets. Three-

dimensional (3D) crystal structures of the targets Akt1, TNF, MMP9, PTGS2, MAPK1, MAPK14, and RELA were obtained from the Protein Data Bank (PDB), and the two-dimensional (2D) SDF format of chlorogenic acid was downloaded from the PubChem database. The docking of chlorogenic acid to each target was performed using Autodock. The specific docking process is described as follows: First, the crystal structures were retrieved and downloaded from the PDB protein database. Secondly, the receptor macromolecule was deleted with ligands and excess water molecules as the receptor structure for molecular docking. Then the rotatable bonds of the small molecule drug are identified and set to add hydrogen atoms to the protein structure. Finally, the blind docking approach we used, the docking box was selected to dock the whole protein. In particular, the docking method we chose was semi-flexible docking with 100 docking times.

2.17 Statistical analysis

The experimental results were expressed as the mean ± standard deviation. Statistical analysis was performed using SPSS software using one-way ANOVA. P values <0.05 were considered statistically significant.

3 Results

3.1 Chlorogenic acid improved cognitive dysfunction in mice with LPS-induced neuroinflammation

We used a neurobehavioral scoring method at different periods to assess the effects of lipopolysaccharide-induced neuroinflammation on the brain. On the 3rd, 5th, and 7th days after LPS injection, the neurobehavioral scores of mice in the LPS group were lower than those in the Sham group, whereas the neurobehavioral scores of mice pretreated with chlorogenic acid were higher than those of mice in the LPS group (Figure 1B). Two days after LPS injection, we observed the voluntary locomotor ability of mice in each group by the open field experiment. Mouse activity trajectory plots showed that the locomotor complexity of mice in the LPS group was obviously reduced compared with that of mice in the sham group, and chlorogenic acid pretreatment was able to reverse the LPS-induced reduction in mouse activity complexity (Figure 1C). In addition, our data showed that the total distance moved, the number of times entering the central area, the percentage of time staying in the central area, and the duration of rapid locomotion were all reduced in the LPS group of mice compared to the Sham group, and pretreatment with chlorogenic acid improved these performances (Figures 1D-H). On the 6th and 7th days after LPS injection, we examined the learning and memory abilities of each group of mice by the Morris water maze test. The results of the positioning navigation test showed that the escape latency of mice in the LPS group was observably longer than that of mice in the sham group, while the escape latency of mice in the LPS+CGA group was shorter than that of mice in the LPS group (Figures 1I, J). The results of the space exploration test showed that LPS mice had lower swimming distance, average speed, and duration in the target quadrant than sham group mice, and chlorogenic acid-treated mice were able to attenuate the abovementioned performance caused by LPS (Figures 1K-M). These results suggest that chlorogenic acid can improve the cognitive dysfunction induced by LPS in mice.

3.2 Chlorogenic acid reduced the expression of LPS-induced inflammatory factors and attenuated neuronal damage in the mouse brain

The expression of inflammatory factors in mouse brain tissue homogenates was measured by ELISA to assess the inflammatory response in the brain. The results showed that LPS induced increased levels of TNF-α secretion in brain tissue, while chlorogenic acid attenuated this inflammatory response (Figure 2A).

The normal survival of neurons in the brain is closely related to cognitive and memory functions in mice. To demonstrate the effect of chlorogenic acid on the survival of neurons in the mouse brain, we performed HE staining on brain tissue sections of mice. The results showed that neurons in the hippocampal region of the sham group were abundant and closely arranged, with normal neuronal morphology, clear nucleus-cytoplasm demarcation, obvious nucleoli, and no obvious pathological changes. The brain tissues of mice in the LPS group showed a decrease in the number of neurons in the CA1 region of the hippocampus, a disorganized arrangement of neurons in the CA2 region, deep staining of neuronal consolidation in the CA3 region, poorly delineated nuclei and cytoplasm, and increased basophilia. The neuronal damage in the brain tissues of mice pretreated with CGA was significantly improved (Figures 2B, C). We further examined the apoptosis of neurons in the mouse brains by immunohistochemistry. The results showed that there was an obvious increase in brownish-yellow particles in the cytoplasm of neuronal cells in the cerebral cortex of LPS mice compared with sham mice, while there was a decrease in brownish-yellow particles in the cytoplasm of neuronal cells in the cerebral cortex of LPS+CGA mice (Figures 2D, E). These results suggest that chlorogenic acid can improve LPS-induced neuronal damage in the mouse brain.

3.3 Chlorogenic acid regulated M1/M2 polarization levels in mouse hippocampal microglia

Microglia, as intrinsic immune cells in the brain, are inextricably linked to central nervous system inflammation. Abnormal activation or dysregulation of the polarization ratio of microglia can cause severe inflammatory responses in the brain. We analyzed the polarization levels of microglia in the brain by immunohistofluorescence. The results showed that IBA-1$^+$ expression was increased in the brains of mice in the LPS group compared to the sham group, the expression of IBA-1$^+$ and CD86$^+$ was increased, and the expression of IBA-1$^+$ and CD206$^+$ was decreased. This indicates that LPS can induce microglial activation

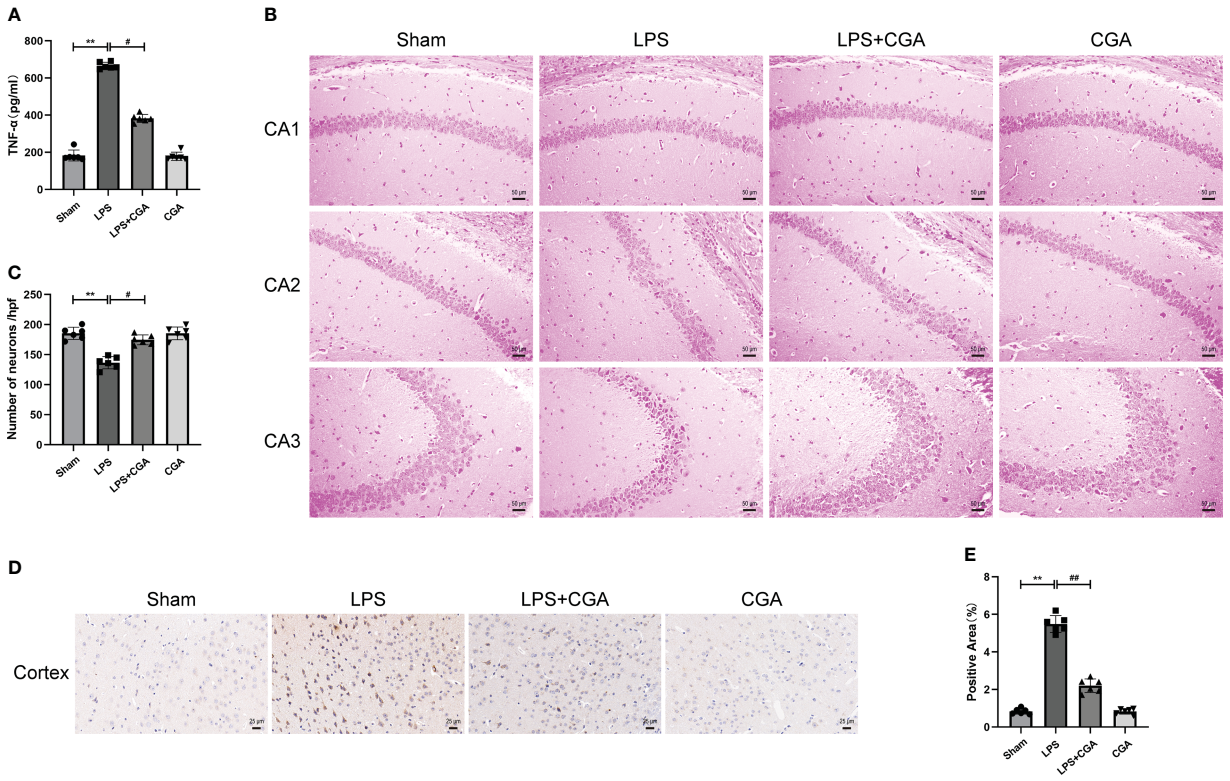

FIGURE 2
Chlorogenic acid pretreatment reduces the expression of inflammatory factors and neuronal damage in LPS-induced mouse brains. **(A)** Measurement of the expression levels of inflammatory factors in mouse brain tissue by ELISA. **(B, C)** HE staining of the hippocampus in different groups of mice. Magnification: ×200. **(D, E)** Immunohistochemical staining of cortical regions in different groups of mice. Magnification: ×400. $^{**}P < 0.01$ vs. the sham group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. the LPS group. (n=6).

and polarization toward the M1 phenotype. Compared with the LPS group, the expression of IBA-1$^+$ in the brains of mice in the LPS +CGA group was decreased, and the expression of IBA-1$^+$ and CD86$^+$ was decreased, while the expression of IBA-1$^+$ and CD206$^+$ was increased. The above results indicated that CGA pretreatment inhibited LPS-induced microglial activation, inhibited microglial polarization toward the M1 phenotype and promoted microglial polarization toward the M2 phenotype (Figures 3A–F).

Next, we analyzed the morphology of microglia by Fiji, including the number of branches, junctions, endpoints, average branch length, and maximum branch length. The results showed that compared with the sham group, the microglia in the LPS group were in an activated state, the number of branches, junctions and endpoints of the cells were reduced, and their average branch length and maximum branch length were also decreased. Pretreatment with chlorogenic acid effectively inhibited microglial activation (Figures 4A–F).

3.4 Chlorogenic acid regulated M1/M2 polarization levels in BV-2 cells

To study the effect of CGA on the polarization level of BV-2 cells, we detected the expression of M1/M2 polarization-related proteins by Western blotting. The results showed that the expression of iNOS and CD86 was elevated in the LPS group compared with the control group, i.e., LPS stimulation polarized the cells toward the M1. While the expression of iNOS and CD86 was decreased after pretreatment with different concentrations of CGA (Figures 5A–C), the expression of Arg-1, IL-10 and CD206 was increased (Figures 5A, D–F). We further detected the expression of M1/M2 polarization markers by flow cytometry. Consistent with the Western blot results, the proportion of CD86$^+$ cells was increased in the LPS group (Figures 5G, H), and the proportion of CD206$^+$ cells was decreased (Figures 5I, J). Compared with the LPS group, the percentage of CD86$^+$ cells decreased and the percentage of CD206$^+$ cells increased after pretreatment with CGA. The above results indicated that chlorogenic acid could inhibit LPS-induced M1 polarization of microglia and promote M2 polarization of microglia.

3.5 Chlorogenic acid reduced the migration level of BV-2 cells

Activated microglia are capable of producing large amounts of inflammatory mediators that cause damage to the surrounding environment. The increased migratory capacity of activated microglia leads to further amplification of the inflammatory response. To investigate the effect of CGA on the migratory capacity of BV-2 cells, we examined the lateral and longitudinal migration levels of BV-2 cells by scratch and Transwell assays. The

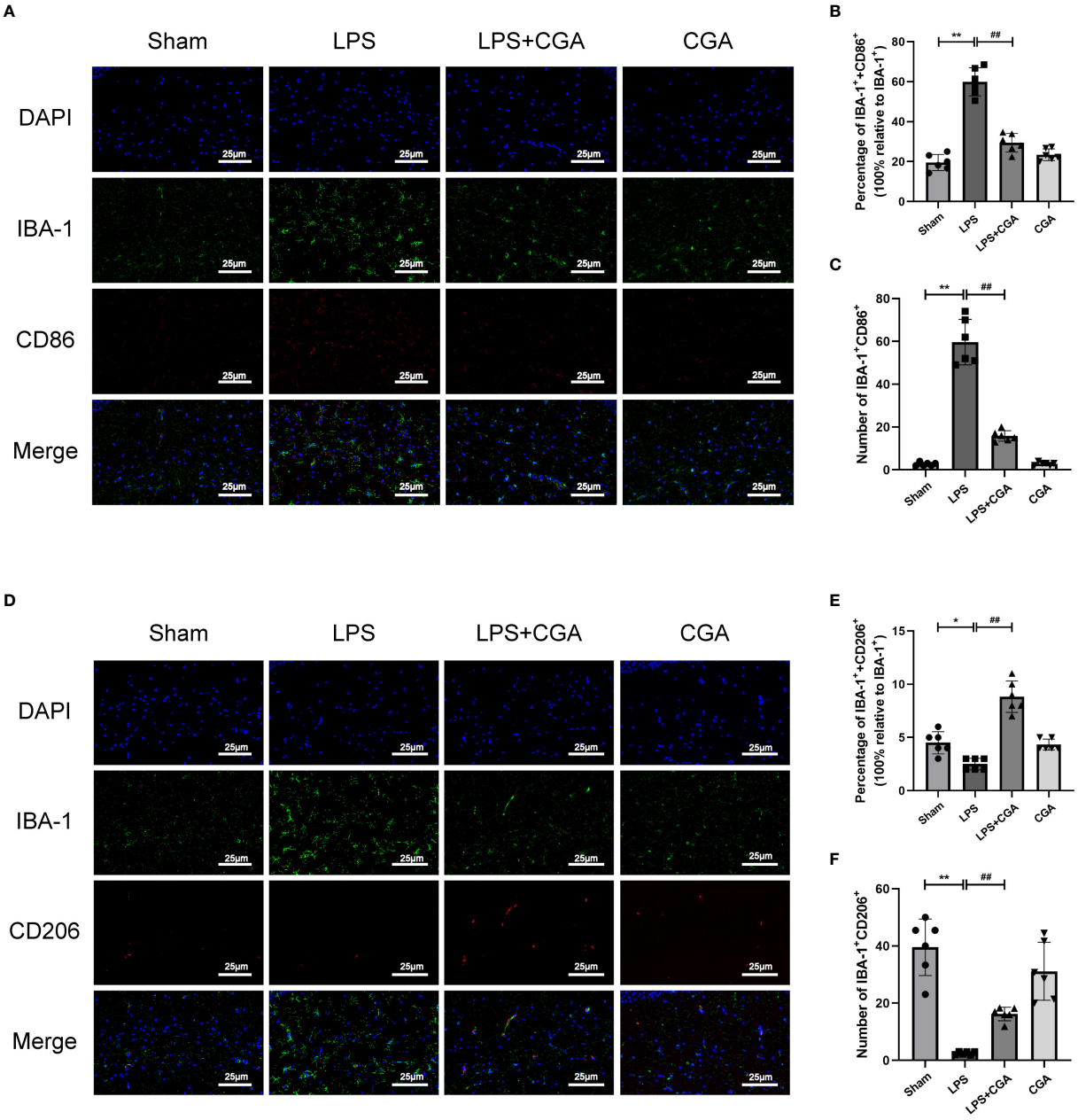

FIGURE 3
Chlorogenic acid inhibited the polarization of mouse hippocampal microglia toward M1 phenotype and promoted the polarization of microglia toward M2 phenotype. **(A-C)** Immunofluorescence staining of IBA-1/CD86 expression in microglia from different groups of mice. **(D-F)** Immunofluorescence staining of IBA-1/CD206 expression in microglia from different groups of mice.*P < 0.05, **P < 0.01 vs. the sham group, ##P < 0.01 vs. the LPS group. (n=6).

results showed that LPS stimulation upregulated the lateral and longitudinal migration levels of BV-2 cells, while CGA pretreatment effectively attenuated the migration ability of BV-2 cells (Figures 6A–D). We further examined the expression of migration-associated proteins by Western blotting. The results showed that LPS induced the upregulation of MMP9 expression, while CGA pretreatment was able to reduce the expression of MMP9 (Figures 6E, F). All these results indicated that microglial migration levels were increased in the inflammatory environment and that CGA was able to inhibit microglial migration.

3.6 Chlorogenic acid improved neuroinflammation-induced cognitive dysfunction *via* the TNF signaling pathway

To further investigate the potential mechanisms by which CGA improves neuroinflammation-induced cognitive dysfunction, we analyzed this using web-based pharmacology-related databases. A total of 650 chlorogenic acid-related targets were obtained from the TCMSP database, Swiss target prediction database, and TargetNet database. A total of 902 targets related to neuroinflammation and

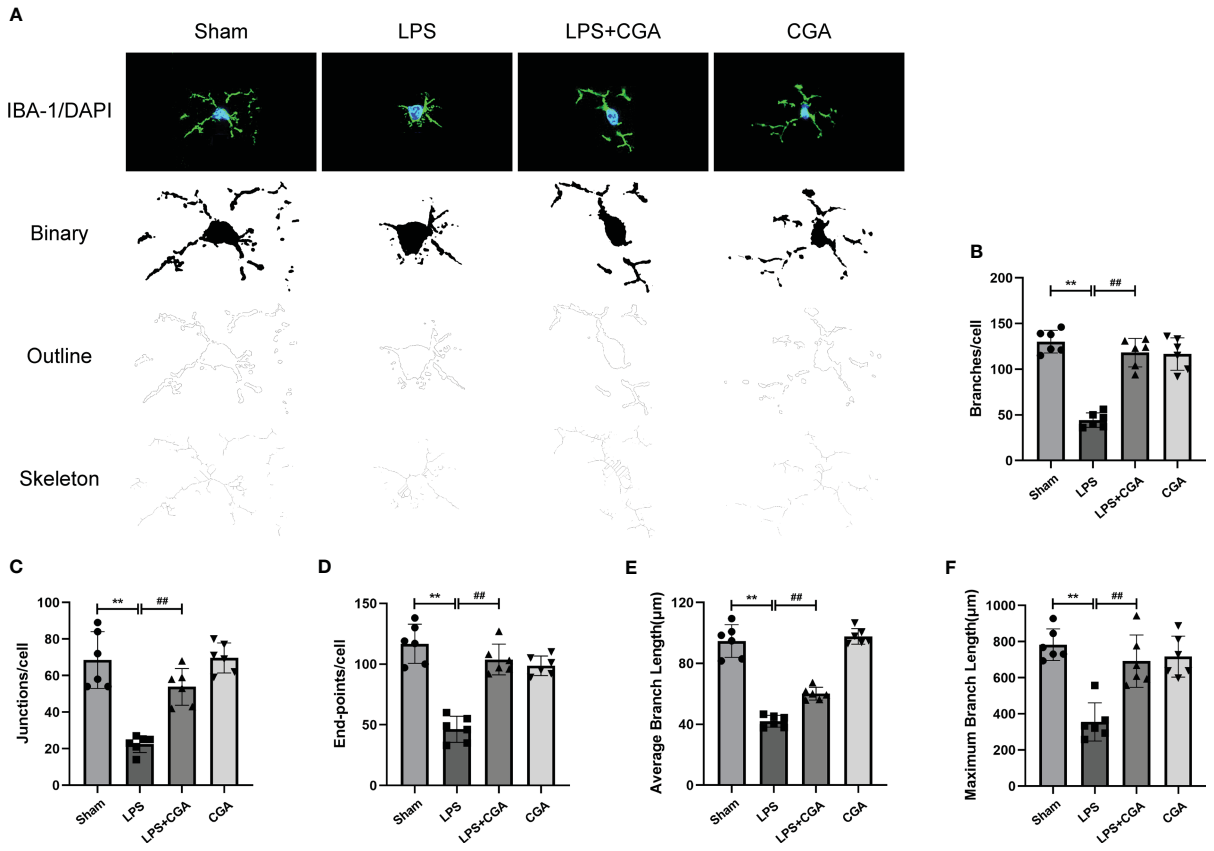

FIGURE 4
Chlorogenic acid reduces LPS-induced microglial activation in the hippocampal region of mice. **(A)** Binary, outline and skeleton images of microglia after Iba-1 labeling. **(B–F)** Statistical analysis of microglial cell branches, junctions, endpoints, average branch length, and maximum branch length. $^{**}P < 0.01$ vs. the sham group, $^{\#\#}P < 0.01$ vs. the LPS group. (n = 6).

650 targets related to cognitive dysfunction were obtained from the GeneCards and OMIM databases, and 117 common targets for chlorogenic acid, neuroinflammation and cognitive dysfunction were identified (Figure 7A). The PPI network of these 117 potential targets was constructed from the String database. The 117 targets were visualized using Cytoscape 3.8.0 according to the degree value in descending order (Figure 7B). The top 20 proteins were Akt1, TNF, CASP3, EGFR, MMP9, PTGS2, MTOR, PPARG, SIRT1, MAPK1, NOS3, APP, ICAM1, CXCR4, MAPK14, VCAM1, CXCL12, RELA, MMP2, and JAK.

We further performed GO and KEGG enrichment analyses on the top 20 targets. The results of GO enrichment analysis suggested that the top 20 targets were mainly involved in immune system regulation and intracellular signaling (Figure 7C). The results of KEGG enrichment analysis showed that the top 20 targets were enriched in the AGE-RAGE signaling pathway, TNF signaling pathway, relaxin signaling pathway, and IL-17 signaling pathways, among which the TNF signaling pathway was closely related to inflammation (Figure 7D). The above results suggest that the TNF signaling pathway may be a key pathway by which CGA improves cognitive dysfunction caused by neuroinflammation (Figure 7E). Moreover, Akt1, TNF, MMP9, PTGS2, MAPK1, MAPK14, and RELA are closely related to the development of inflammation, and they may become key targets for chlorogenic acid to exert its protective effects.

3.7 Chlorogenic acid inhibited LPS-induced activation of Akt1, TNF, MMP9, PTGS2, MAPK1, MAPK14, and RELA targets in the TNF signaling pathway

We evaluated the interaction between chlorogenic acid and Akt1, TNF, MMP9, PTGS2, MAPK1, MAPK14, and RELA by molecular docking. CGA docked with Akt1, RELA, MAPK14, MAPK1, TNFα, MMP9, and PTGS2 (Figures 8A–G). A small molecule-protein docking fraction <-1.2 indicates good binding between the two, so chlorogenic acid binds well to the above target molecules in docking and corroborates that chlorogenic acid may act through the above targets (Table 1).

Then, we further examined the effects of chlorogenic acid on Akt1, TNF, MMP9, PTGS2, MAPK1, MAPK14, and RELA targets by Western blotting. The results revealed that LPS stimulation increased the protein expression levels of p-Akt1, p-NF-κB, p-p38, p-ERK1/2, MMP9, PTGS2, and TNFα in BV-2 cells, and pretreatment with chlorogenic acid inhibited the upregulation of

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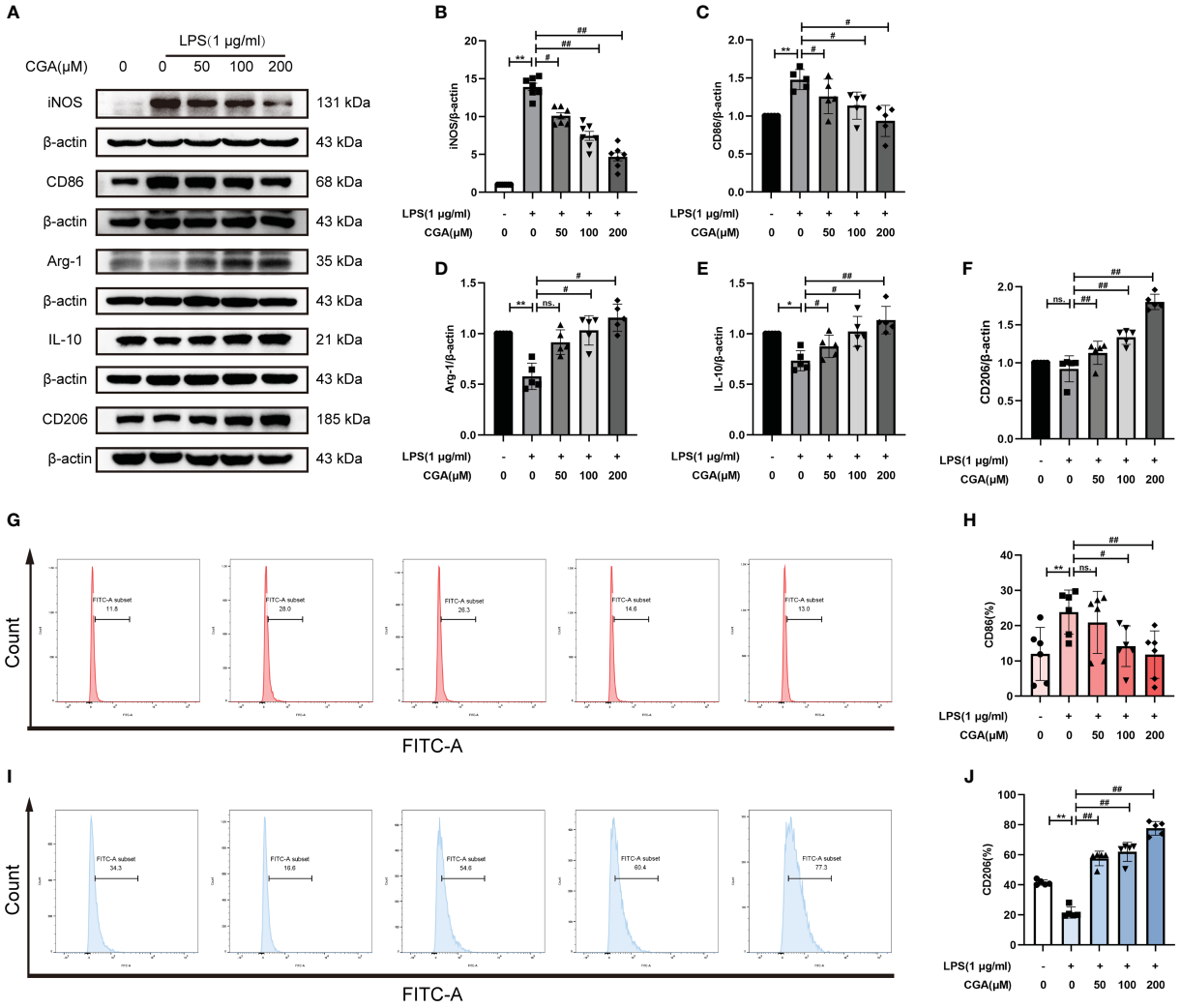

FIGURE 5
Chlorogenic acid regulates M1/M2 polarization levels in BV-2 cells. BV-2 cells were exposed to LPS (1 μg·ml^{-1}) for 24 h with or without CGA pretreatment for 24 h. **(A–F)** Detection of the protein expression levels of iNOS, CD86, Arg-1, IL-10 and CD206 by Western blotting. **(G, H)** Detection of CD86 expression levels by flow cytometry. **(I, J)** Detection of CD206 expression levels by flow cytometry. $^{**}P < 0.01$ vs. the control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. the LPS group. (n=5).

these proteins (Figures 9A–G, Figures 6E, F). The above results indicated that chlorogenic acid was able to inhibit LPS-induced activation of Akt1, TNF, MMP9, PTGS2, MAPK1, MAPK14, and RELA targets in the TNF signaling pathway.

4 Discussion

Neuroinflammation is an important feature in the pathogenesis and progression of many neurodegenerative diseases, and inhibition of neuroinflammation can be a key target for the treatment of neurodegenerative diseases (25). The establishment of neuroinflammation models in mice facilitates the screening of drugs with ameliorating effects on neuroinflammation. Chlorogenic acid, found in a variety of plants, has been shown to have anti-inflammatory effects. However, its specific role in microglial cell polarization in neuroinflammation has rarely been reported. The aim of this study was to investigate the effects and mechanisms of chlorogenic acid on mouse microglia and neurons during neuroinflammation. Previously, it has been shown that either intraperitoneal or intracerebral injection of LPS leads to the production of inflammatory factors in the brains of mice (26, 27). Our results likewise showed that intraperitoneal injection of LPS resulted in elevated expression of inflammatory factors in mouse brain tissue homogenates, whereas chlorogenic acid decreased the expression of inflammatory factors in the mouse brain, indicating that chlorogenic acid was able to inhibit LPS-induced neuroinflammation. Neuroinflammation is not only reflected in elevated expression of inflammatory factors alone but also leads to a series of changes in the brain, such as neuronal degeneration and death an microglia activation (28). Gao suggested that LPS-induced neuroinflammation leads to an increase in the number of damaged neurons in the CA1, CA2, CA3 and DG regions of the hippocampus in mice (29). Wang proposed that immunofluorescence results in

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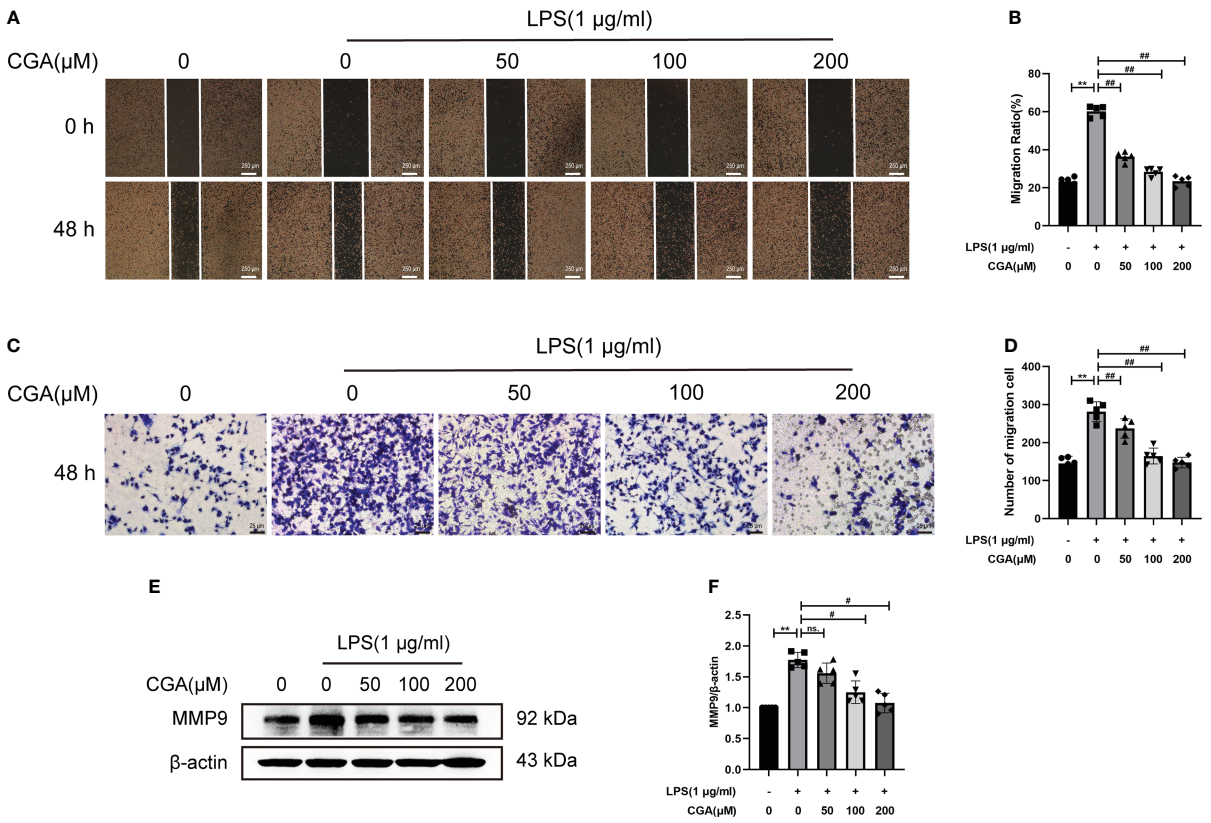

FIGURE 6
Chlorogenic acid inhibits the LPS-induced increase in the migration ability of BV-2 cells. BV-2 cells were exposed to LPS (1 μg·ml^{-1}) for 24 h with or without CGA pretreatment for 24 h. **(A, B)** Wound healing assay of BV-2 cells migration at 48 h. **(C, D)** Transwell assay of BV-2 cells migration at 48 h. **(E, F)** Detection of MMP9 by Western blotting. $^{**}P < 0.01$ vs. the control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. the LPS group. (n=5).

cortical areas 6 s after LPS intracerebral injection showed reduced NeuN expression levels, indicating a decrease in the number of cortical neurons in mice (30). Similarly, we also observed neurons in the brain. The results showed that neurons in both the hippocampal and cortical regions of mice in the LPS group showed different degrees of damage and apoptosis, while chlorogenic acid-pretreated mice were protected from neuronal damage caused by LPS.

Abnormal neuronal function and death can lead to abnormal behavior in mice. Previous studies have shown that intraperitoneal injection of LPS leads to the production of IFN-γ and TNF-α in the mouse brain, and these two inflammatory factors have been shown to play an important role in LPS-induced depression-like behavior (31). The sucrose preference test in this study showed the same results. In addition, the results of Morri's water maze in a previous study showed that LPS causes deficits in learning and memory function in animals (22, 32). Our results showed that mice subjected to LPS stimulation had reduced autonomic activity. The water maze experiment showed that the learning memory ability of mice was decreased after being subjected to LPS. All of the above behavioral changes in mice triggered by LPS were improved in the LPS+CGA group mice.

Microglia are a class of intrinsic immune cells found in the central nervous system and are involved in immune surveillance, signaling, injury response, phagocytosis of cellular debris and repair of synapses (33, 34). Since microglia are a major source of inflammatory factors, their overactivation leads to neuroinflammation, which is key to the development of many neurological diseases (35, 36). Activated microglia are generally divided into two phenotypes, M1 and M2, which exert proinflammatory and anti-inflammatory effects, respectively. Cytokines and chemokines released from M1 microglia induce the release of inflammatory factors and cytotoxic substances from leukocytes and macrophages, mediating neuroinflammation and neurotoxicity, leading to blood–brain barrier disruption and glial cell death (37); M2 microglia secrete anti-inflammatory factors such as IL-10 and IL-4 and neurotrophic factors such as transforming growth factor -β (TGF-β), insulin growth factor (IGF) and vascular endothelial growth factor (VEGF), which play a role in reducing inflammation and restoring homeostasis in the body (38). Therefore, an increasing number of studies are targeting the microglial phenotype to inhibit neuroinflammation to improve or reverse the pathological process of neurological diseases (39, 40). In this study, double immunofluorescence staining of IBA-1 with CD86/CD206 showed that chlorogenic acid inhibited LPS-induced M1 polarization and promoted M2 polarization of microglia in the hippocampal region of mice. The results of *in vitro* experiments showed that chlorogenic acid could inhibit LPS-induced M1 polarization in BV-2 cells and promote M2 polarization in microglia. The above results suggest that

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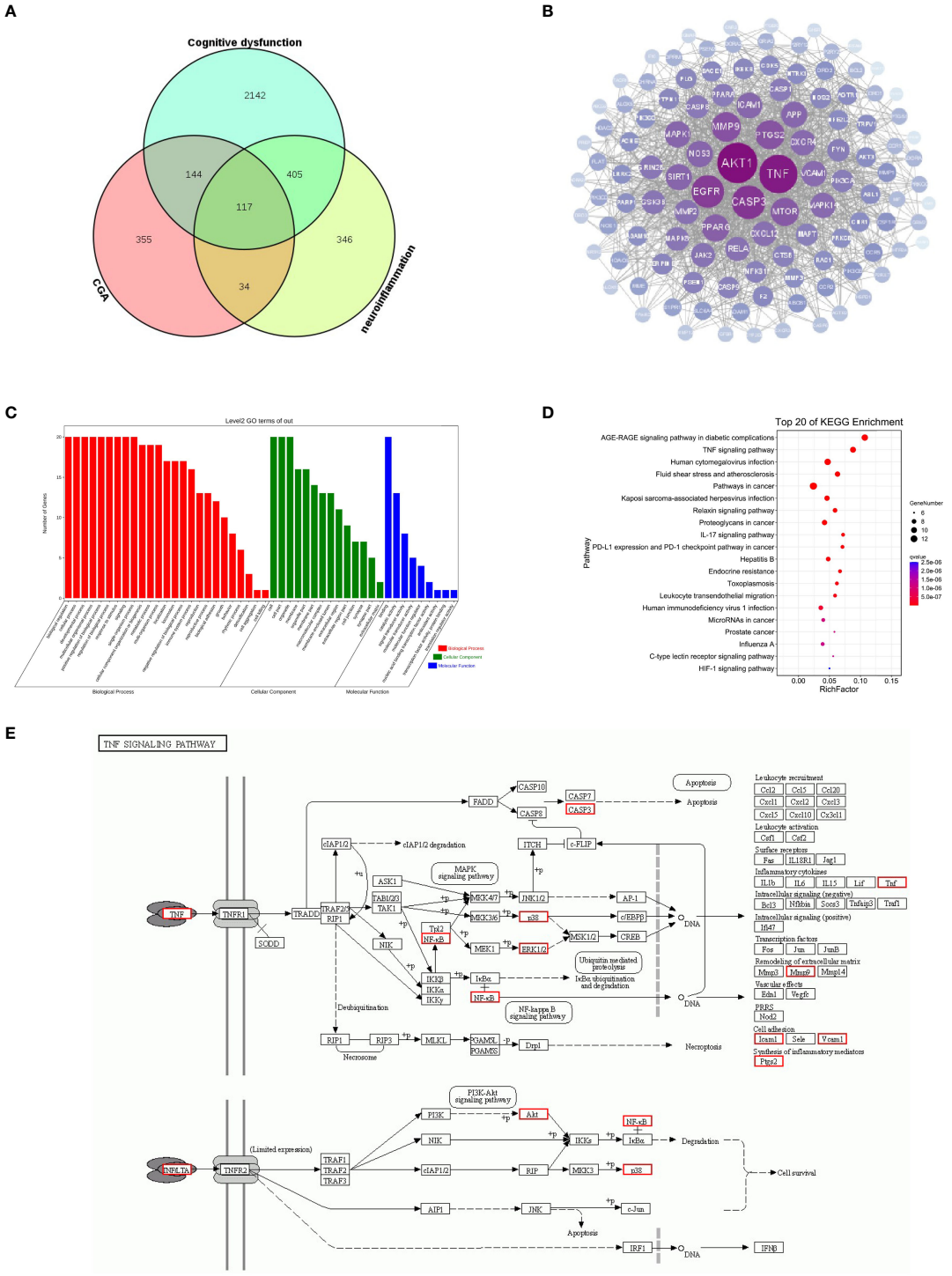

FIGURE 7
Results of network pharmacology analysis. **(A)** Cognitive dysfunction targets and neuroinflammation targets were obtained from the GeneCards database, and chlorogenic acid targets were obtained from the TCMSP database, Swiss target prediction database and QSAR database. A total of 117 targets were obtained after intersection of the Venn diagram. **(B)** PPI was constructed using 117 targets based on the degree values. **(C)** GO enrichment analysis of 20 targets. **(D)** KEGG pathway analysis of 20 targets. **(E)** Schematic diagram of TNF signaling pathway.

chlorogenic acid can ameliorate LPS-induced neuroinflammation by regulating microglial polarization. Previously, the role of chlorogenic acid in microglial polarization has not been reported, and our study provides new ideas and evidence for chlorogenic acid in the treatment of neuroinflammation.

Network pharmacology is a novel research method for identifying putative targets and pharmacological mechanisms (41). To investigate the mechanism by which chlorogenic acid improves neuroinflammation-induced cognitive dysfunction, we used network pharmacology for further analysis. A total of 117 targets were found for

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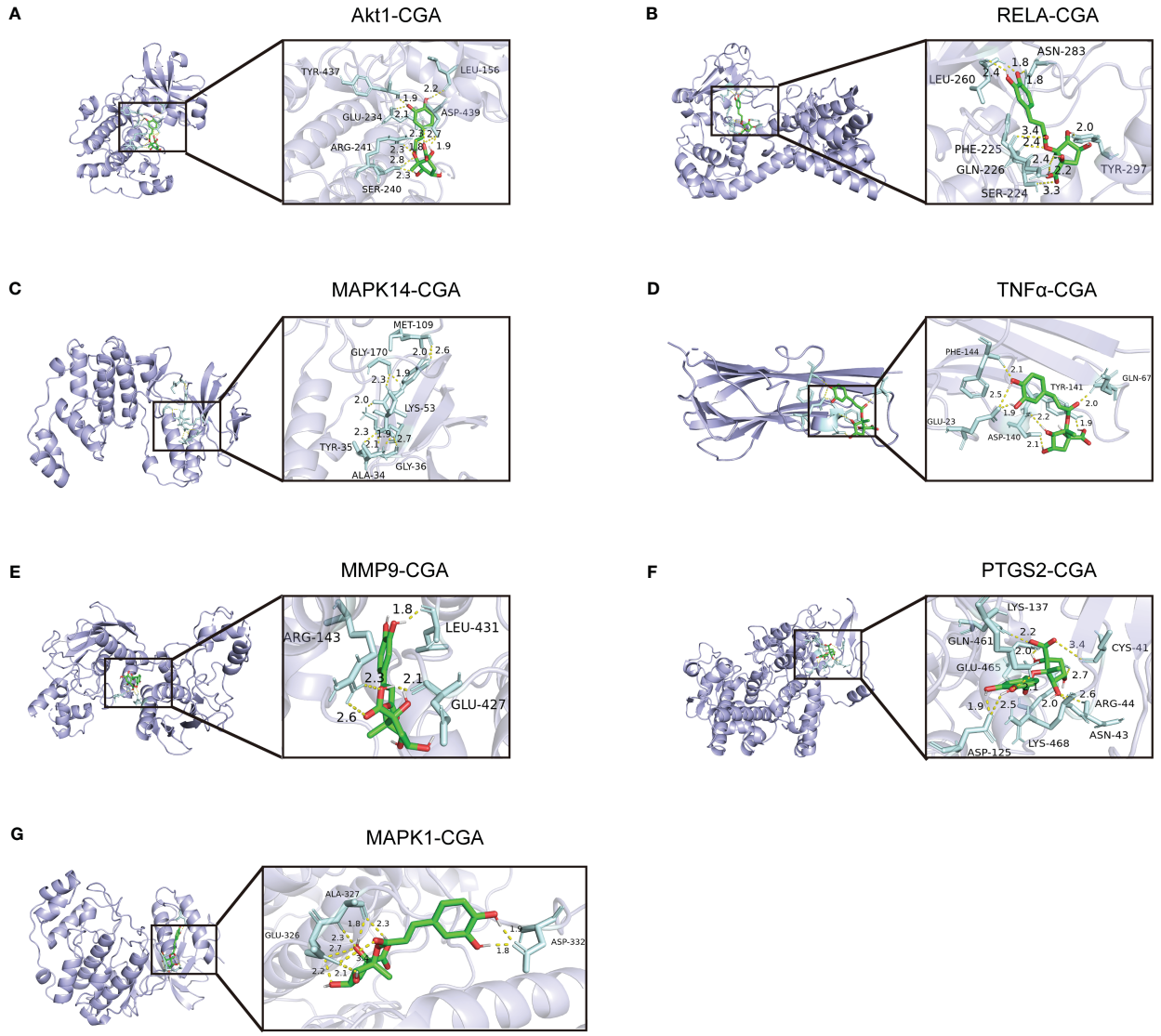

FIGURE 8
Molecular mechanisms by which chlorogenic acid improves neuroinflammation-induced cognitive dysfunction. **(A)** Three-dimensional docking of CGA and Akt1. **(B)** Three-dimensional docking of CGA and RELA. **(C)** Three-dimensional docking of CGA and MAPK14. **(D)** Three-dimensional docking of CGA and TNFα. **(E)** Three-dimensional docking of CGA and MMP9. **(F)** Three-dimensional docking of CGA and PTGS2. **(G)** Three-dimensional docking of CGA and MAPK1.

TABLE 1 Molecular docking binding of chlorogenic acid to different targets.

Target	PDB	Score (Kcal/mol)
Akt1	3MVH	-6.96
RELA	3rc0	-5.45
MAPK14	6hwt	-6.99
MAPK1	6g54	-6.55
TNFα	6x86	-5.56
MMP9	5th6	-7.33
PTGS2	5IKT	-6.61

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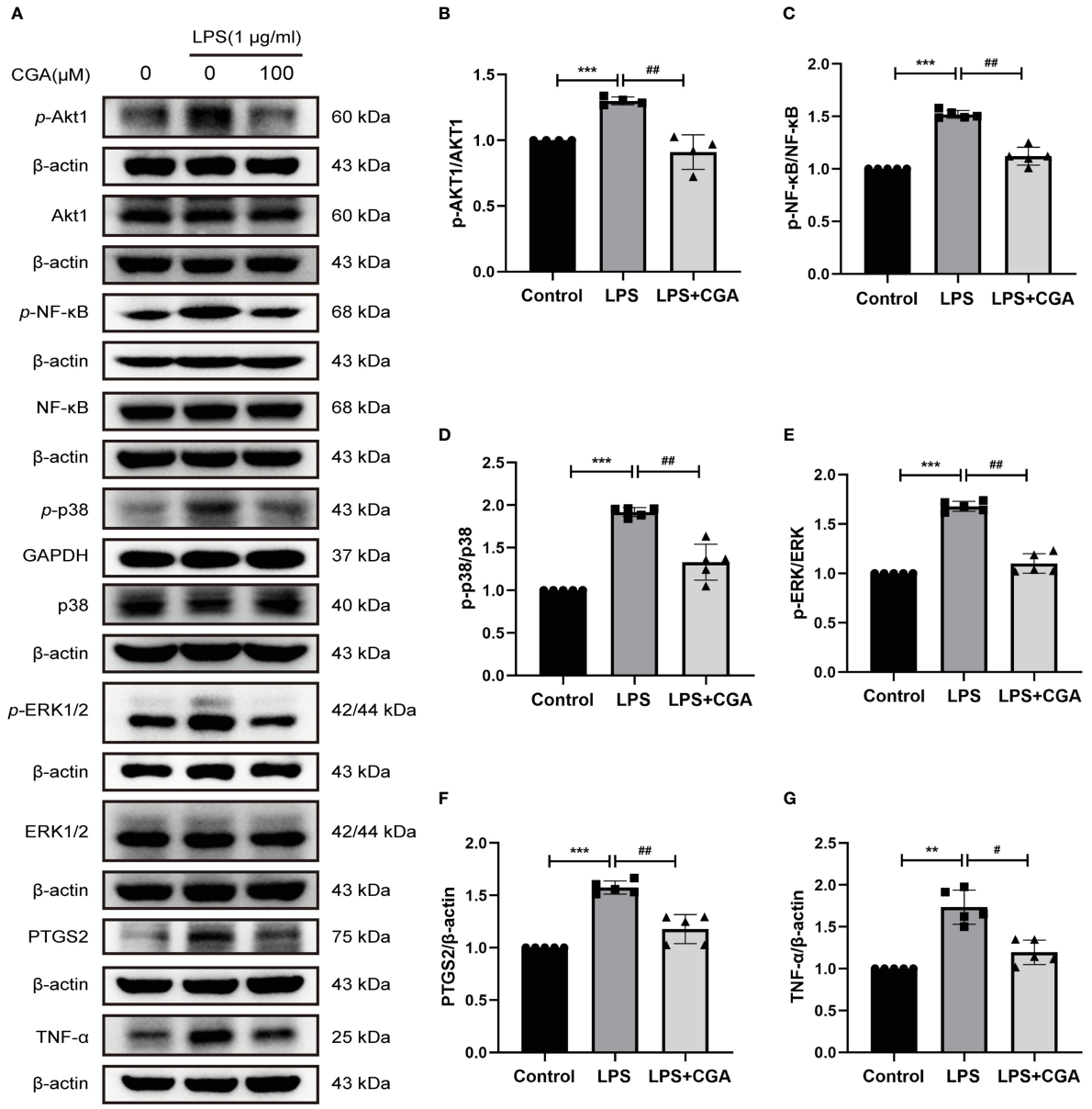

FIGURE 9
Chlorogenic acid downregulated the expression of core targets in the TNF signaling pathway. BV-2 cells were exposed to LPS (1 μg·ml^{-1}) for 24 h with or without CGA pretreatment for 24 h. **(A–G)** Western blotting analysis of the protein expression of p-Akt1, Akt1, p-NF-κB, NF-κB, p-p38, p38, p-ERK1/2, ERK1/2, PTGS2, and TNF-α. **P < 0.01, ***P < 0.001 vs. the control group, #P < 0.05, ##P < 0.01 vs. the LPS group.(n=5)

chlorogenic acid, neuroinflammation and cognitive dysfunction, and GO and KEGG analyses were performed on the top 20 targets ranked by degree value. KEGG enrichment analysis showed a high enrichment score for the TNF signaling pathway. The targets enriched in the TNF signaling pathway were Akt1, TNF, MMP9, PTGS2, MAPK1, MAPK14, and RELA. Akt1 was the first target ranked by degree value in the network pharmacology results, suggesting that Akt1 may be an important target for chlorogenic acid action in neuroinflammation. Akt is an important signaling molecule that regulates many cellular processes, such as cell growth, survival and metabolism (42). Inflammation-associated proteins are often located downstream of Akt, which plays an important role in the development of inflammation. Inactivation of the JNK/Akt/NF-κB signaling pathway inhibits microglia-mediated inflammation in mice with experimental autoimmune encephalomyelitis (43). Ethanol and LPS were able to induce liver injury and activate the Akt signaling pathway with elevated expression of three Akt isoform phosphorylations (44). Our data show that the level of Akt1 phosphorylation is increased in activated microglia and that chlorogenic acid is able to reduce p-Akt1 expression.

Previous studies have shown that microglial activation is closely associated with the MAPK pathway. MAPK belongs to a family of highly conserved serine/threonine protein kinases, consisting of extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK)/stress-activated protein kinase, and p38 MAPK (45). Among them, ERK is the signal transduction protein that transmits

mitogenic signals, and ERK1/2 is the most classical. Treatment of lipopolysaccharide-activated BV-2 cells with the ERK inhibitor SCH772984 resulted in an inhibition of NO release and a decrease in phosphorylated ERK (46). p38 MAPK is a stress-activated protein kinase. Activated p38 MAPK activates protein kinases, cytosolic proteins, cytoplasmic proteins, and transcription factors, which are involved in cell differentiation, apoptosis, senescence, inflammatory responses, and cytokine production (45, 47, 48). One study found that phosphorylated p38 MAPK was predominantly expressed in activated spinal microglia 1 d after surgery for lumbar disc herniation in rats (49). Our results indicate that chlorogenic acid suppresses neuroinflammation by inhibiting the phosphorylation of ERK1/2 and P38. In addition, microglial activation is closely related to the NF-κB pathway. NF-κB consists of five members, RElA (p65), RElB, c-REl, NF-κB1 (p50) and NF-κB2 (p52) (50). BV-2 cells activated by lipopolysaccharide *in vitro* showed increased release of inflammatory factors such as TNF-α and ROS-like, increased levels of phosphorylated NF-κB, and promoted p65 nuclear translocation (51, 52). Blocking the NF-κB pathway inhibits neurotoxin secretion, inflammatory factor release, and microglial activation, reducing the neuroinflammatory response. Our work showed that chlorogenic acid inhibits the release of the inflammatory factor TNF-α by inhibiting the phosphorylation of NF-κB.

In addition, the PTGS2 and MMP9 targets in the network pharmacology results are equally relevant to neuroinflammation. Previous research suggested that inhibition of mTOR can reduce microglial proliferation and regulate microglial activation by reducing the expression of iNOS and COX-2 in inflammatory cytokines (53). Therefore, PTGS2 may be a key therapeutic target for inflammation-mediated neurological disorders. Our results are consistent with previous reports that chlorogenic acid inhibited inflammation-mediated PTGS2 production.

MMP9 belongs to the matrix metalloprotein (MMP) family, whose main function is to degrade and remodel the extracellular matrix. Lin proposed that intracerebral injection of LPS increased MMP9 expression in the mouse brain (26). Overproduction of MMP9 leads to disruption of the blood–brain barrier and death-inducing ligand release, propagating neuroinflammatory responses through the recruitment of immune cells. Inhibition of MMP9 has been shown to protect against neuronal death and improve neurological function (54, 55). Our findings are consistent with a previous study showing that LPS promotes increased levels of microglial MMP9 expression, which increases the migration level of microglia and causes an increased inflammatory response. Moreover, wound healing and transwell assays further corroborated the change in the migration level of BV-2 cells after stimulation by LPS. In contrast, chlorogenic acid treatment inhibited the increased level of microglial migration in the inflammatory environment, inhibiting further amplification of inflammation to some extent.

In summary, chlorogenic acid exerted anti-inflammatory effects by inhibiting the activation of Akt1, NF-κB, ERK1/2, and p38 in the TNF signaling pathway and suppressing the expression of MMP9, PTGS2, and TNF-α. Our study provides new ideas for the treatment of neuroinflammation-induced cognitive dysfunction and strong evidence for the specific mechanism by which chlorogenic acid exerts its anti-inflammatory effects.

5 Conclusion

In summary, this study used network pharmacology analysis, molecular docking techniques, and *in vivo* and *in vitro* experiments to elucidate the role played by chlorogenic acid in a model of LPS-induced neuroinflammation and to explore the potential mechanisms by which chlorogenic acid acts. Our results showed that chlorogenic acid inhibited LPS-induced microglia activation and M1 phenotype polarization, promoted microglia polarization to M2 phenotype, reduced the release of inflammatory factor TNF-α, and decreased the degree of neuronal damage in hippocampal and cortical regions, thereby improving neuroinflammation as well as neuroinflammation-induced cognitive dysfunction in mice.Network pharmacology results suggest that the mechanism by which chlorogenic acid ameliorates neuroinflammation-induced cognitive dysfunction mainly involves the TNF signaling pathway. Molecular docking and *in vitro* experimental results show that the specific mechanism by which chlorogenic acid exerts its protective effect is through the inhibition of the activation of NF-κB and MAPK signaling in the TNF signaling pathway and the inhibition of the expression of inflammatory mediators TNF-α, MMP9, and PTGS2.Although the validation of the mechanism is not fully in-depth, our findings provide a partial research basis for the application of chlorogenic acid and offer new ideas for clinical prevention and treatment of neuroinflammation-induced cognitive dysfunction.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by Experimental Animal Ethics Committee of the First Affiliated Hospital, School of Medicine, Shihezi University (license number A2018-052-01).

Author contributions

SX completed the experiments, wrote and charted the paper; XS wrote and revised the paper; YK assisted with mouse modeling and behavioral assays; JS completed data analysis and article revision; LW completed the network pharmacology analysis and molecular docking; XL and KM guided the research and revised the paper. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1178188/full#supplementary-material

References

1. Armstrong R. What causes neurodegenerative disease? *Folia Neuropathologica* (2020) 582:93–112. doi: 10.5114/fn.2020.96707

2. Loffredo L, Ettorre E, Zicari AM, Inghilleri M, Nocella C, Perri L, et al. Oxidative stress and gut-derived lipopolysaccharides in neurodegenerative disease: role of NOX2. *Oxid Medicine Longevity Cellular* (2020) 2020:8630275. doi: 10.1155/2020/8630275

3. Zusso M, Lunardi V, Franceschini D, Pagetta A, Lo R, Stifani S, et al. Ciprofloxacin and levofloxacin attenuate microglia inflammatory response *via* TLR4/NF-kB pathway. *J Neuroinflammation* (2019) 161:148. doi: 10.1186/s12974-019-1538-9

4. Mrak RE, Griffin WS. Glia and their cytokines in progression of neurodegeneration. *Neurobiol Aging* (2005) 263:349–54. doi: 10.1016/j.neurobiolaging.2004.05.010

5. Lu YC, Yeh WC, Ohashi PS. LPS/TLR4 signal transduction pathway. *Cytokine* (2008) 422:145–51. doi: 10.1016/j.cyto.2008.01.006

6. Choi DY, Lee JW, Lin G, Lee YK, Lee YH, Choi IS, et al. Obovatol attenuates LPS-induced memory impairments in mice *via* inhibition of NF-κB signaling pathway. *Neurochemistry Int* (2012) 601:68–77. doi: 10.1016/j.neuint.2011.11.005

7. Zhao J, Bi W, Xiao S, Lan X, Cheng X, Zhang J, et al. Neuroinflammation induced by lipopolysaccharide causes cognitive impairment in mice. *Sci Rep* (2019) 91:5790. doi: 10.1038/s41598-019-42286-8

8. Jeong S. Molecular and cellular basis of neurodegeneration in alzheimer's disease. *Molecules Cells* (2017) 409:613–20. doi: 10.14348/molcells.2017.0096

9. Ye Y, Jin T, Zhang X, Zeng Z, Ye B, Wang J, et al. Meisoindigo protects against focal cerebral ischemia-reperfusion injury by inhibiting NLRP3 inflammasome activation and regulating Microglia/Macrophage polarization *via* TLR4/NF-κB signaling pathway. *Front Cell Neurosci* (2019) 13:553. doi: 10.3389/fncel.2019.00553

10. Kwon HS, Koh SH. Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. *Trans Neurodegeneration* (2020) 91:42. doi: 10.1186/s40035-020-00221-2

11. Tang Y, Le W. Differential roles of M1 and M2 microglia in neurodegenerative diseases. *Mol Neurobiology* (2016) 532:1181–94. doi: 10.1007/s12035-014-9070-5

12. Wang J, Xing H, Wan L, Jiang X, Wang C, Wu Y. Treatment targets for M2 microglia polarization in ischemic stroke. *Biomedecine Pharmacotherapy* (2018) 105:518–25. doi: 10.1016/j.biopha.2018.05.143

13. Naveed M, Hejazi V, Abbas M, Kamboh AA, Khan GJ, Shumzaid M, et al. Chlorogenic acid (CGA): a pharmacological review and call for further research. *BioMed Pharmacother* (2018) 97:67–74. doi: 10.1016/j.biopha.2017.10.064

14. Lin MT, Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature* (2006) 4437113:787–95. doi: 10.1038/nature05292

15. Liu Y, Wang F, Li Z, Mu Y, Yong VW, Xue M. Neuroprotective effects of chlorogenic acid in a mouse model of intracerebral hemorrhage associated with reduced extracellular matrix metalloproteinase inducer. *Biomolecules* (2022) 12 (8):1020. doi: 10.3390/biom12081020

16. Ascherio A, Chen H, Schwarzschild MA, Zhang SM, Colditz GA, Speizer FE. Caffeine, postmenopausal estrogen, and risk of parkinson's disease. *Neurology* (2003) 605:790–5. doi: 10.1212/01.wnl.0000046523.05125.87

17. Arendash GW, Schleif W, Rezai-Zadeh K, Jackson EK, Zacharia LC, Cracchiolo JR, et al. Caffeine protects alzheimer's mice against cognitive impairment and reduces brain beta-amyloid production. *J Neurosci* (2006) 1424:941–52. doi: 10.1016/j.neuroscience.2006.07.021

18. Hermawati E, Arfian N, Mustofa M, Partadiredja G. Chlorogenic acid ameliorates memory loss and hippocampal cell death after transient global ischemia. *Eur J Neurosci* (2020) 512:651–69. doi: 10.1111/ejn.14556

19. Ma ZY, Chen F, Xiao P, Zhang XM, Gao XX. Silence of MiR-9 protects depression mice through notch signaling pathway. *Eur Rev Med Pharmacol Sci* (2019) 23(11):4961–70. doi: 10.26355/eurrev_201906_18087

20. Dai J, Pan JY, Liao N. Influence of miR-155 on behaviors of depression mice through regulating wnt/β-catenin signaling pathway. *Eur Rev Med Pharmacol Sci* (2020) 24(3):1398–407. doi: 10.26355/eurrev_202002_20197

21. Lee CH, Yoon SJ, Lee SM. Chlorogenic acid attenuates high mobility group box 1 (HMGB1) and enhances host defense mechanisms in murine sepsis. *Mol Med* (2013) 181:1437–48. doi: 10.2119/molmed.2012.00279

22. Lin SP, Wei JX, Hu JS, Bu JY, Zhu LD, Li Q, et al. Artemisinin improves neurocognitive deficits associated with sepsis by activating the AMPK axis in microglia. *Acta Pharmacologica Sinica* (2021) 427:1069–79. doi: 10.1038/s41401-021-00634-3

23. Zhou R, Yang X, Li X, Qu Y, Huang Q, Sun X, et al. Recombinant CC16 inhibits NLRP3/caspase-1-induced pyroptosis through p38 MAPK and ERK signaling pathways in the brain of a neonatal rat model with sepsis. *J Neuroinflammation* (2019) 161:239. doi: 10.1186/s12974-019-1651-9

24. Li L, Wan G, Han B, Zhang Z. Echinacoside alleviated LPS-induced cell apoptosis and inflammation in rat intestine epithelial cells by inhibiting the mTOR/STAT3 pathway. *BioMed Pharmacother* (2018) 104:622–8. doi: 10.1016/j.biopha.2018.05.072

25. Wei W, Wang S, Xu C, Zhou X, Lian X, He L, et al. Gut microbiota, pathogenic proteins and neurodegenerative diseases. *Front Microbiol* (2022) 13:959856. doi: 10.3389/fmicb.2022.959856

26. Lin FL, Yen JL, Kuo YC, Kang JJ, Cheng YW, Huang WJ, et al. HADC8 inhibitor WK2-16 therapeutically targets lipopolysaccharide-induced mouse model of neuroinflammation and microglial activation. *Int J Mol Sci* (2019) 20:410. doi: 10.3390/ijms20020410

27. Rojas-Colón LA, Dash PK, Morales-Vías FA, Lebrón-Dávila M, Ferchmin PA, Redell JB, et al. 4R-cembranoid confers neuroprotection against LPS-induced hippocampal inflammation in mice. *J Neuroinflammation* (2021) 181:95. doi: 10.1186/s12974-021-02136-9

28. Zhang R, Song Y, Su X. Necroptosis and alzheimer's disease: pathogenic mechanisms and therapeutic opportunities. *J Alzheimer's Dis* (2022) 10.3233/JAD-220809. doi: 10.3233/jad-220809

29. Gao Y, Cui M, Zhong S, Feng C, Nwobodo AK, Chen B, et al. Dihydroartemisinin ameliorates LPS-induced neuroinflammation by inhibiting the PI3K/AKT pathway. *Metab Brain Disease* (2020) 354:661–72. doi: 10.1007/s11011-020-00533-2

30. Wang Y, Ge X, Yu S, Cheng Q. Achyranthes bidentata polypeptide alleviates neurotoxicity of lipopolysaccharide-activated microglia *via* PI3K/Akt dependent NOX2/ROS pathway. *Ann Trans Med* (2021) 920:1522. doi: 10.21037/atm-21-4027

31. He H, Geng T, Chen P, Wang M, Hu J, Kang L, et al. NK cells promote neutrophil recruitment in the brain during sepsis-induced neuroinflammation. *Sci Rep* (2016) 6:27711. doi: 10.1038/srep27711

32. Zhao W, Xu Z, Cao J, Fu Q, Wu Y, Zhang X, et al. Elamipretide (SS-31) improves mitochondrial dysfunction, synaptic and memory impairment induced by lipopolysaccharide in mice. *J Neuroinflammation* (2019) 161:230. doi: 10.1186/s12974-019-1627-9

33. Prinz M, Jung S, Priller J. Microglia biology: one century of evolving concepts. *Cell.* (2019) 1792:292–311. doi: 10.1016/j.cell.2019.08.053

34. Wang M, Pan W, Xu Y, Zhang J, Wan J, Jiang H. Microglia-mediated neuroinflammation: a potential target for the treatment of cardiovascular diseases. *J Inflammation Res* (2022) 15:3083–94. doi: 10.2147/jir.S350109

35. Chen H, Deng C, Meng Z, Meng S. Research progress of targeting neuro-immune inflammation in the treatment of alzheimer's disease. *Front Bioscience* (2022) 2711:312. doi: 10.31083/j.fbl2711312

36. Gopinath A, Mackie PM, Phan LT, Tansey MG, Khoshbouei H. The complex role of inflammation and gliotransmitters in parkinson's disease. *Neurobiol Disease* (2022) 176:105940. doi: 10.1016/j.nbd.2022.105940

37. Zhou Y, Wang Y, Wang J, Anne Stetler R, Yang QW. Inflammation in intracerebral hemorrhage: from mechanisms to clinical translation. *Prog Neurobiology* (2014) 115:25–44. doi: 10.1016/j.pneurobio.2013.11.003

38. Colonna M, Butovsky O. Microglia function in the central nervous system during health and neurodegeneration. *Annu Rev Immunol* (2017) 35:441–68. doi: 10.1146/annurev-immunol-051116-052358

39. Nakagawa Y, Chiba K. Diversity and plasticity of microglial cells in psychiatric and neurological disorders. *Pharmacology Ther* (2015) 154:21–35. doi: 10.1016/j.pharmthera.2015.06.010

40. Bernier LP, York EM, MacVicar BA. Immunometabolism in the brain: how metabolism shapes microglial function. *Trends Neurosciences* (2020) 4311:854–69. doi: 10.1016/j.tins.2020.08.008

41. Wei S, Niu M, Wang J, Wang J, Su H, Luo S, et al. A network pharmacology approach to discover active compounds and action mechanisms of San-cao granule for treatment of liver fibrosis. *Drug Design Dev Ther* (2016) 10:733–43. doi: 10.2147/dddt.S96964

42. O'Neill BT, Abel ED. Akt1 in the cardiovascular system: friend or foe? *J Clin Invest* (2005) 1158:2059–64. doi: 10.1172/jci25900

43. Yang L, Han X, Xing F, Wu H, Shi H, Huang F, et al. Total flavonoids of astragalus attenuates experimental autoimmune encephalomyelitis by suppressing the activation and inflammatory responses of microglia *via* JNK/AKT/NFκB signaling pathway. *Phytomedicine* (2021) 80:153385. doi: 10.1016/j.phymed.2020.153385

44. Reyes-Gordillo K, Shah R, Arellanes-Robledo J, Cheng Y, Ibrahim J, Tuma PL. Akt1 and Akt2 isoforms play distinct roles in regulating the development of inflammation and fibrosis associated with alcoholic liver disease. *Cells* (2019) 8:1337. doi: 10.3390/cells8111337

45. Morrison DK. MAP kinase pathways. *Cold Spring Harbor Perspect Biol* (2012) 4 (11):a011254. doi: 10.1101/cshperspect.a011254

46. Liu M, Jiang L, Wen M, Ke Y, Tong X, Huang W, et al. Microglia depletion exacerbates acute seizures and hippocampal neuronal degeneration in mouse models of epilepsy. *Am J Physiology-Cell Physiol* (2020) 3193:C605–10. doi: 10.1152/ajpcell.00205.2020

47. Segalés J, Perdiguero E, Muñoz-Cánoves P. Regulation of muscle stem cell functions: a focus on the p38 MAPK signaling pathway. *Front Cell Biol Developmental* (2016) 4:91. doi: 10.3389/fcell.2016.00091

48. Akaishi T, Yamamoto S, Abe K. The synthetic curcumin derivative CNB-001 attenuates thrombin-stimulated microglial inflammation by inhibiting the ERK and p38 MAPK pathways. *Biol Bull Pharmaceutical* (2020) 431:138–44. doi: 10.1248/bpb.b19-00699

49. Wang YM, Gao FJ, Lin SQ, Yi ZX, Zhang JM, Wu HX, et al. Activation of p38MAPK in spinal microglia contributes to autologous nucleus pulposus-induced mechanical hyperalgesia in a modified rat model of lumbar disk herniation. *Brain Res* (2020) 1742:146881. doi: 10.1016/j.brainres.2020.146881

50. Gilmore TD, Herscovitch M. Inhibitors of NF-kappaB signaling: 785 and counting. *Oncogene* (2006) 2551:6887–99. doi: 10.1038/sj.onc.1209982

51. Lim HS, Kim YJ, Kim BY, Park G, Jeong SJ. The anti-neuroinflammatory activity of tectorigenin pretreatment *via* downregulated NF-κB and ERK/JNK pathways in BV-2 microglial and microglia inactivation in mice with lipopolysaccharide. *Front Pharmacol* (2018) 9:462. doi: 10.3389/fphar.2018.00462

52. Li Y, Liu L, Sun P, Zhang Y, Wu T, Sun H, et al. Nitzschia LaevisFucoxanthinol from the diatom ameliorates neuroinflammatory responses in lipopolysaccharide-stimulated BV-2 microglia. *Mar Drugs* (2020) 18:116. doi: 10.3390/md18020116

53. Dello Russo C, Lisi L, Tringali G, Navarra P. Involvement of mTOR kinase in cytokine-dependent microglial activation and cell proliferation. *Biochem Pharmacol* (2009) 789:1242–51. doi: 10.1016/j.bcp.2009.06.097

54. Wang X, Jung J, Asahi M, Chwang W, Russo L, Moskowitz MA, et al. Effects of matrix metalloproteinase-9 gene knock-out on morphological and motor outcomes after traumatic brain injury. *J Neurosci* (2000) 2018:7037–42. doi: 10.1523/jneurosci.20-18-07037.2000

55. Rosenberg GA. Matrix metalloproteinases in neuroinflammation. *Glia* (2002) 393:279–91. doi: 10.1002/glia.10108

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Lipopolysaccharide-induced depression-like model in mice: meta-analysis and systematic evaluation

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Depression is a complex and biologically heterogeneous disorder. Recent studies have shown that central nervous system (CNS) inflammation plays a key role in the development of depression. Lipopolysaccharide (LPS)-induced depression-like model in mice is commonly used to studying the mechanisms of inflammation-associated depression and the therapeutic effects of drugs. Numerous LPS-induced depression-like models in mice exist and differ widely in animal characteristics and methodological parameters. Here, we systematically reviewed studies on PubMed from January 2017 to July 2022 and performed cardinal of 170 studies and meta-analyses of 61 studies to support finding suitable animal models for future experimental studies on inflammation-associated depression. Mouse strains, LPS administration, and behavioral outcomes of these models have been assessed. In the meta-analysis, forced swimming test (FST) was used to evaluate the effect size of different mouse strains and LPS doses. The results revealed large effect sizes in ICR and Swiss mice, but less heterogeneity in C57BL/6 mice. For LPS intraperitoneal dose, the difference did not affect behavioral outcomes in C57BL/6 mice. However, in ICR mice, the most significant effect on behavioral outcomes was observed after the injection of 0.5 mg/kg LPS. Our results suggests that mice strains and LPS administration play a key role in the evaluation of behavioral outcomes in such models.

KEYWORDS

mouse model, lipopolysaccharide, systematic review, meta analysis, depression

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Highlights

- Neuroinflammation has been strongly implicated in the pathogenesis of depression.
- Lipopolysaccharide (LPS) administration induced depression-like model in mice is commonly used to studying the mechanisms of inflammation-associated depression and the therapeutic effects of drugs.
- We systematically reviewed studies and performed cardinal of 170 studies and meta-analyses of 64 studies to support finding suitable animal models for future experimental studies on inflammation-associated depression.
- Mouse strains, LPS administration, and behavioral outcomes of these models have been assessed in meta-analysis.

1 Introduction

Depression is a global disease with a high incidence and suicide rate. The World Health Organization indicates that ~300 million people worldwide suffer from varying degrees of depression, affecting about 20% of the population and thereby creating a heavy social burden (1). The Global Burden of Disease, Injury, and Risk Factors Study showed that depression caused 34.1 million years of living with disability (YLD), ranking fifth among the causes of YLD (2017) (2). Mental health problems are more prominent in COVID-19 pandemic. Depressive symptoms and clinically significant depression are commonly reported among individuals as part of the post-COVID-19 syndrome (3). Therefore, research advances and application of new discoveries or techniques are imminent. The understanding of the pathophysiological mechanisms of depression has evolved over the last decades. Due to the complexity and heterogeneity of depression, it is challenging to determine its exact biological mechanisms, which are largely determined by genetic and environmental factors. So far, many hypotheses have been proposed for the pathogenesis of depression, including heritability, neurotransmitter systems, brain-derived neurotrophic factor (BDNF), and overactivity of the hypothalamic pituitary-adrenal axis (4), etc. Whereas existing antidepressants are mainly based on the monoamine hypothesis, a proportion of depressed patients (20% to 30%) do not respond to pharmacological treatment.

Abbreviations: LPS, Lipopolysaccharide; CNS, central nervous system; FST, forced swimming test; BDNF, brain-derived neurotrophic factor; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; SMDs, standardized mean differences; IP, intraperitoneal injection; ICV, intracerebroventricular injection; IG, intragastric; TST, suspension tail test; OFT, open field test; SPT, sucrose preference test; PAMP, pathogen-associated molecular patterns; IL-8, interleukin-8; MWM, morris water maze; EPM, elevated plus maze; NORT, novel object recognition test; NSFT, novelty suppressed-feeding test; SIT, social interaction test; ST, splash test.

Since 1991, Smith (5) first proposed the neuroinflammation hypothesis of depression, increasingly more studies provide strong evidence to suggest that neuroinflammation is critical in clinical depression. Recent reviews have investigated that patients with depression are often accompanied by elevated levels of inflammatory markers, and persistent inflammatory activation can make susceptible people suffer from depression (6, 7). Grigoleit has reported that injection of the bacterial LPS in humans can cause an increase in plasmal pro-inflammatory cytokines and dose-related decreased mood (8), which suggests that inflammation is a causal factor in the subgroup of depression and not just an epiphenomenon (9). Meanwhile, the finding that the severity of depressive symptoms after COVID-19 syndrome has been shown to be proportional to the systemic inflammation measured at baseline during acute infection provides support for this hypothesis (10). Pharmacological studies have also confirmed the interconnection between depression and inflammation. Several antidepressants have been shown to reduce inflammation in both preclinical (11) and clinical (12, 13) models of neuropsychiatric disorders. Antidepressants such as selective serotonin reuptake inhibitors fluoxetine (14) and paroxetine, tricyclic antidepressants amitriptyline and clomipramine, and monoamine oxidase inhibitors tranylcypromine can prevent LPS-induced microglial changes and the production of inflammatory markers interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) (15). These studies suggest that antidepressants may modulate neuroinflammation by modulating the microglial phenotype, which may be one of the mechanisms by which antidepressants work.

Based on the etiology of depression, a variety of unavoidable or uncontrollable stress exposure have been explored on animals to exhibit phenotypes that are similar to the symptoms of depressed patients (16). During the last 50 years, depression-like animal models have improved our understanding of the disease and have played a key role in the development of antidepressants (17). However, these models still have limitations in understanding the inflammatory mechanisms of depression and related antidepressant drug studies. *In vivo*, LPS acts as an inflammatory inducer that activates monocytes, macrophages, endothelial cells, and epithelial cells, which in turn activates cellular signaling systems leading to increased levels of various cytokines and inflammatory mediators (18). These peripheral inflammatory signals reach the central nervous system (CNS) through endothelial cells or second messengers *via* the blood–brain barrier, triggering neuroinflammation (19). Hence, peripheral or central injection of LPS can activate microglia and trigger a series of inflammatory responses to induce depression (15). In addition, the disruption of a subset of neurons that express parvalbumin interneuron mediates systemic inflammation–induced depression-like behavior and working memory impairment by LPS challenge (20). Animal models of inflammation-related depression are most commonly induced by administration of LPS. In the past 5 years, the number of these animal models has increased considerably. Among these mouse models, variations in factors such as animal strain, administration of LPS determine the face, construct and predictive validity offered in each model. However, this differs widely between models. It is critical to assess the validity of these

models in terms of whether the animal strains and methodologies studied in these models are constructively valid for depression-like behavior. Therefore, we reviewed the experimental literature of the past five years to provide a comprehensive report of the different protocols and parameters used in these models.

2 Materials and methods

2.1 Search methodology and inclusion criteria

The present meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (21). Two authors (Yingming Li and Zilei Tang) searched the PubMed database for articles published between January 1, 2017, and July 31, 2022, the following search terms were used "Depression [Mesh] OR depressive OR depressant AND lipopolysaccharide* AND mice* OR " mouse"[Mesh]. In order to count the experimental parameters of LPS-induced depression in mice, the authors developed the following screening conditions:

(1) Articles are published as original articles.
(2) Experimental protocol of the LPS-induced depression model in mice is described.
(3) Mice without other co-morbidities, such as depression + obesity, depression + diabetes.
(4) Mice did not experience other stresses or injuries, such as early maternal separation, cerebral hemorrhage, etc.
(5) Mice used were not genetically edited.
(6) No LPS combined with other methods was used to induce depression model in mice, such as LPS + chronic unpredictable mild stress.

For the articles that met the above six criteria, the authors extracted the mouse strain, LPS dose, routes and times of administration, and behavioral test data for Chi-square test.

Based on serotype the consensus and the previous step of screening criteria, the authors developed the following inclusion criteria for meta-analysis:

(1) Forced swimming test (FST) was used to assess depressive behavior in mice.
(2) Experimental protocol using intraperitoneal injection of LPS.
(3) Experimental protocols used 0.5mg/kg or 0.83mg/kg or 1mg/kg as the induction dose of LPS.

2.2 Extraction of study data

For studies that met the inclusion criteria, the two authors (Run Yin and Yue Ma) extracted the following data in a table to facilitate subsequent frequency statistics and meta-analysis: (1) name of the first author and year of publication; (2) strain of mice; (3) dose and times of LPS administration; (4) behavioral test method selected; and (5) mean, standard deviation, and sample size (n) of the duration of immobilization in FST for mice in each model and control group. Data were extracted directly from the graphs or figures by using a digital scale. Any disagreements in data extraction were resolved by discussion with a third reviewer (kailing Zhang) until a consensus was reached. The meta-analysis was performed after collecting at least three studies from each group and, when available, providing the mean immobility time, the standard deviation, and sample size (22).

2.3 Chi-square test

The data obtained from the counting process is known as the count data. To test either the counts are differed significantly or not, the Chi-square test is applied (23). Statistical analyses were performed using IBM SPSS Statistics. Data (e.g., mouse strain, routes of LPS administration, times and dose of LPS administration, and LPS serotype) were summarized using frequencies and percentages and arranged in descending order of the number of times they were used. To identify significant of the experimental parameters chosen by the investigators between variables, Chi-squared analysis was performed (Separately tested for the presence of selection differences between two adjacent parameters). When an expected count less than 5, the Fisher's Exact Test was used instead of the Chi-squared analysis. At a 95% confidence interval, values of $P < 0.05$ were deemed as statistically significant.

2.4 Assessment of risk of bias in included studies and publication bias

The risk of bias was assessed for each of the included studies by two authors independently using SYRCLE's risk of bias tool. The answer to the assessment questions was either "yes" (indicating low risk of bias) or "no" (indicating high risk of bias). For unclear items, an "unclear" tag was assigned: random sequence generation (selection bias), baseline characteristics (selection bias), allocation concealment (selection bias), random housing (performance bias), blinding of participants (performance bias), random outcome assessment (detection bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias) and other bias. The publication bias was assessed *via* Begg's test by Stata software (Version 12.0). Duval and Tweedie'trim and fill' was used to adjust the analysis for the effects of publication bias.

2.5 Data synthesis

To assess the effect of LPS inducing depression-like behavior in mice, we pooled the immobility time of FST in mice from all the included studies. Forest plots generated from the data were graphically analyzed and visualized using RevMan 5.3 software. A random-effect model was used for the analysis and the standard mean difference (SMD) was considered. To evaluate the effect of

treatment on each parameter, 95% confidence interval (CI) was used and significance set at $P < 0.10$. SMD is a measure of the effect size, and the effect size reflects the degree of difference between the LPS group and the control group. Heterogeneity values were also calculated to determinate if included studies were suitable for meta-analysis. I^2 has been used to quantify heterogeneity and $I^2 > 50$ was considered substantial and significant if $P < 0.10$. Sensitivity analysis was also performed to assess heterogeneity of the study results by excluding each study in turn, for each of the parameters considered.

3 Results

3.1 Literature search and study characteristics

A total of 469 studies published from 2017 to 2022 were retrieved from the PubMed database and 170 studies were left after excluding those that did not meet the inclusion criteria. The literature was based on mouse strain, behavioral tests, LPS (dosage, routes and times of administration) statistics, and meta-analysis of FST in depressive-like mice. The flowchart of screening and choosing eligible articles are shown in Figure 1.

3.2 Counts results for mice strains, LPS administration and behavior test

3.2.1 Mice strains

Mouse strains such as C57BL/6, ICR, Swiss, and Balb/c have been widely used in preclinical studies of depression. Among the 170 studies, there are 2 studies did not specify the mouse strain. Statistical results (Table 1) showed that C57BL/6 mice were the most frequently used strain in the LPS-induced depression model (frequency 86, accounting for 51.19%; vs. ICR, $P = 0.000$), followed by ICR mice (frequency 40, accounting for 23.81%; vs. Swiss, $P = 0.011$) and Swiss mice (frequency 22, accounting for 13.10%; vs. Balb/c, $P = 0.070$), Balb/c mice, CD-1, Kunming, and Wistar albino mice were the last in order; we did not perform statistical analyses because few studies have used these strains. Therefore, we subsequently selected studies that used C57BL/6 mice, ICR mice, and Swiss mice for further meta-analysis.

3.2.2 Routes of LPS administration

The onset of depression may be induced by different molecular pathways due to the route of administration. The statistical results showed the following three ways of LPS administration: intraperitoneal injection (IP), intracerebroventricular injection (ICV), and intragastric (IG), among which IP was the most commonly used administration method (frequently 164, accounting for 96.47%, vs. ICV, $P = 0.000$) (as shown in Table 2).

3.2.3 Times of LPS administration

The statistical results showed that the administration times of LPS-induced depressive-like behavior in mice included single administration and multiple injections of LPS to induce depression. A total of 2 administration regimens were used in 3 studies. Among them, single induction of depression in mice was the most common experimental method (frequency 139, accounting for 80.35%, vs. repeatedly, $P = 0.000$) (As shown in Table 3).

3.2.4 LPS dose and serotype

Different doses of LPS may have an effect on the degree of depression in mice. Based on the statistical results of the LPS administration route and times, we divided the dose of LPS into an IP/single injection, IP/multiple injections/single dose, IP/multiple injections/multi-dose, and ICV/single injection to determine the frequency statistics.

The results of IP/single injection showed that 0.83 mg/kg was the most frequently used dose (frequency 60, accounting for 43.48%, vs. 0.5 mg/kg, $P = 0.000$), followed by 0.5 mg/kg (frequency 23, accounting for 16.67%) and 1 mg/kg (frequency 18, accounting for 13.04%), and no significant difference was found in the dose selection between the two ($P = 0.397$), details of other doses are provided in Table 4.

The results of the IP/multiple injections/single dose (Table 5) was similar to those of a single administration. The three most frequently used doses were 0.5 mg/kg (frequency 8, accounting for 29.63%, vs. 0.83 mg/kg, $P = 0.535$), 0.83 mg/kg (frequency 6, accounting for 22.22%, vs. 1 mg/kg, $P = 1.000$), and 1 mg/kg (frequency 6, accounting for 22.22%). No significant difference was found among these three doses. For the experimental design of multiple injections in a single dose, the most used protocol was "1 mg/kg, once a day for 5 days."

A total of four methods were used for LPS multi-dose modeling (Table 6), and the most commonly used methods were "0.052, 0.104, 0.208, and 0.415, 0.83 mg/kg; one dose per day for 5 days." Finally, we obtained 5 studies that used the intracerebral injection of LPS. The location and volume of injections are described in detail in Table 7.

The doses to be used should depend on the LPS serotype as different serotypes have different endotoxin activity. A total of 6 serotypes of LPS were used in the included studies (Table 8), and 68 studies did not specify the LPS serotype. Among them, 055: B5 was the most frequently used LPS serotype (frequency 35, accounting for 34.31%, vs. 0127: B8, P = 0.766), followed by 0127: B8 (frequency 33, accounting for 32.35%, vs. 0111: B4, $P = 0.444$) and 0111: B4(frequency 28, accounting for 27.45%), which indicated that there was no statistically significant difference in the selection of the LPS model among these three types. The most frequently used dosage of 0127: B8, 0111: B4, 026: B6 was 0.83mg/kg, while 055: B5 was 0.5mg/kg.

3.2.5 Behavior test

Behavioral test is acknowledged and intuitive method to test whether the animal model of depression was successfully established. A total of 18 behavioral methods were used in our results, among which the most commonly used methods were FST (frequency 140), suspension tail test (TST) (frequency 115), open

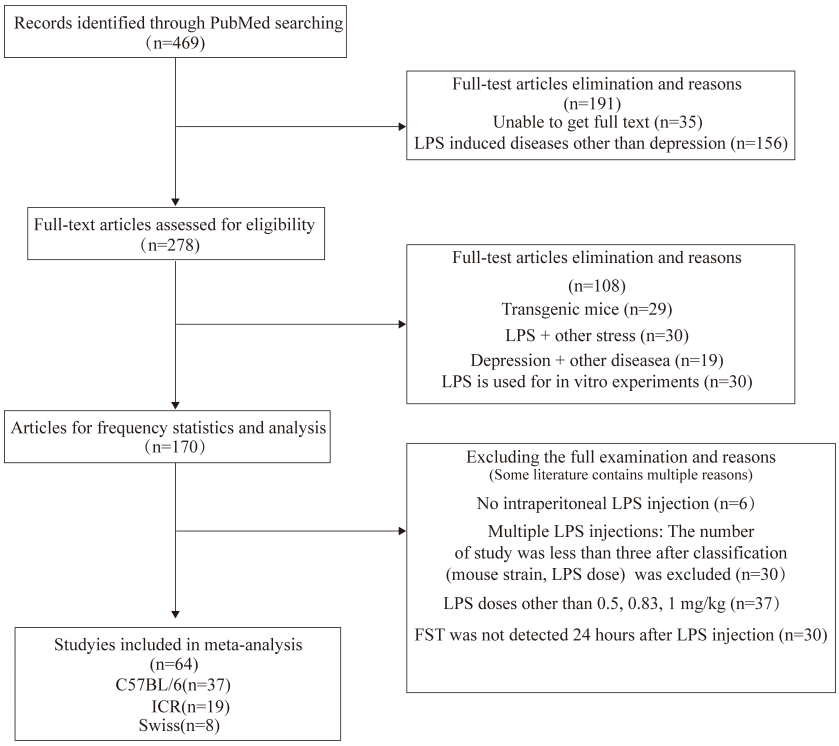

FIGURE 1
Flowchart of screening and choosing eligible articles.

field test (OFT) (frequency 95), and sucrose preference test (SPT) (frequency 78), the remaining items are presented in Table 9.

3.3 Meta-analysis

However, the highest frequency of use is not considered to be the optimal choice of experimental parameters. A meta-analysis was performed to assess the effects of different LPS doses on depressive behavior in different mouse strains using FST. Based on the frequency results shown in 3.2, we used C57BL/6 mice, ICR mice, and Swiss mice as observational subjects to determine the immovability time of FST after IP injection of LPS (0.5 mg/kg, 0.83 mg/kg, and 1 mg/kg). The results indicated that the mice showed a state of behavioral despair after LPS injection. This was evidenced by an increased FST immobility time in the LPS group compared with the control group.

3.3.1 Risk of bias in included studies and publication bias

The risk of bias for each included study is summarized in Figure 2. Of the 64 included studies, 32 described the methods used to generate the allocation sequence and one study (24) explicitly described no randomization was performed, while the remaining studies lacked information about this process. Only 1 study reported baseline characteristics other than age, sex, and weight,

TABLE 1 Mice strains.

Mice strain	NO. of studies using mice	Use this strain			*p*-value
		YES	NO	Percentage(%)	
C57BL/6	168	86	82	51.19	0.000
ICR	168	40	128	23.81	0.011
Swiss	168	22	146	13.10	0.070
Balb/c	168	12	156	7.14	0.017
NMRI	168	3	165	1.79	1.000
CD-1	168	2	166	1.19	1.000
Kunming	168	2	166	1.19	1.000
Wistar albino	168	1	167	0.6	

TABLE 2 Routes of lipopolysaccharide administration.

Route of administration	NO. of studies	Use this route			*p*-value
		YES	NO	Percentage (%)	
IP	170	164	6	96.47	0.000
ICV	170	5	165	2.94	0.215
IG	170	1	169	0.59	

such as locomotion pattern, unusual respiratory, piloerection, etc. (25). No study clarified whether the allocation of different groups was sufficiently hidden. The breeding conditions and environment of all experimental animals included in the study were the same; therefore, we considered that the animal placement complied with the principle of randomization (26). Only one study reported that animal breeders and/or researchers were blinded to the study groups, although it did not report the specific processes (27). Three and 19 studies reported randomization and blinding of outcome evaluation respectively. In terms of incomplete outcome data, in 13 studies, animals were withdrawn during the experimental procedure, although the impact of withdrawals on the results was not examined. Selective outcome reporting bias was assessed as unclear for all studies because none reported using a research protocol defining primary and secondary outcomes. No study was identified with other problems that could result in high risk of bias.

The risk of publication bias is shown in a funnel plot graph (Figure 3). The Begg's bias test showed that, except for"C57BL/6-0.83 mg/kg LPS" (Pr > |z| = 0.074 > 0.05) (Figure 3A), publication bias was detected for "C57BL/6 - 1 mg/kg LPS" (Pr > |z| = 0.032 < 0.05) (Figure 3B) and "ICR - 0.83 mg/kg LPS" (Pr > |z| = 0.001 < 0.05) (Figure 3C). However, when these publication biases were corrected using trim and fill method by adding theoretically missing studies, the results did not change significantly [(Figures 3D, E). We did not conduct a publication bias test for the other outcome measures because of the small number of studies (<10)].

3.3.2 Mice strains

Under the protocol of 0.5 mg/kg LPS single injection, the study includes nine articles from the C57BL/6 strain, five articles from the ICR strain, and three articles from the Swiss strain. Overall, 0.5 mg/kg LPS injection significantly increased the immobility time in the FST of C57BL/6, ICR, and Swiss mice. In addition, C57BL/6 mice (Figure 4A) (SMD = 1.78 [1.30, 2.27], Z =7.19 ($P < 0.00001$) and heterogeneity $\chi^2 = 12.87$, $P = 0.12$, $I^2 = 38\%$) exhibit smaller overall effect size compared to ICR (Figure 4B) (SMD = 4.73 [2.41, 7.06], Z =3.99 ($P < 0.00001$) and heterogeneity $\chi^2 = 31.61$, $P < 0.00001$, $I^2 = 87\%$) and Swiss mice (Figure 4C) (SMD = 2.87 [1.94, 3.79], Z =6.06 ($P < 0.00001$) and heterogeneity $\chi^2 = 1.41$, $P = 0.5$, $I^2 = 0\%$). Sensitivity analysis showed that none of the study reversed the effect identified by the meta-analysis, and excluding individual studies in order did not descend heterogeneity in ICR strain.

Similarly, for the protocol of 0.83 mg/kg LPS single injection, C57BL/6 strain showed even lower effect sizes. The overall effect size for LPS-induced mice after FST was the following: C57BL/6 mice (Figure 5A) (SMD = 1.88 [1.42, 2.35], Z =7.94 ($P < 0.00001$) and heterogeneity $\chi^2 = 28.16$, $P = 0.009$, $I^2 = 54\%$), ICR mice (Figure 5B) (SMD = 2.24 [1.78, 2.70], Z =9.53 ($P < 0.00001$) and heterogeneity $\chi^2 = 27.39$, $P = 0.01$, $I^2 = 53\%$), Swiss mice (Figure 5C) (SMD = 5.05 [2.41, 7.68], Z =3.76 ($P = 0.0002$) and heterogeneity $\chi^2 = 23.4$, $P = 0.00001$, $I^2 = 83\%$). Among them, C57BL/6, ICR and Swiss mice showed significant heterogeneity. The sensitivity analysis proved that the heterogeneity originated from a single study (28–30) of C57BL/6 and ICR, respectively. and after excluding this study heterogeneity $\chi^2 = 20.46$, $P = 0.06$, $I^2 = 41\%$, $\chi^2 = 10.95$, $P = 0.53$, $I^2 = 0\%$, and did not have a significant effect on the overall effect value (SMD = 1.65 [1.26, 2.05], Z =8.20 ($P < 0.00001$); SMD = 2.06 [1.75, 2.37], Z =13.03 ($P < 0.00001$)) in C57BL/6 and ICR mice. However, no source of heterogeneity was found in Swiss mice.

3.3.3 LPS doses

Through further analysis, different LPS doses did not exhibit induction differences in the FST of C57BL/6 mice, but in ICR and Swiss mice. When injected with 0.5 mg/kg (Figure 4A) (SMD = 1.78 [1.30, 2.27], Z =7.19 ($P < 0.00001$)), 0.83mg/kg (Figure 5A) (SMD = 1.88 [1.42, 2.35], Z =7.94 ($P < 0.00001$)) and 1mg/kg (Figure 6A) (SMD = 1.78 [1.42, 2.15], Z =9.51 (P < 0.00001) and heterogeneity $\chi^2 = 6.40$, P = 0.70, $I^2 = 0\%$) doses of LPS, the meta-analysis showed that the sizes of the overall effect of FST immobility time were at the same level in C57BL/6 mice. Whereas 0.5 mg/kg LPS-induced performance a greater effect size than 0.83 mg/kg in ICR (Figures 4B, 5B) (SMD = 4.73 [2.41, 7.06], Z =3.99 ($P < 0.00001$), SMD = 2.24 [1.78, 2.70], Z =9.53 ($P < 0.00001$)). However, Swiss mice showed opposite results, 0.83 mg/k (Figure 5C) (SMD = 5.05 [2.41, 7.68], Z =3.76 ($P = 0.0002$)) LPS-induced performance a greater effect size than 0.5 mg/kg (Figure 4C) (SMD = 2.87 [1.94, 3.79], Z =6.06 ($P < 0.00001$)). Meta-analysis was not performed on

TABLE 3 Times of lipopolysaccharide administration.

Times of administration	NO. of protocols	Use this protocol			*p*-value
		YES	NO	Percentage (%)	
Once	173	139	34	80.35	0.000
Repeatedly	173	34	139	19.65	

TABLE 4 Dose of lipopolysaccharide for a single intraperitoneal injection.

LPS Dose (mg/kg)	NO. of studies using dose	Use this dose			*p*-value
		YES	NO	Percentage (%)	
0.83	138	60	78	43.48	0.000
0.5	138	23	115	16.67	0.397
1	138	18	120	13.04	0.068
0.8	138	9	129	6.52	0.273
2	138	5	133	3.62	1.000
0.1	138	4	134	2.90	1.000
0.3	138	4	134	2.90	1.000
5	138	4	134	2.90	1.000
1.2(including 1.25)	138	3	135	2.17	1.000
0.4	138	2	136	1.45	1.000
0.083	138	1	137	0.72	1.000
0.25	138	1	137	0.72	1.000
0.6	138	1	137	0.72	1.000
0.85	138	1	137	0.72	1.000
1.5	138	1	137	0.72	1.000
1.8	138	1	137	0.72	

TABLE 5 Single dose of multiple intraperitoneal injections of lipopolysaccharide.

LPS Dose (mg/kg)	NO. of studies using dose	Use this dose			*p*-value	Study Design
		YES	NO	Percentage (%)		
0.5	27	8	19	29.63	0.535	Once a day for 10 days
						Once a day for 6 days
						Once a day for 7 days
						Once a day for 4 days
0.83	27	6	21	22.22	1.000	Once a day for 5 days
						Once a day for 3 days
						Once a day for 2 days
						The interval between injections was 16 h
1	27	6	21	22.22	0.250	Once a day for 5 days
						Once a day for 2 days
0.1	27	2	25	7.41	1.000	Once a day for 4 days
						Twice a week for 21 days
2	27	2	25	7.41	1.000	Once a day for 3 days
1.2	27	1	26	3.70	1.000	Once a day for 7 days
0.083	27	1	26	3.70	1.000	Once a day for 4 days
0.25	27	1	26	3.70		Once a day for 7 days

TABLE 6 Multiple doses of lipopolysaccharide for intraperitoneal injection.

Study design
(1) Day 1: 0.75 mg/kg; day 2: 1 mg/kg; day 3: 1.25 mg/kg; day 4: 1 mg/kg; day 5: 0.75mg/kg (2) 0.052, 0.104, 0.208, 0.415, 0.83 mg/kg; One dose per day for 5 days; (3) Day 1: 0.2 mg/kg; day 6: 8.3 mg/kg; (4) Week 1-2: 0.33 mg/kg; week 3: 0.53 mg/kg; week 4: 0.63 mg/kg; week 5: 0.73 mg/kg; week 6: 0.83 mg/kg;

ICR mice, Swiss mice induced by 1 mg/kg due to the limited number of articles.

Furthermore, we calculate the overall effect size of the LPS total dose applied during the intervention period. However, meta-analysis was only achieved under "C57BL/6-1mg/kg-Once a day for 5 days" due to the limited number of articles meeting the inclusion criteria. The result showed that five consecutive injections of 1mg/kg LPS (Figure 6B) (SMD = 2.76 [1.67, 4.21], Z =6.68 (P < 0.00001) and heterogeneity χ^2 = 1.87, P = 0.60, I^2 = 0% produced a greater effect size than a single injection of LPS in C57BL/6 mice (Figure 6A) (SMD = 1.78 [1.42, 2.15], Z =9.51 (P < 0.00001) and heterogeneity χ^2 = 6.40, P = 0.70, I^2 = 0%).

3.4 Summary

For mouse strains, C57BL/6 mice seem to be the optimal choice because they have lower heterogeneity and can more stably reflect behavioral changes in depression. As for the dose of LPS, accurate conclusions cannot be drawn due to the limited number of literatures, but it is clear that 0.5mg/kg, 0.83mg/kg and 1mg/kg LPS can stably induce depression behavior in mice.

4 Discussion

4.1 Mouse strains

Rodent research has long been utilized to model affective and immune deficits, as well as their interplay. Mice as experimental subjects have the sensitivity, validity, and reliability suitable for depression modeling, which can greatly provide a feasible experimental reference and develop relevant standards. Mice appear to be more suitable than rats for application in models of neuroinflammation-induced depression. The selection of appropriate mouse strains is important for determining the antidepressant activity of drugs. After the frequency analysis of the mouse strains selected in the 168 included studies, we found that C57BL/6 mice (inbred strain) were used in many studies, followed by ICR mice (closed colony), Swiss mice (outbred strain), and Balb/c mice (inbred strain). Baseline immobility time can more appropriately describe innate vulnerability to stressors and the tendency to despair under stress (31). Different genetic backgrounds can modify responses through different baseline levels of behavior. Lucki compared outbred and inbred mice by FST and suggested that mouse strains with higher immobility values, such as C57BL/6, can be more susceptible to stress-induced depressive behavior (30). Importantly, many studies have shown that mouse strains differ in their sensitivity to antidepressants and that the behavioral effects and mechanisms of action of drugs differ between strains (30, 32, 33). Study have shown that outbred mice are more variable in their baseline performance than inbred strains (30, 34), which can contribute to some of the high heterogeneity in our experimental results. Moreover, strains with less variation in baseline C57BL/6 can provide more accurate changes in drug efficacy (30). Different strains of mice showed similarities in baseline immobility in TST and FST, and Nadège Ripoll's study showed that C57BL/6 mice had a longer baseline immobility time in the hanging tail test compared with other strains (Swiss, NMRI, DBA/2) (35). However, some studies have shown the opposite results. David D J P did not observe baseline differences in FST among Swiss, NMRI, DBA/2, and C57BL/6J Rj strains (36).

Based on the results of the meta-analysis, C57BL/6 mice produced lower effect values under LPS induction than ICR and Swiss mice. However, in general, C57BL/6 mice had lower heterogeneity and could consistently model depression and respond to drug effects, which is consistent with the conclusions drawn earlier. Alternatively, it is well known that there are important differences in the behavior of mice of the same strain purchased from different providers. Therefore, we recommend that the selection of a suitable animal strain for establishing a depression-like model should be taken into account based on various factors such as the purpose and content of the study, drug properties, and experimental conditions, and ensure that the mice were derived from the same providers or laboratory production.

4.2 LPS administration

The induction of cytokines by the low-dose systemic administration of LPS can induce depressive features. Initially,

TABLE 7 Dose of lipopolysaccharide for a single intracerebral injection.

Concentration	Injection volume/quality	Injection location
10 mg/mL	1 μL	ML: -1.0 mm; AP: -0.5 mm; DV: -2.5 mm;
10 ng/μL	1 μL/side	/
/	10 μg	AP: -0.22 mm; ML: + 1.0 mm
/	100 ng	ML: +1.0 mm; AP: 0.5 mm; DV: 2.0 mm
10 mg/mL	1 μL	ML: -1.0 mm; DV: -2.5 mm; AP: -0.5 mm

TABLE 8 Lipopoysaccharide serotypes.

LPS serotypes	NO. of studies using serotypes	Use this serotypes			*p*-value
		YES	NO	Percentage (%)	
055: B5	102	35	67	34.31	0.766
0127: B8	102	33	69	32.35	0.444
0111: B4	102	28	74	27.45	0.000
026: B6	102	3	99	2.94	1.000
RH51487	102	2	100	1.96	1.000
L-7985	102	1	101	0.98	

LPS acts as a pathogen-associated molecular patterns (PAMP) that induces peripheral inflammation to produce pro-inflammatory cytokines. PAMP and circulating cytokines act on toll-like receptors in macrophage-like cells to transmit peripheral inflammatory signals to the center by synthesizing and releasing central cytokines (37, 38). The effects of peripheral pro-inflammatory cytokines can also be transmitted to the brain, leading to microglia activation and pro-inflammatory cytokine release (39). At about 6 h, the production of pro-inflammatory cytokines leads to disease behaviors, including fever, anorexia, decreased exercise capacity, and reduced social interaction; however, it is usually terminated by endogenous anti-inflammatory molecules (40). When the production of pro-inflammatory cytokines continues, and the amount of anti-inflammatory cytokines produced is insufficient to counteract the amount of pro-inflammatory cytokines, depression-like behaviors are induced and peaked at about 24 h of LPS injection (41). Genetic background and social environment affect depression-like behaviors induced by a single IP of LPS (42).

Intraperitoneal injection of LPS induces peritoneal inflammation and can modulate the activation of the brainstem, hypothalamus, and limbic structures *via* vagal afferents in response to peripherally administered LPS, thus inducing depression-like behaviors at about 24 hours. Moreover, IP injection is simple and reproducible and is therefore selected as the most commonly used method for LPS-induced depression. The direct injection of LPS into the brain can cause intracerebral inflammation, which is closely associated with psychiatric disorders, cognitive dysfunction, and motor impairment. The ICV injection of LPS caused mice to exhibit depressive states in the Y-maze to detect spatial memory and TST to detect despair, induced dendritic atrophy in the prefrontal cortex and hippocampal pyramidal neurons (43), and increased the levels of the pro-inflammatory cytokines IL-1β and TNF-α in the hippocampus, leading to neuronal damage. The intracerebral injection of LPS could stably establish a depression model. However, there are shortcomings in this complicated operation which cause extensive damage to experimental animals and have high mortality.

4.2.1 Routes of LPS administration

Studies in the literature included in the present study have shown three common types of LPS administration: IP, ICV, and IG, with IP being the most commonly used mode of administration.

4.2.2 Times and doses of LPS administration

Single or multiple LPS injections represent acute or chronic LPS-induced depression, respectively. Various studies have shown that both acute and chronic LPS excitation can successfully induce

TABLE 9 Behavioral test.

Behavioral tests	Frequency	Behavioral tests	Frequency
FST	140	SIT	3
TST	115	LDB	3
OFT	95	YMT	3
SPT	78	MWM	2
LMA	19	Rotarod	2
EPM	12	PAT	2
NSFT	11	FUST	2
ST	8	ATM	2
NORT	4	TFC	1

FST, Forced Swimming Test; TST, Tail Suspension Test; OFT, Open Field Test; SPT, Sucrose Preference Test; LMA, The locomotor activity; EPM, Elevated plus maze test; NSFT, Novelty suppressed-feeding test; ST, Splash Test; NORT, Novel object recognition task; SIT, Social interaction test; LDB, Light-dark box; YMT, Y maze test; MWM, Morris water maze test; PAT, Passive avoidance test; FUST, Female urine sniffing test; ATM, Autonomic activity tests; TFC, Trace fear conditioning.

FIGURE 2
Risk of bias summary for the included studies. 1, Random sequence generation (selection bias). 2, Baseline characteristics (selection bias). 3, Allocation concealment (selection bias). 4, Random housing (performance bias). 5, Blinding of participants (performance bias). 6, Random outcome assessment (detection bias). 7, Blinding of outcome assessment (detection bias). 8, Incomplete outcome data (attrition bias). 9, Selective reporting (reporting bias). 10, Other bias.

depressive behaviors in rodents (44–47). Acute LPS administered systemically in mice or rats release pro-inflammatory cytokines that induce strong but transient disease behaviors as evidenced by reduced motor activity and weight loss (48, 49). FST, SPT, and OFT are generally performed to assess depression-like models 24 hours after LPS injection. study has shown that depression-like behaviors can still be observed in mice 48 h after LPS injection (50). Moreover, depression is a chronic and recurrent disease characterized by persistent levels of inflammatory markers (48); hence, Depression models were derived by multiple repeated LPS injections to mimic persistent inflammation. Multiple LPS injections were further divided into single-dose multiple

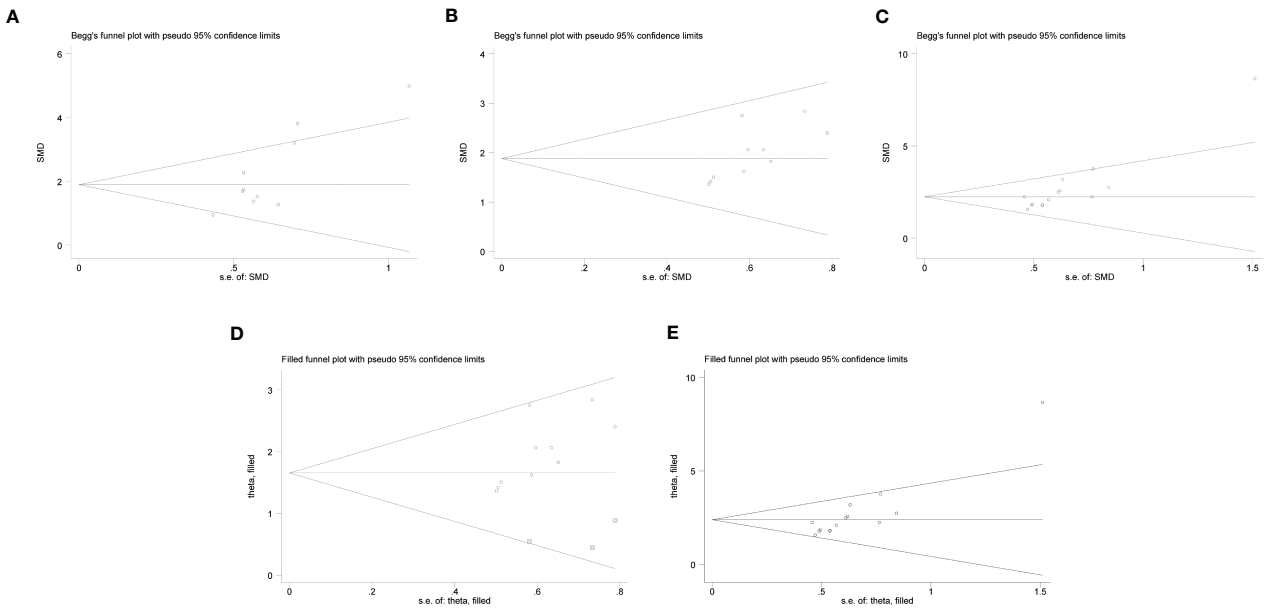

FIGURE 3
Begg's bias test of **(A)** C57BL/6 -0.83 mg/kg LPS. **(B)** C57BL/6 - 1 mg/kg LPS. **(C)** ICR - 0.83 mg/kg LPS. Trim-and- fill evaluation of **(D)** C57BL/6 - 1 mg/kg LPS. **(E)** ICR - 0.83 mg/kg LPS.

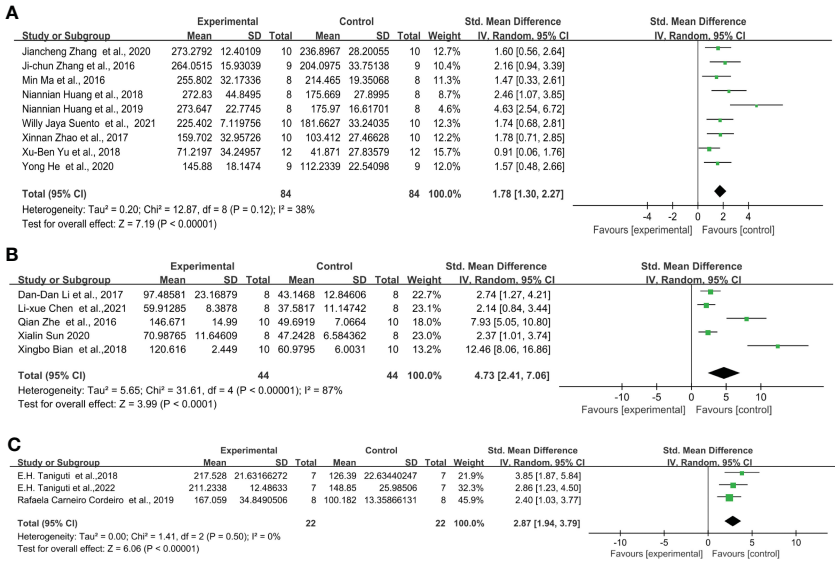

FIGURE 4
Forest plots of standardized mean differences in forced swimming test (s) in **(A)** C57BL/6 mouse - 0.5 mg/kg LPS. **(B)** ICR mouse-0.5 mg/kg LPS. **(C)** Swiss mouse - 0.5 mg/kg LPS.

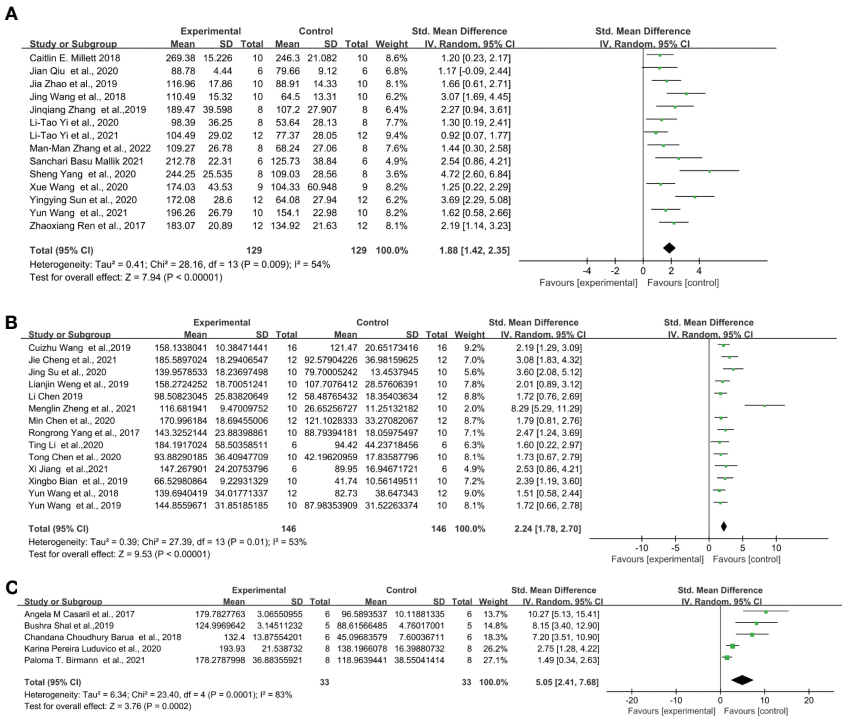

FIGURE 5
Forest plots of standardized mean differences in forced swimming test (s) in **(A)** C57BL/6 mouse - 0.83 mg/kg LPS. **(B)** ICR mouse- 0.83 mg/kg LPS. **(C)** Swiss mouse-0.83 mg/kg LPS.

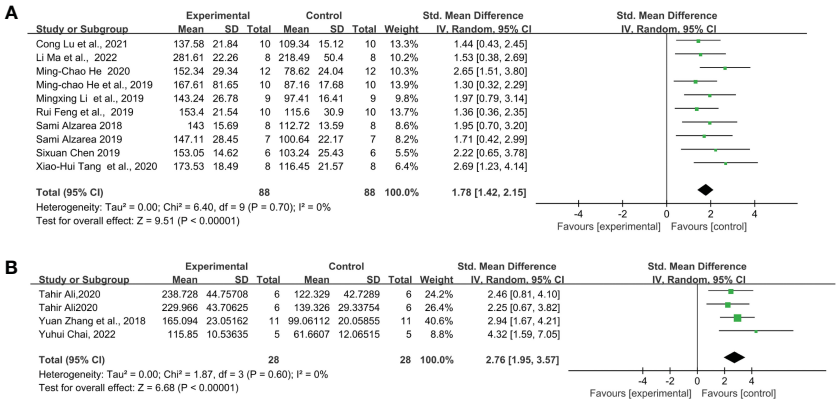

FIGURE 6
Forest plots of standardized mean differences in forced swimming test (s) in **(A)** C57BL/6 mouse - 1 mg/kg LPS. **(B)** C57BL/6-1mg/kg-Once a day for 5 days.

injections and multiple-dose injections. Yong He (49) compared acute and chronic LPS depression mouse models with a protocol of single and multiple injections (once daily for 7 days) of 0.5 mg/kg LPS and showed that both administration methods were successful in establishing depression models; however, the acute LPS-induced mice showed significantly more depression-like behaviors in SPT and FST than the chronic LPS-induced mice (49). Robin A. Wickens (48) compared repeatedly increasing doses of LPS (0.1, 0.42, and 0.83 mg/kg) with a constant dose (0.83 mg/kg) for 3 days, and with increasing doses, mice developed disease behaviors; however, they also developed tolerance to repeated constant doses of LPS, resulting in diminished behavioral responses (48).

The serotype has an important influence on the dose selection of LPS. We analyzed the frequency of the use of LPS serotypes and found that 055: B5 was the most frequently used model, followed by 0127: B8 and 0111: B4. We found no significant difference in the frequency of selecting these three serotypes. However, a selective difference in the dose of LPS selected for different serotypes and a significant effect on depressive behaviors or biochemical indicators must be systematically evaluated. Different LPS serotypes induce the differential activation of inflammatory transcription factors, such as IL-1β, IL-6, interleukin-8 (IL-8), and TNF-α, leading to differential protein expression, which may be related to the structure of LPS models, and the selective use of LPS serotypes may help investigate activation-specific inflammatory mechanisms (51, 52).

The meta-analysis results showed that the three doses of the single injection of LPS at 0.5 mg/kg, 0.83 mg/kg, and 1 mg/kg induced depression models in mice and were closely related to the despairing behaviors of animals. Mice in the model group were immobilized for a longer period than those in the FST compared with those in the control group. High heterogeneity was observed in some single-group analyses, probably indicating difficulties in replicating the LPS depression model using these programs. The effects of other factors, such as mouse strain, individual differences (weight, age, and sex), environment (water and food, light, temperature, and noise), experimental design (sequence of FST and other behavioral tests, water temperature of FST test, and test time), on the experimental results were considered.

4.3 Behavior test

Among the 168 papers included in the present study, there were 18 behavioral tests, and the most commonly used methods to evaluate depression-like behaviors in mice were FST, TST, OFT, and SPT, in that order. FST and TST have a similar theoretical basis and measure of time spent stationary in an inescapable environment; the immobility state reflects despair-like inhibitory learning behaviors and is an important indicator of depressive psychomotor retardation (53). The effects of LPS usually disappear at higher sucrose concentrations, which are commonly used in 1% sucrose solutions (54); OFT simulates an unsafe environment to assess the autonomous behavior of an animal to reveal the level of tension in the animal. When animals are afraid of a new environment, they tend to move around the edge area of the open field box and rarely in the central area. Additionally, locomotor activity and autonomic activity tests were performed to assess the motor ability of mice (55). Y maze, Morris water maze (MWM) (56), and elevated plus maze (EPM) (57) tests were performed to assess spatial learning memory and the ability to explore new environments, whereas the novel object recognition test (NORT) assessed non-spatial memory in mice (58). The novelty suppressed-feeding test (NSFT) is an observation of the conflicting behavior of animals in a hunger state with the desire to ingest food and the fear of entering a bright central area, and it assesses depressive or anxious behaviors as an indicator of food intake and latency to find food. The social interaction test (SIT) is performed to evaluate social competence in mice, and the splash test (ST) is a valid marker for assessing self-care behaviors in depressed mice.

FST is the most commonly performed behavioral experiment for evaluating depression models and screening antidepressants in preclinical studies because of its ease of operation and sensitivity.

Many published papers have described the experimental protocols and considerations of FST (59–61). However, the FST results were susceptible to many external (experimental design and environmental conditions) and biological factors (strain, weight, age, and individual differences). The FST experimental protocol, including the size of the test bucket, water depth, water temperature, and test time, affected the experimental results. The size of the test barrel affected FST immobility. Sunal et al. placed mice into vertical Plexiglas cylinders (height: 30 cm, diameter: 10, 20, 30, and 50 cm) in 20-cm deep water to swim, and as the diameter of the barrel increased, FST immobility in mice decreased (62). Compared with other barrels, the total duration of immobility was shorter, and the latency period was longer in the 10-cm test barrel (63), the most commonly used test barrel size for mice. The water temperature during the test was also an important affecting factor, with mice exhibiting greater immobility when swimming at 25°C than at 35°C (64), and reduced swimming speed was observed in the MWM test at low temperatures (65). However, the effect of water temperature on the FST results was different in different mouse strains, as the immobility of C57BL/6 mice increased when the water temperature increased; however, BALB/C mice showed the opposite performance (66). Our extensive literature analysis showed that most investigators selected a water temperature of 21–25°C for the FST. In addition, the mice showed a clear 24-h rhythm of immobility in the FST, with a shorter duration of immobility in mice at noon (12:00 PM–2 PM) than at midnight (12:00 AM–2:00 AM). If more than one behavioral test is required, we recommend the FST as the last test. In addition, studies have shown that environmental factors such as light, light/dark cycle time, noise, odor, and living environment can affect the immobility time of animals (63).

It has to be mentioned that the interpretation of duration of immobility as measure of behavioral despair has been criticized repeatedly (67). Hawkins et al. (68) agree with the use of the FST as an innovative antidepressant screening tool, but refute the notion that immobility in the FST represents hopelessness. The researchers (69) believe that immobility is an adaptive response to an inescapable environment, rather than depression-like behavior. Nonetheless, its high efficacy has made it a popular behavioral test for selecting antidepressants.

5 Conclusion

Depression is a complex biological heterogeneous disorder. Due to differences between animal models and human disease groups, it is challenging to replicate the human depression phenotype in a depression-like animal model. LPS-induced models of depression in mice are subject to genetic, environmental, and experimental parameters that may ultimately have a qualitative impact on the study results obtained by applying this model. Therefore, reaching consensus on modeling methods or experimental parameters in depressive-like animal models is essential to avoid unnecessary external effects. Our results suggests that mice strains and LPS administration play a key role in the assessment of behavioral outcomes in these models. This provides an important reference for the rational application of such models. In addition, the design, implementation, measurement criteria, and reporting of this animal model need to be further improved and standardized to facilitate the development of better animal experiments and clinical studies of inflammation induced depression. Compared with the traditional animal model of depression, LPS model has some disadvantages in that it cannot more fully replicate the depression phenotype. Rodents were used to identify animal models of antidepressants commonly associated with stress or genetic factors. However, we believe that the use of depression-like animal models based on neuroinflammation is beneficial for further elucidating the mechanism of action of antidepressants. Finally, although there has been controversy surrounding the use of animal models, their utility and value in depression research over the past decades cannot be ignored. Optimizing experimental protocols is one of the means to reduce the harm to animals.

Author contributions

RY, KZ, and YX contributed to the conception, design, and preparation of the manuscript. YL, ZT, and RZ outlined the initial literature review and extracted literature data. YM, NL contributed significantly to drafting the manuscript and critically revising it, GL, ZC, PG, NL meticulously guided the application of the methodology. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1181973/full#supplementary-material

References

1. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PloS Med* (2006) 3(11):e442. doi: 10.1371/journal.pmed.0030442

2. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries 1990-2016: a systematic analysis for the global burden of disease study 2016. *Lancet* (2017) 390(10100):1211–59.

3. Renaud-Charest O, Lui LMW, Eskander S, Ceban F, Ho R, Di Vincenzo JD, et al. Onset and frequency of depression in post-COVID-19 syndrome: a systematic review. *J Psychiatr Res* (2021) 144:129–37. doi: 10.1016/j.jpsychires.2021.09.054

4. Peng GJ, Tian JS, Gao XX, Zhou YZ, Qin XM. Research on the pathological mechanism and drug treatment mechanism of depression. *Curr Neuropharmacol* (2015) 13(4):514–23. doi: 10.2174/1570159x1304150831120428

5. Smith RS. The macrophage theory of depression. *Med Hypotheses* (1991) 35 (4):298–306. doi: 10.1016/0306-9877(91)90272-Z

6. Wang AK, Miller BJ. Meta-analysis of cerebrospinal fluid cytokine and tryptophan catabolite alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder, and depression. *Schizophr Bull* (2018) 44(1):75–83. doi: 10.1093/schbul/sbx035

7. Wang M, Huang W, Gao T, Zhao X, Lv Z. Effects of xiao yao San on interferon-α-induced depression in mice. *Brain Res Bull* (2018) 139:197–202. doi: 10.1016/j.brainresbull.2017.12.001

8. Grigoleit J-S, Kullmann JS, Wolf OT, Hammes F, Wegner A, Jablonowski S, et al. Dose-dependent effects of endotoxin on neurobehavioral functions in humans. *PloS One* (2011) 6(12):e28330. doi: 10.1371/journal.pone.0028330

9. Suneson K, Lindahl J, Chamli Hårsmar S, Söderberg G, Lindqvist D. Inflammatory depression-mechanisms and non-pharmacological interventions. *Int J Mol Sci* (2021) 22(4):1640. doi: 10.3390/ijms22041640

10. Mazza MG, Palladini M, De Lorenzo R, Magnaghi C, Poletti S, Furlan R, et al. Persistent psychopathology and neurocognitive impairment in COVID-19 survivors: effect of inflammatory biomarkersat three-month follow-up. *Brain Behavior Immun* (2021) 94:138–47. doi: 10.1016/j.bbi.2021.02.021

11. Lyu D, Wang F, Zhang M, Yang W, Huang H, Huang Q, et al. Ketamine induces rapid antidepressant effects *via* the autophagy-NLRP3 inflammasome pathway. *Psychopharmacology* (2022) 239(10):3201–12. doi: 10.1007/s00213-022-06201-w

12. Kofod J, Elfving B, Nielsen EH, Mors O, Köhler-Forsberg O. Depression and inflammation: correlation between changes in inflammatory markers with antidepressant response and long-term prognosis. *Eur Neuropsychopharmacol* (2022) 54:116–25. doi: 10.1016/j.euroneuro.2021.09.006

13. Sukhram SD, Yilmaz G, Gu J. Antidepressant effect of ketamine on inflammation-mediated cytokine dysregulation in adults with treatment-resistant depression: rapid systematic review. *Oxid Med Cell Longevity* (2022) 2022:1061274. doi: 10.1155/2022/1061274

14. Mao M, Li S, Zong M, Qiu L, Yang J, Xia J, et al. Two-hit model of postintensive care syndrome induced by lipopolysaccharide challenge and subsequent chronic unpredictable stress in mice. *Int Immunopharmacol* (2019) 70:446–58. doi: 10.1016/j.intimp.2019.03.012

15. Mariani N, Everson J, Pariante CM, Borsini A. Modulation of microglial activation by antidepressants. *J Psychopharmacol (Oxford England)* (2022) 36(2):131–50. doi: 10.1177/02698811211069110

16. Wang Q, Timberlake MA, Prall K, Dwivedi Y. The recent progress in animal models of depression. *Prog In Neuropsychopharmacol Biol Psychiatry* (2017) 77:99–109. doi: 10.1016/j.pnpbp.2017.04.008

17. Czéh B, Fuchs E, Wiborg O, Simon M. Animal models of major depression and their clinical implications. progress. *Neuropsychopharmacol Biol Psychiatry* (2016) 64:293–310. doi: 10.1016/j.pnpbp.2015.04.004

18. Cavaillon J-M. Exotoxins and endotoxins: inducers of inflammatory cytokines. *Toxicon* (2018) 149:45–53. doi: 10.1016/j.toxicon.2017.10.016

19. Yan T, Wang N, Liu B, Wu B, Xiao F, He B, et al. Schisandra chinensis ameliorates depressive-like behaviors by regulating microbiota-gut-brain axis *via* its anti-inflammation activity. *Phytother Res* (2021) 35(1):289–96. doi: 10.1002/ptr.6799

20. Ji M-H, Zhang L, Mao M-J, Zhang H, Yang J-J, Qiu L-L. Overinhibition mediated by parvalbumin interneurons might contribute to depression-like behavior and working memory impairment induced by lipopolysaccharide challenge. *Behav Brain Res* (2020) 383:112509. doi: 10.1016/j.bbr.2020.112509

21. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Int J Surg* (2010) 8 (5):336–41. doi: 10.1016/j.ijsu.2010.02.007

22. Antoniuk S, Bijata M, Ponimaskin E, Wlodarczyk J. Chronic unpredictable mild stress for modeling depression in rodents: meta-analysis of model reliability. *Neurosci Biobehav Rev* (2019) 99:101–16. doi: 10.1016/j.neubiorev.2018.12.002

23. Aslam M. Chi-square test under indeterminacy: an application using pulse count data. *BMC Med Res Methodol.* (2021) 21(1):201. doi: 10.1186/s12874-021-01400-z

24. Suento WJ, Kunisawa K, Wulaer B, Kosuge A, Iida T, Fujigaki S, et al. Prefrontal cortex miR-874-3p prevents lipopolysaccharide-induced depression-like behavior through inhibition of indoleamine 2,3-dioxygenase 1 expression in mice. *J Neurochem.* (2021) 157(6):1963–78. doi: 10.1111/jnc.15222

25. Casaril AM, Domingues M, Fronza M, Vieira B, Begnini K, Lenardão EJ, et al. Antidepressant-like effect of a new selenium-containing compound is accompanied by a reduction of neuroinflammation and oxidative stress in lipopolysaccharide-challenged mice. *J Psychopharmacol (Oxford England)* (2017) 31(9):1263–73. doi: 10.1177/0269881117711713

26. Feng H, Wu T, Zhou Q, Li H, Liu T, Ma X, et al. Protective effect and possible mechanisms of artemisinin and its derivatives for diabetic nephropathy: a systematic review and meta-analysis in animal models. *Oxid Med Cell Longevity* (2022) 2022:5401760. doi: 10.1155/2022/5401760

27. Millett CE, Phillips BE, Saunders EFH. The sex-specific effects of LPS on depressive-like behavior and oxidative stress in the hippocampus of the mouse. *Neuroscience* (2019) 399:77–88. doi: 10.1016/j.neuroscience.2018.12.008

28. Yang S, Chen X, Xu Y, Hao Y, Meng X. Effects of metformin on lipopolysaccharide-induced depressive-like behavior in mice and its mechanisms. *Neuroreport* (2020) 31(4):305–10. doi: 10.1097/WNR.0000000000001401

29. Zheng M, Li K, Chen T, Liu S, He L. Geniposide protects depression through BTK/JAK2/STAT1 signaling pathway in lipopolysaccharide-induced depressive mice. *Brain Res Bull* (2021) 170:65–73. doi: 10.1016/j.brainresbull.2021.02.008

30. Lucki I, Dalvi A, Mayorga AJ. Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. *Psychopharmacology* (2001) 155 (3):315–22. doi: 10.1007/s002130100694

31. Yoshikawa T, Watanabe A, Ishitsuka Y, Nakaya A, Nakatani N. Identification of multiple genetic loci linked to the propensity for "behavioral despair". *mice. Genome Res* (2002) 12(3):357–66. doi: 10.1101/gr.222602

32. Guzzetti S, Calcagno E, Canetta A, Sacchetti G, Fracasso C, Caccia S, et al. Strain differences in paroxetine-induced reduction of immobility time in the forced swimming test in mice: role of serotonin. *Eur J Pharmacol* (2008) 594(1-3):117–24. doi: 10.1016/j.ejphar.2008.07.031

33. Jin Z-L, Chen X-F, Ran Y-H, Li X-R, Xiong J, Zheng Y-Y, et al. Mouse strain differences in SSRI sensitivity correlate with serotonin transporter binding and function. *Sci Rep* (2017) 7(1):8631. doi: 10.1038/s41598-017-08953-4

34. Nikulina EM, Skrinskaya JA, Popova NK. Role of genotype and dopamine receptors in behaviour of inbred mice in a forced swimming test. *Psychopharmacology* (1991) 105(4):525–9. doi: 10.1007/BF02244374

35. Ripoll N, David DJP, Dailly E, Hascoët M, Bourin M. Antidepressant-like effects in various mice strains in the tail suspension test. *Behav Brain Res* (2003) 143(2):193–200. doi: 10.1016/S0166-4328(03)00034-2

36. David DJP, Renard CE, Jolliet P, Hascoët M, Bourin M. Antidepressant-like effects in various mice strains in the forced swimming test. *Psychopharmacology* (2003) 166(4):373–82. doi: 10.1007/s00213-002-1335-4

37. Dantzer R. Cytokine, sickness behavior, and depression. *Neurol Clin* (2006) 24 (3):441–60. doi: 10.1016/j.ncl.2006.03.003

38. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci* (2008) 9(1):46–56. doi: 10.1038/nrn2297

39. Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry* (2009) 65 (9):732–41. doi: 10.1016/j.biopsych.2008.11.029

40. Dantzer R. Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity. *Eur J Pharmacol* (2004) 500(1-3):399–411. doi: 10.1016/j.ejphar.2004.07.040

41. Shirayama Y, Yang C, Zhang JC, Ren Q, Yao W, Hashimoto K. Alterations in brain-derived neurotrophic factor (BDNF) and its precursor proBDNF in the brain regions of a learned helplessness rat model and the antidepressant effects of a TrkB agonist and antagonist. *Eur Neuropsychopharmacol* (2015) 25(12):2449–58. doi: 10.1016/j.euroneuro.2015.09.002

42. Painsipp E, Köfer MJ, Sinner F, Holzer P. Prolonged depression-like behavior caused by immune challenge: influence of mouse strain and social environment. *PloS One* (2011) 6(6):e20719. doi: 10.1371/journal.pone.0020719

43. Ano Y, Ohya R, Kita M, Taniguchi Y, Kondo K. Theaflavins improve memory impairment and depression-like behavior by regulating microglial activation. *Mol. (Basel Switzerland)* (2019) 24(3):467. doi: 10.3390/molecules24030467

44. Arioz BI, Tastan B, Tarakcioglu E, Tufekci KU, Olcum M, Ersoy N, et al. Melatonin attenuates LPS-induced acute depressive-like behaviors and microglial NLRP3 inflammasome activation through the SIRT1/Nrf2 pathway. *Front In Immunol* (2019) 10:1511. doi: 10.3389/fimmu.2019.01511

45. Li Y, Song W, Tong Y, Zhang X, Zhao J, Gao X, et al. Isoliquiritin ameliorates depression by suppressing NLRP3-mediated pyroptosis *via* miRNA-27a/SYK/NF-κB axis. *J Neuroinflamm* (2021) 18(1):1. doi: 10.1186/s12974-020-02040-8

46. Li W, Ali T, He K, Liu Z, Shah FA, Ren Q, et al. Ibrutinib alleviates LPS-induced neuroinflammation and synaptic defects in a mouse model of depression. *Brain Behavior Immun* (2021) 92:10–24. doi: 10.1016/j.bbi.2020.11.008

47. Li W, Ali T, Zheng C, Liu Z, He K, Shah FA, et al. Fluoxetine regulates eEF2 activity (phosphorylation) *via* HDAC1 inhibitory mechanism in an LPS-induced mouse model of depression. *J Neuroinflamm* (2021) 18(1):38. doi: 10.1186/s12974-021-02091-5

48. Wickens RA, Ver Donck L, MacKenzie AB, Bailey SJ. Repeated daily administration of increasing doses of lipopolysaccharide provides a model of sustained inflammation-induced depressive-like behaviour in mice that is independent of the NLRP3 inflammasome. *Behav Brain Res* (2018) 352:99–108. doi: 10.1016/j.bbr.2017.07.041

49. He Y, Li W, Wang Y, Tian Y, Chen X, Wu Z, et al. Major depression accompanied with inflammation and multiple cytokines alterations: evidences from clinical patients to macaca fascicularis and LPS-induced depressive mice model. *J Affect Disord* (2020) 271:262–71. doi: 10.1016/j.jad.2020.03.131

50. Qiu J, Liu R, Ma Y, Li Y, Chen Z, He H, et al. Lipopolysaccharide-induced depression-like behaviors is ameliorated by sodium butyrate *via* inhibiting neuroinflammation and oxido-nitrosative stress. *Pharmacology* (2020) 105(9-10):550–60. doi: 10.1159/000505132

51. Migale R, Herbert BR, Lee YS, Sykes L, Waddington SN, Peebles D, et al. Specific lipopolysaccharide serotypes induce differential maternal and neonatal inflammatory responses in a murine model of preterm labor. *Am J Pathol* (2015) 185(9):2390–401. doi: 10.1016/j.ajpath.2015.05.015

52. Mastrodonato A, Cohensedgh O, LaGamma CT, McGowan JC, Hunsberger HC, Denny CA. Prophylactic (R,S)-ketamine selectively protects against inflammatory stressors. *Behav Brain Res* (2020) 378:112238. doi: 10.1016/j.bbr.2019.112238

53. Unal G, Canbeyli R. Psychomotor retardation in depression: a critical measure of the forced swim test. *Behav Brain Res* (2019) 372:112047. doi: 10.1016/j.bbr.2019.112047

54. Liu M-Y, Yin C-Y, Zhu L-J, Zhu X-H, Xu C, Luo C-X, et al. Sucrose preference test for measurement of stress-induced anhedonia in mice. *Nat Protoc* (2018) 13 (7):1686–98. doi: 10.1038/s41596-018-0011-z

55. Seibenhener ML, Wooten MC. Use of the open field maze to measure locomotor and anxiety-like behavior in mice. *J Vis Exp* (2015) 96):e52434. doi: 10.3791/52434

56. Bromley-Brits K, Deng Y, Song W. Morris water maze test for learning and memory deficits in alzheimer's disease model mice. *J Vis Exp* (2011) 53:2920. doi: 10.3791/2920

57. Komada M, Takao K, Miyakawa T. Elevated plus maze for mice. *J Vis Exp* (2008) (22):1088. doi: 10.3791/1088-v

58. Lueptow LM. Novel object recognition test for the investigation of learning and memory in mice. *J Vis Exp* (2017) (126):55718. doi: 10.3791/55718-v

59. Castagné V, Moser P, Roux S, Porsolt RD. Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. *Curr Protoc Neurosci* Chapter 8:Unit 8.10A. (2011). doi: 10.1002/0471142301.ns0810as55

60. Yankelevitch-Yahav R, Franko M, Huly A, Doron R. The forced swim test as a model of depressive-like behavior. *J Vis Exp* (2015) (97):52587. doi: 10.3791/52587-v

61. Armario A. The forced swim test: historical, conceptual and methodological considerations and its relationship with individual behavioral traits. *Neurosci Biobehav Rev* (2021) 128:74–86. doi: 10.1016/j.neubiorev.2021.06.014

62. Sunal R, Gümüşel B, Kayaalp SO. Effect of changes in swimming area on results of "behavioral despair test". *Pharmacol. Biochem. Behav* (1994) 49(4):891–6. doi: 10.1016/0091-3057(94)90239-9

63. Bogdanova OV, Kanekar S, D'Anci KE, Renshaw PF. Factors influencing behavior in the forced swim test. *Physiol Behav* (2013) 118:227–39. doi: 10.1016/j.physbeh.2013.05.012

64. Arai I, Tsuyuki Y, Shiomoto H, Satoh M, Otomo S. Decreased body temperature dependent appearance of behavioral despair in the forced swimming test in mice. *Pharmacol Res* (2000) 42(2):171–6. doi: 10.1006/phrs.2000.0672

65. Iivonen H, Nurminen L, Harri M, Tanila H, Puoliväli J. Hypothermia in mice tested in morris water maze. *Behav Brain Res* (2003) 141(2):207–13. doi: 10.1016/S0166-4328(02)00369-8

66. Bächli H, Steiner MA, Habersetzer U, Wotjak CT. Increased water temperature renders single-housed C57BL/6J mice susceptible to antidepressant treatment in the forced swim test. *Behav Brain Res* (2008) 187(1):67–71. doi: 10.1016/j.bbr.2007.08.029

67. Molendijk ML, de Kloet ER. Forced swim stressor: trends in usage and mechanistic consideration. *Eur J Neurosci* (2022) 55(9-10):2813–31. doi: 10.1111/ejn.15139

68. Hawkins J, Hicks RA, Phillips N, Moore JD. Swimming rats and human depression. *Nature* (1978) 274(5670):512–3. doi: 10.1038/274512a0

69. de Kloet ER, Molendijk ML. Coping with the forced swim stressor: towards understanding an adaptive mechanism. *Neural Plastic.* (2016) 2016:6503162. doi: 10.1155/2016/6503162

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Levodopa-induced dyskinesia: interplay between the N-methyl-D-aspartic acid receptor and neuroinflammation

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Parkinson's disease (PD) is a common neurodegenerative disorder of middle-aged and elderly people, clinically characterized by resting tremor, myotonia, reduced movement, and impaired postural balance. Clinically, patients with PD are often administered levodopa (L-DOPA) to improve their symptoms. However, after years of L-DOPA treatment, most patients experience complications of varying severity, including the "on-off phenomenon", decreased efficacy, and levodopa-induced dyskinesia (LID). The development of LID can seriously affect the quality of life of patients, but its pathogenesis is unclear and effective treatments are lacking. Glutamic acid (Glu)-mediated changes in synaptic plasticity play a major role in LID. The N-methyl-D-aspartic acid receptor (NMDAR), an ionotropic glutamate receptor, is closely associated with synaptic plasticity, and neuroinflammation can modulate NMDAR activation or expression; in addition, neuroinflammation may be involved in the development of LID. However, it is not clear whether NMDA receptors are co-regulated with neuroinflammation during LID formation. Here we review how neuroinflammation mediates the development of LID through the regulation of NMDA receptors, and assess whether common anti-inflammatory drugs and NMDA receptor antagonists may be able to mitigate the development of LID through the regulation of central neuroinflammation, thereby providing a new theoretical basis for finding new therapeutic targets for LID.

KEYWORDS

N-methyl-D-aspartate receptor, neuroinflammation, Parkinson's disease, levodopa, levodopa-induced dyskinesia

1 Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder that affects middle-aged and elderly populations and is characterized by an insidious onset and slow progression. Its main clinical manifestations are resting tremor, bradykinesia, rigidity, and postural gait disorders (1). The main pathological change underlying PD is the

degenerative death of dopaminergic neurons in the midbrain substantia nigra, which results in a significant decrease in striatal dopamine and thereby causes disease (2, 3). The exact etiology of this pathological change remains unclear, but genetic factors, environmental factors, aging, oxidative stress, and mitochondrial dysfunction have all been suggested to be involved in the degenerative death of dopaminergic neurons in PD (2, 4, 5). Drug therapy is the primary treatment for patients with PD, and levodopa (L-DOPA) is still the most effective drug (5), despite its side effects (6). Most patients with PD undergoing long-term dopamine drug treatment develop motor complications after an average of 6.5 years, the most common of which is L-DOPA-induced dyskinesia (LID) (7, 8, 9). LID manifests in abnormal movements such as stereotypic, choreiform, and throwing movements as well as in dystonia that mainly involves the head, face, limbs, and trunk and greatly affects the quality of life of patients with PD (9). The exploration of non-dopaminergic causative factors and the question of how L-DOPA drugs can be combined with other drugs that reduce these side effects have therefore attracted the attention of many scientists. The role of the neuroimmune inflammatory response in the pathogenesis of LID has also become the focus of research in recent years. In experimental work in animal models, we have identified that the release of neuroinflammatory factors, the activation and subtype transformation of microglia, and the activation of astrocytes all lead to the degenerative death and loss of dopaminergic neurons (10–12). The loss of dopaminergic neurons causes PD, and the mechanisms by which neuroinflammation may be involved in PD and LID are unknown and may be different (13, 14). Current preclinical studies support a role for neuroinflammation in LID, and it is not possible to determine whether exacerbation of neuroinflammation may contribute to the development of LID in patients with PD (15, 16). As patients with PD are often treated with L-DOPA in the later stages of the disease, it cannot be ruled out that this treatment may be related to neuroinflammation (17, 18). The mechanism of how L-DOPA therapy may augment the neuroinflammatory response, and how this may affect LID outcomes, remains to be studied (14, 16). A link between neuroinflammation and the N-methyl-D-aspartic acid receptor (NMDAR) has been found (19, 20), namely the activation of glial cells and the release of inflammatory factors such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), inducible nitric oxide (NO), nitric oxide synthase (iNOS), and chemokines, which regulate the release of glutamic acid (Glu) from presynaptic neurons and the expression of Glu receptors in postsynaptic neurons (21, 22). NMDARs are common ionotropic Glu receptor channels, and expression and phosphorylation of their subunits GluN1 and GluN2 can contribute to the development of LID by regulating synaptic plasticity (23, 24), suggesting that the interaction between neuroinflammation and NMDARs may have an important role in the progression of LID. Synaptic plasticity refers to the regulation of the strength of synaptic connections (25, 26), by means of formation, elimination, enhancement, and weakening (25, 27). L-DOPA can lead to the development of LID through alterations in synaptic plasticity at the neurobiological level in the context of the pathologic changes underlying PD (25, 28). The corticostriatal synapses physiologically can undergo long-term potentiation (LTP), long-term depression (LTD) and depotentiation ("de-enhancement") (27, 28). Normally, signals from the cortex reach the striatum, where they are filtered and integrated via LTP and LTD, and appropriate signals are exported from the basal ganglia to regulate movement and learning (27). In rodent models of PD, the cortex still sends signals to the striatum, but the striatum loses LTP and LTD, hence the ability to screen and integrate these signals and thus failing to appropriately regulate the output signals from the basal ganglia (26). In long-term L-DOPA-treated rodent models of PD, striatal synapses lose their ability to "de-enhance," leading to uncontrolled enhancement in the pathway, which further contributes to the development of LID (26, 27). In this article, we review the possible mechanisms through which the interplay of NMDARs and neuroinflammation is involved in LID (29).

2 Overview of LID

As populations are aging, the global numbers of people with PD are increasing, placing a heavy financial and emotional burden on individuals, families, and society at large (30, 31). L-DOPA is currently recognized as a drug that offers good symptom control in patients with PD, but most patients will develop LID after long-term application, with mechanisms that are complex and not yet understood. LID is characterized by choreiform movements, dystonia, tardive dyskinesia, or simple repetitive involuntary movements, the severity of which often correlates with the degree of degeneration of dopaminergic neurons. In recent years, new research on presynaptic mechanisms, changes in postsynaptic plasticity, γ-aminobutyric acid (GABA)-ergic and glutamatergic neurons, and non-dopaminergic regulatory factors, as well as microvascular permeability, have provided new ideas and directions for the study of LID (32, 33). The pathogenesis of LID is complex and unclear. In this article, we mainly chose to review the effects of the interaction between neuroinflammation and NMDARs on the synaptic plasticity of neurons and to explore the mechanism and treatment of its role in LID.

3 Role of NMDARs in LID

In patients with PD, dopamine depletion and long-term treatment with L-DOPA trigger adaptive changes in glutamatergic transmission from the cortex to the striatum, leading to abnormalities in striatal NMDAR function(34). NMDARs are voltage- and ligand-gated cation channels, and their intracellular subunit composition, distribution, and phosphorylation level are related to their function. In particular, phosphorylation is an important mechanism for regulating the transport and channel properties of NMDARs(35, 36). In the brain, GluN1 and GluN2 (GluN2A, GluN2B, GluN2C, GluN2D) are the predominant NMDAR subunits (37, 38). As a calcium channel, it is mainly located in the postsynaptic membrane and recognizes the neurotransmitter Glu, the binding of which causes the excitation of postsynaptic neurons (39–41). Over-stimulation of NMDARs leads to a large inflow of extracellular Ca^{2+}, which in turn

activates Ca^{2+}-dependent enzymes involved in protein, nucleic acid, and phospholipid catabolism, and NO synthesis. This leads to cell membrane rupture and cytoskeletal changes in dopaminergic neurons in brain tissue, thereby causing dopaminergic neurons to die (42).

Abnormal NMDAR expression alters the synaptic plasticity of neurons in the striatal region and further imbalances the basal ganglia circuit, thereby inducing motor complications, most commonly LID (43–45). In experimental work on animal models, NMDAR signaling via the extracellular regulatory protein kinase (ERK1/2) and mitogen-activated protein kinase (MAPK) pathways regulates neuronal development and refinement of synaptic connections to regulate the progression of LID (46, 47). Phosphorylation levels of several NMDAR subunits are involved in changes in synaptic plasticity, such as the induction of LTP, and play a role in learning and memory formation (48). LTP responds to the storage of various types of information at the synaptic level and is inextricably linked to aspects of learning and memory formation (48). LTP can also be reversed to normal levels by low-frequency stimulation, a phenomenon known as synaptic de-enhancement. This bidirectional regulation of synaptic plasticity is important in regulating motor information storage in the basal ganglia (49). The bidirectional regulation and plasticity of striatal synapses in rats with LID are more severely impaired and the loss of this de-enhancement leads to the storage of abnormal redundant motor information, which triggers the production of redundant movements, a process that is largely dependent on NMDAR-mediated over-activation of LTP (27, 50, 51). Phosphorylation levels of the GluN1 subunit and its carboxy-terminal 890, 896, and 897 serine sites were found to be elevated in the striatum of rats with LID (52). The GluN2 subunit also has an important role in LID. Individuals with PD and LID induced by long-term treatment with L-DOPA exhibit increased expression of GluN2B compared to individuals without LID (53, 54). On the other hand, since the occurrence of LID is related to GluN2A expression and the phosphorylation of ERK/MAPK, the probability of LID in PD rats was reduced by a peptide penetrating the cell membrane and cutting off nine amino acids from the C-terminus of GluN2A, thus inhibiting the expression and phosphorylation of GluN2A (55). In addition, the massive release of Glu upon activation of NMDAR increases excitatory postsynaptic calcium currents, induces LTP, exacerbates abnormal behavior, and promotes the development of LID (56, 57).

Among the NMDAR subunit types expressed by striatal projection neurons, GluN1, GluN2A, and GluN2B are most commonly regulated by phosphorylation modifications; however, GluN2C and GluN2D expression have recently been found to be associated with the development of LID as well (58). The synaptic abundance of GluN2D subunits is selectively increased in the rat striatum, allowing for a dramatic increase in the binding of the postsynaptic protein scaffold PSD-95. PSD-95 expression controls LID via dopamine D1 receptor trafficking (59, 60). Aberrant levels of NMDAR expression and phosphorylation thus play an important role in LID progression and act primarily through the induction of LTP following NMDAR activation, which alters synaptic plasticity between neurons.

4 Role of microglia in LID

Microglia are the main immune cells in brain tissue and are an important component of neuroinflammation when activated. In the resting state, microglia are characterized by small cell bodies and elongated protrusions and are important in immune surveillance (61). Microglia are particularly susceptible to activation during brain injury or in response to inflammatory stimuli, which trigger the development of a neuroinflammatory response in brain tissue. Two main types of activated microglia have been identified: M1 and M2 (62, 63, 64). In basic experiments, M1 microglia release various neurotoxic mediators, including inflammatory cytokines IL-1β, TNF-α, iNOS, chemokines, NO, and oxygen radicals (62). These toxic mediators activate surrounding microglia and astrocytes in an autocrine or paracrine manner, creating a positive feedback loop that promotes the development of a neuroinflammatory response. These processes damage dopaminergic neurons and accelerate the progression of LID. M2 microglia are referred to as "anti-inflammatory" cells and mainly release anti-inflammatory mediators, including IL-4, IL-10, IL-13, and neuroprotective factors (brain-derived neurotrophic factor [BDNF], nerve growth factor [NGF], epidermal growth factor [EGF], glial-cell line-derived neurotrophic factor [GDNF]), which inhibit neuroinflammatory responses in brain tissue and promote tissue repair, thereby slowing disease progression (62). These phenomena are considered evidence for the activation of microglia in response to different environmental stimuli, through a series of organismic responses (64). Figure 1 depicts the mechanism of microglia-mediated damage to dopaminergic neurons. Since microglia can switch between pro-inflammatory neurotoxic and anti-inflammatory neuroprotective phenotypes, this switch has been suggested to play a very important role in the development of LID (65, 66).

Microglia plays an important role in the regulation of synaptic plasticity. Recent and growing evidence suggests that changes in synaptic plasticity, particularly in LTP and LTD, may manifest through microglia (67). Although synaptic plasticity can manifest in many forms, the most carefully studied process with respect to learning and memory is LTP. LTP can be induced by the activation of NMDA-type Glu receptors, usually through the activity of presynaptic and postsynaptic neurons. Activated glial cells promote the development of LID by releasing inflammatory factors such as IL-1β as well as by upregulating NMDAR expression, which induces LTP and alters the plasticity of synapses between neurons. Current basic research has revealed that inflammatory factors released from microglia in brain tissue activate GluN2B, increase the levels of phosphorylated GluN2B subunit, and activate the downstream PKC/MEK/ERK signaling pathways, thus exacerbating the development of LID (16). The administration of continuous chronic lipopolysaccharide (LPS) stimulation to the rats with LID and analysis of the various NMDAR subtypes in their hippocampal and cortical regions revealed that chronic inflammatory stimulation further increases the release of inflammatory factors, resulting in increased expression of striatal GluN2B (16). It has therefore been speculated that the activation of microglia and astrocytes and the release of inflammatory factors in the striatum could influence the

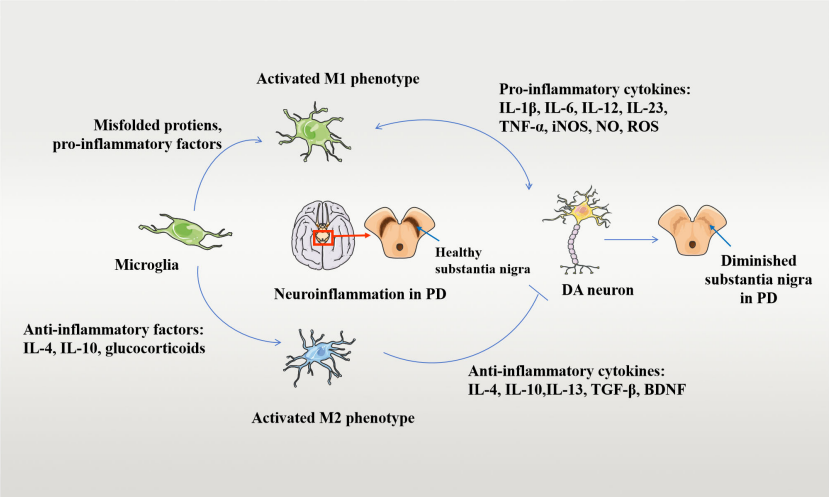

FIGURE 1
Mechanisms of damage to DA neurons by activated microglia. Microglia-mediated neuroinflammation and neuroprotective mechanisms in the pathogenesis of PD. Microglia turns into an activated M1 phenotype when exposed to stimuli such as infection, trauma, and intoxication. M1 phenotype microglia secrete pro-inflammatory factors, which further induce neuroinflammatory and neurotoxic mechanisms in the human brain through processes such as enhanced phagocytic activity and increased IL-1β, TNF-α, and ROS generation, damaging DAergic neurons. On the other hand, the presence and stimulation of anti-inflammatory factors can lead to an activated M2 phenotype. The neuroprotective mechanism of M2 microglia against PD involves the release of anti-inflammatory cytokines into the brain and the upregulation of neuroprotective trophic factors. The anti-inflammatory cytokines include IL-4, IL-10, and so on, which inhibit persistent neuroinflammation and consequently protect the DAergic neurons. Disease occurs when this balance in the organism is disrupted.

development of LID by affecting the activation or expression of this NMDAR. The release of inflammatory factors in an inflammatory rat model drove the activation of GluN2B, thereby allowing the Glu neurotransmitter to bind to GluN2B receptors, which leads to the opening of GluN2B receptor channels and the inward flow of Ca^{2+} ions (68, 69). These processes increase intracellular Ca^{2+} concentrations and subsequently trigger a series of biochemical reactions: G protein mediates the activation of phospholipase C (PLC), which catalyzes the hydrolysis of phosphatidylinositol (PI) to inositol triphosphate (IP3) and diacetylglycerol (DAG), which in turn activates protein kinase C (PKC) in the presence of Ca^{2+} (70–72). PKC not only enhances Ca^{2+}-dependent Glu release and increases the sensitivity of the postsynaptic membrane to the transmitter, but also further enhances the Ca^{2+} influx into the cell via voltage-dependent channels. PKC phosphorylates MEK, which in turn affects the phosphorylation of ERK (73–75). In addition, there is evidence of enhanced phosphorylation of GluN1 in NMDARs in rats with inflammation, but there is a lack of definitive experimental results and literature on the specific signaling pathways involved in GluN1 phosphorylation (76). Thus, the neuroinflammatory response may have a further effect on LID by increasing the expression of subunits of striatal NMDARs (GluN1, GluN2) or by activating their function. In other words, whether neuroinflammation promotes and exacerbates LID by affecting the expression of GluN1 and GluN2 remains an open question. Only one basic experiment exploring these processes has ever been reported (6), and further studies are needed to elucidate the relationships between neuroinflammation, NMDARs, and LID and to provide a basis for finding therapeutic targets for LID.

5 Role of astrocytes in LID

Activated astrocytes are an important component of the neuroinflammatory response in brain tissue and can be classified into A1 and A2 phenotypes, similar to microglia (77, 78, 64). A1-reactive astrocytes are widely seen in neurodegenerative diseases such as Alzheimer's disease and PD and exhibit neurotoxic effects (77, 78). In contrast, the A2 phenotype has neuroprotective effects. A2-reactive astrocytes upregulate the expression of many neurotrophic factors to promote neuronal survival, growth, and differentiation (77, 79). A2-reactive astrocytes also upregulate the expression of anti-inflammatory cytokines, such as transforming growth factor beta (TGF-β), which are involved in synapse formation. However, the mechanism of conversion between the two phenotypes is currently unknown (77, 79). Activated astrocytes, the most abundant neuronal support cells in the human body, can also produce inflammatory cytokines such as IL-1β and TNF-α, while aggravating the damage to dopaminergic neurons and promoting the progression of LID (80). In mice, microglia can also promote the activation of astrocytes. Upon activation, the astrocyte nuclear factor kB (NF-kB) signaling pathway releases large amounts of TNF-α and IL-1β, which amplify the neuroinflammatory response and exacerbate dopaminergic neuronal damage (81). At the same time, high expression of glial fibrillary acidic protein (GFAP), cyclooxygenase-2 (COX-2), and iNOS increase TNF-α and IL-1β in cellular experiments and animal models (82), which can then activate astrocytes and microglia and trigger a neuroinflammatory response (83, 84). It is evident that astrocytes and inflammatory cytokines are closely related, and inflammatory cytokines have been shown to regulate the

activation of astrocytes and microglia through positive feedback in animal models and in patients (85, 86). The morphology and spatial localization of mitochondria in astrocytes are affected by neuroinflammatory stimuli, which may result in excitotoxicity by interfering with the uptake of Ca^{2+}-coupled Glu, affecting dopaminergic neuron survival and promoting the onset of LID. Figure 2 depicts the mechanism of astrocyte damage to dopaminergic neurons.

Astrocytes can regulate intersynaptic D-type serine content, thereby regulating NMDAR activity and neurosynaptic plasticity (87, 88). Astrocyte proliferation via NMDAR signaling in neurons may play an important role in learning memory functions, neurodegenerative diseases, and psychiatric disorders (89, 90). Basic research has shown that astrocytes can sense the synaptic activity of neurons through membrane surface receptors and respond to information from neurons by altering intracellular Ca^{2+} concentrations and releasing gliotransmitters, Glu, ATP, and D-type serine, which regulate adjacent neurons and synaptic plasticity (91). There is also evidence that microglia affect astrocyte activity through ATP release (67). These findings from both *in vitro* and *in vivo* models suggest that astrocytes play a proactive role in the transmission of information between neurons (91, 92). D-type serine released from astrocytes had an active effect on NMDARs in cellular and animal experiments, especially on the GluN2A and GluN2B subunits (93). D-type serine released by astrocytes functions as a co-transmitter or co-agonist of NMDARs in mice (93). In addition, changes in Ca^{2+} concentration in astrocytes may also play an important role in the regulation of NMDAR activity in adjacent neurons (94). Glu enhances TNF-α secretion and GFAP expression in astrocytes and promotes the expression of ionized Ca^{2+}-binding adapter molecule 1 (Iba-1) in microglia (95, 14). In experimental work on animal models, activated astrocytes release TNF-α, possibly through a self-reinforcing mechanism, which enhances the excitability and maintains astrocytes in an activated state, thus promoting LID (14, 95, 96). Therefore, astrocytes act on the development of LID through modulating NMDAR activation and expression, which in turn acts on synaptic plasticity in neurons.

6 Role of NMDARs and microglia-astrocyte interactions in LID

Neuron–microglia–astrocyte interactions play a major role in synaptic plasticity in the neuronal response of postsynaptic neurons to L-DOPA (94, 97). Microglia and astrocytes are major players in the neuroinflammatory response, taking on a dual role between the immune and neuroinflammatory responses (98). The clinical development of LID relies on a cascade of altered pre- and postsynaptic messaging steps, leading to abnormalities in cortical neuronal messaging and abnormal changes in striatal projection neurons. In recent years, the inflammatory response induced by L-DOPA has been further explored. Both microglia and astrocytes express a variety of neurotransmitter receptors and regulate synaptic function at pre- and postsynaptic level by releasing a variety of soluble molecules. L-DOPA can over-activate glial cells, and the long-term presence of abnormally activated microglia and astrocytes leads to abnormal neuron–glia communication, which affects synaptic activity and neuroplasticity and exacerbates LID (14, 99). In response to external stimuli, such as aggregation of α-synuclein or LPS, microglia will rapidly transform into an activated state while releasing large amounts of inflammatory factors which,

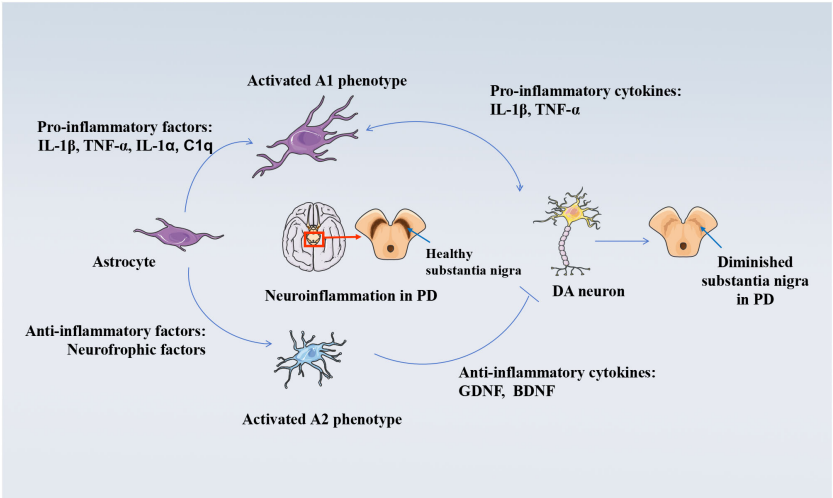

FIGURE 2
Mechanisms of damage to DA neurons by activated astrocytes. Astrocyte-mediated neuroinflammation and neuroprotective mechanisms in the pathogenesis of PD. Upon exposure to inflammatory stimuli, astrocytes become activated A1 phenotype. A1 phenotype astrocytes secrete pro-inflammatory factors such as IL-1β, TNF-α, and other inflammatory factors, which further induce neuroinflammation and neurotoxicity mechanisms in the human brain, damaging DAergic neurons. A1 phenotype astrocytes secrete pro-inflammatory factors such as IL-1β, TNF-α, and other inflammatory factors. On the other hand, the neuroprotective mechanism of activated A2 phenotype astrocytes against PD includes the release of anti-inflammatory cytokines into the brain as well as the upregulation of neuroprotective trophic factors, anti-inflammatory cytokines including TGF-β, which inhibit persistent neuroinflammation and thus protect DAergic neurons. Disease occurs when this balance in the organism is disrupted.

under inflammatory conditions at the site of injury, will further act on another type of glial cell—the astroglia (100). Stimulated astrocytes can activate and release inflammatory factors. These factors, as well as those released by microglia, act simultaneously on dopaminergic neurons and thereby lead to degeneration. Meanwhile, diseased neurons can release large amounts of toxic factors that continuously activate microglia, leaving the body in a state of marked inflammation. This positive feedback process ultimately exacerbates the development of LID both *in vivo* and *in vitro* (100, 101). In brain tissue of mouse models, it has been suggested that there are two main signals for microglia-mediated neurotoxicity: Glu and TNF-α (102). When Toll-like receptors (TLRs) stimulate microglia, they trigger pro-inflammatory programming, TNF-α production, and glutaminase expression, leading to increased Glu secretion (81, 103, 104). High levels of TNF-α and Glu can in turn induce neuronal death by stimulating NMDAR expression in neurons (105, 106). Thus, TNF-α and Glu act as two synergistic inflammatory mediators produced by microglia. TNF-α may also act on astrocytes to induce further production of TNF-α and other inflammatory molecules such as IL-6 and monocyte chemotactic protein 1 (MCP-1) (107). In addition, TNF-α inhibits the uptake of Glu by astrocytes, while astrocytes are the main mediators of Glu clearance in steady-state conditions (108). Abnormally increased Glu in the synaptic gap exacerbates LID by promoting NMDAR overexpression and altering synaptic plasticity (109, 110). Figure 3 depicts the interaction of microglia and astrocytes and the mechanism of damage to dopaminergic neurons.

7 Role of NMDARs and cytokines in LID

Several studies have shown that fluctuating stimulation by L-DOPA will exacerbate inflammation in the striatum when dopamine is in a depleted state (3). The development of LID is accompanied by increased levels of IL-6, IL-12, IL-1β, TNF-α, iNOS, chemokines, NO, COX-2, and reactive oxygen species (ROS) in the dorsolateral striatum (97). Notably, IL-4, IL-10, and neuroprotective factors produced by glial cells are anti-inflammatory and play a very important role in maintaining glial cell and neuronal homeostasis (111). There is evidence that IL-4 and IL-10 can downregulate TNF-α and other pro-inflammatory cytokines and growth factors, which may reduce the occurrence of LID (14). Therefore, promoting the release of anti-inflammatory and neuroprotective factors *in vivo* will be a key research direction for the treatment of LID in the future. As mentioned above, there is increasing evidence that cytokines play a key role in the pathologic process of LID (12), and inhibition of neuroinflammation can downregulate NMDAR and thereby modulate the development of LID. The development of LID is inextricably linked to the activation of glial cells in the striatum and the release of inflammatory factors (TNF-α, IL-1β, and IL-6) (112). The development of neuroinflammation and the release of cytokines (IL-6, IL-12, IL-1β, TNF-α, iNOS, chemokines, NO, COX-2, and ROS) in brain tissues accelerate the development and progression of LID by activating the Glu2/PKC/MEK/ERK signaling pathway and the NF-κB signaling pathway (16).

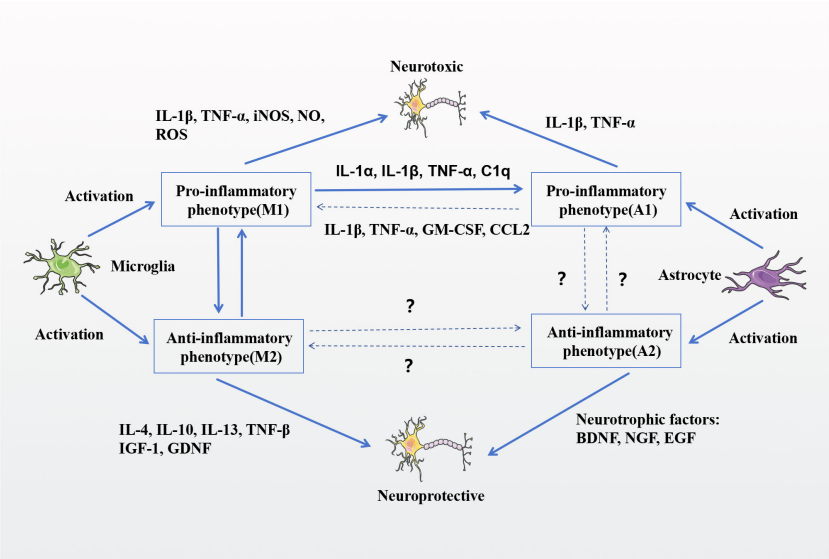

FIGURE 3
Interactions between microglia and astrocytes and mechanisms of damage to DA neurons. Both microglia and astrocytes have differentiated pro-inflammatory phenotypes that are neurotoxic and injurious to DAergic neurons, whereas the neuroprotective phenotypes are neuroprotective and protective of DAergic neurons. Under certain specific circumstances, the pro-inflammatory and anti-inflammatory phenotypes of microglia can be switched. Pro-inflammatory microglia secrete IL-1α, IL-1β, TNF-α, and complement component 1q (C1q), which can convert astrocytes to a pro-inflammatory phenotype. Pro-inflammatory astrocytes secrete IL-1β, TNF-α, Granulocyte-macrophage colony-stimulating factor (GM-CSF), and chemokine C-C motif ligand 2 (CCL2), which in turn activate pro-inflammatory microglia. The phenotypic transition of astrocytes remains to be clarified. Dashed lines with question marks indicate possible relationships, but evidence of a direct association is lacking.

The accumulation of inflammatory factors in brain tissue exacerbates the activation of microglia and astrocytes, releasing more inflammatory factors and neurotransmitters, forming a vicious cycle. Microglia are essentially resident immune cells of the brain and nervous system (113). The release of Glu from microglia is a key component of neuronal damage (114, 115). Cytokines released by microglia, such as TNF-α, promote the release of Glu from astrocytes, which enhances excitotoxicity in neurons (116, 117). Once free Glu is increased in the brain, Glu receptors, such as NMDARs, are overstimulated, and large amounts of Ca^{2+} flow into the cells, thus leading to neuronal injury and death. The increased activity of Glu is thought to play an important role in the development of LID (118). LID may be attenuated by a decrease in glutamatergic function (118). Figure 4 shows interactions of neuroinflammation and NMDARs and the possible sequence of events leading to LID.

8 Role of anti-inflammatory drugs and NMDAR antagonists in anti-LID

Thalidomide and 3,6'-dithiodide have anti-inflammatory effects and are thus able to reduce the neuroinflammatory response in the striatal region of the brain to a certain extent, including reducing abnormal involuntary movement (AIM) behavior in LID rats (11, 119). To date, no studies have confirmed that drugs with anti-inflammatory effects have a direct or indirect effect on NMDAR expression, and the only drug that shows a general clinical improvement in LID and is approved for clinical use is amantadine (120). Amantadine is a low-affinity, non-selective NMDAR antagonist that inhibits striatal NMDAR expression by reducing microglia proliferation, astrocyte GFAP expression, and cytokine release, thereby alleviating the onset of LID (121). Another NMDAR antagonist, ketamine, has recently been found to have a stronger binding affinity than amantadine (122, 123). Ketamine can regulate synaptic transmission and synaptic plasticity by inhibiting NMDAR activity, thereby restoring synaptic function in cortical and hippocampal regions caused by chronic stress. It also exhibits a neuroprotective effect during treatment, accompanied by increased microglia phagocytosis and increased anti-inflammatory factor IL-6 in the striatum, which suggests low-dose ketamine as a potential treatment for LID (124, 125, 126, 123). In addition, the treatment of simvastatin improves cognitive function, anxiety, and depression in MPTP-treated LID mice, restores nerve growth factor IB (Nur77) downstream by reversing the increase in GluN2B expression, and reduces COX-2 and TNF-α (127, 128). A new experimental drug, agmatine, can improve the behavior and AIM scores of LID rats by inhibiting NMDAR expression in the substantia nigra, thereby suppressing the inflammatory and oxidative stress cascades, activating erythroid 2-related factor 2 (Nrf2), and inhibiting the NF-κB signaling pathway to improve the antioxidant, anti-inflammatory, and anti-apoptotic properties of LID (129, 130).

Except for amantadine, there is no medical evidence to suggest that drugs like ketamine, simvastatin, or guanfacine can induce

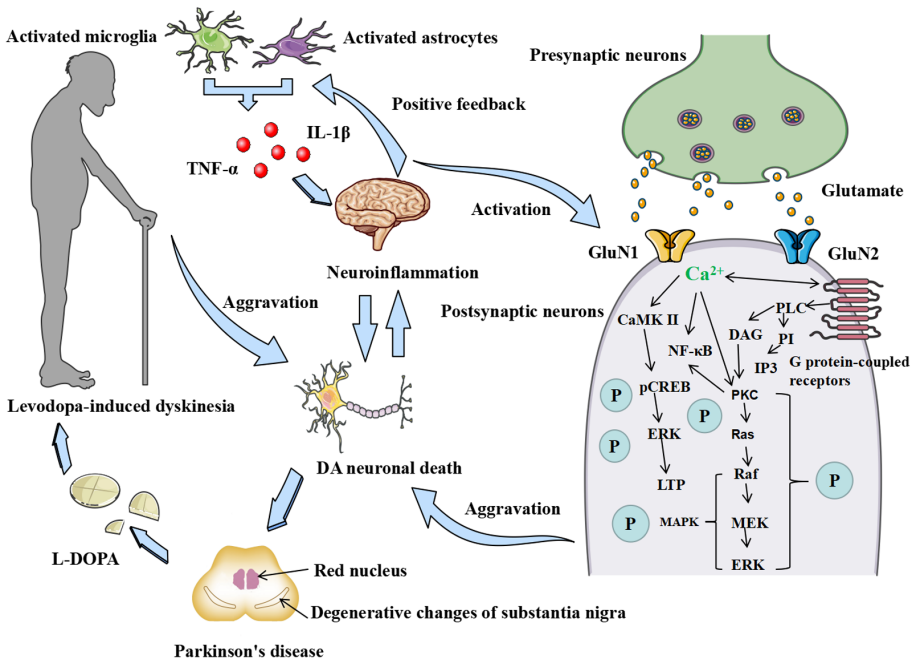

FIGURE 4
Correlations between neuroinflammation, NMDAR, and LID. Mutual activation of microglia and astrocytes and the release of inflammatory factors such as TNF-α and IL-1β. The release of inflammatory factors promotes the expression of the GluN1 and GluN2 subunits of the NMDAR in postsynaptic neurons, allowing the binding of excitatory neuronal Glu and NMDAR released from presynaptic neurons and the inward flow of Ca^{2+} to act on neuronal synaptic plasticity by regulating the LTP and MAPK/ERK phosphorylation pathways through calmodulin-dependent kinase II (CaMKII). The release of inflammatory factors, on the other hand, directly promotes the development of neuroinflammation in the brain, secondary to the death of DA neurons, which leads to the development of PD and the development of LID in patients with PD after years of L-DOPA administration by exacerbating the inflammatory response in the brain tissue and thus creating a vicious circle.

further improvement in neuroinflammation and LID after NMDAR antagonization. Amantadine is therefore the only drug currently used for the clinical treatment of LID. A clinical cohort might help explore the selective use of the aforementioned anti-LID drugs and provide more clinical evidence of their efficacy against LID. Further drugs that can antagonize NMDAR need to be identified, and the mechanisms underlying the relationships between NMDAR, neuroinflammation, and LID need to be examined to optimize the treatment of patients with LID. Table 1 showcases the main references and findings that demonstrate the connection between LID, NMDARs, and neuroinflammation.

9 Summary and outlook

The regular release of dopamine in brain tissue starts fluctuating after long-term administration of L-DOPA to patients with PD. These fluctuations lead to changes in receptors on a variety of neurons in the striatum involved in intracellular information transmission, synaptic plasticity, and other processes, which further disrupt the already unbalanced basal ganglia system. This results in a progressive impairment of motor control and, ultimately, the development of LID. There is a lack of research into the mechanisms underlying LID and associated potential

TABLE 1 Study characteristics. It showcases the main references and findings that demonstrate the connection between LID-NMDAR-neuroinflammation.

Author	Year	Country	Type of Study	Designof Study	Results
Angelopoulou et al.	2021	Greece	_	Review	Fyn kinase may regulate LID, enhanced neuroinflammation and glutamate excitotoxicity by mediating NMDAR axes.
Azar et al.	2022	Egypt	Rat	RCT	Agmatine-mediated inhibition of NMDAR expression and amelioration of dyskinesia with a focus on its anti-inflammatory potentiality.
Bartlett et al.	2020	USA	Rat	RCT	Ketamine is an NMDAR antagonist.The long-term effects of ketamine depend on BDNF signaling in the striatum. And it attenuates the development of LID in rodents.
Bortolanza et al.	2015	Brazil	Rat	RCT	Glu are bound to influence microglial activation states. And LID leads to upregulation of iNOS, GFAP and OX-42-ir.
Carta et al.	2017	Italy	_	Review	LID and neuroinflammation: microglia and astrocytes play a key role.
Cerovic et al.	2015	Italy	Mice	RCT	The Ras-ERK signaling has a central role in striatal LTP, depotentiation, and LTP restored after L-DOPA treatment.
Chen et al.	2022	China	Rat	RCT	Neuroinflammation caused sustained downregulation of synaptic NR2A and NR2B subunits. And anti-inflammatory treatment reversed the downregulation and hypofunction of synaptic NR2A and NR2B.
Gurrera	2019	USA	Human	RCT	Patients with anti-NMDA receptor encephalitis can develop motor dysfunction.
Innes et al.	2019	UK	_	Review	Changes to synaptic plasticity may be mediated by microglial modifications. Microglial production of cytokines may regulate LTP and LTD, thereby underlying the development of disease.
Koh et al.	2022	South Korea	Mice	RCT	Astrocytes regulate NMDAR tone via BEST1-mediated corelease of D-serine and Glu.
Morissette et al.	2022	Canada	Monkeys	RCT	Increased inflammatory markers in the basal ganglia associated with LID and revealed that MPEP inhibition of glutamate activity reduced LID and levels of inflammatory markers.
Pereira et al.	2021	Brazil	Mice	RCT	The release of TNF-α by glutamate-activated astrocytes may contribute to LID by exacerbating corticostriatal glutamatergic inputs excitability and maintaining astrocytes in an activated state through a self-reinforcing mechanism.
Rahman et al.	2022	Australia	Human	RCT	Neuroinflammation may alter NMDAR stoichiometry, and future studies could aim to determine if anti-inflammatory treatment can alleviate this aspect of NMDAR-related pathology.
Rentsch et al.	2020	Australia	Mice	RCT	Amantadine, an NMDAR antagonist, may explore novel features of microglia and astrocyte physiology and pathophysiology and their direct and/or indirect impact on neuronal synaptic signalling in LID.
Thiele et al.	2014	Canada	Mice	RCT	In the LID state, the direct pathway exhibits only LTP, becoming generally overactive, and the indirect pathway exhibits only LTD.
Trudler et al.	2021	California	Mice	RCT	Glutamate release from astrocytes and excessive extrasynaptic NMDAR activity in neurons, thus contributing to Synapse and neuron Loss.
Varley et al.	2019	UK	Human	RCT	The Movement disorder associated with NMDAR antibody-encephalitis is complex and characteristic.

(Continued)

TABLE 1 Continued

Author	Year	Country	Type of Study	Designof Study	Results
Wang et al.	2018	China	Rat	RCT	CaMKIIa-GluN2B interaction had an important role in the development of LID. CaMKII is also associated with inflammatory pathways.
Yan et al.	2021	China	Rat	RCT	Systemic inflammation increases the susceptibility to LID in 6-OHDA lesioned rats by targeting the NR2B-Medicated PKC/MEK/ERK Pathway.
Yuan et al.	2023	China	Rat	RCT	Interventions targeting astrocytes and glutamate transporters may delay LID.

(Randomized Controlled Trial: RCT.).

treatment approaches. Excessive Glu, exerting neurotoxic effects, has been found to be closely related to LID, and NMDAR plays a major role in the pathogenesis of LID due to its high Ca^{2+} permeability, abnormal subunit expression, and phosphorylation levels. In recent years, neuroinflammation has emerged as a hot topic in the study of the pathogenesis of LID. In response to external stimuli, glial cells can be activated into pro- and anti-inflammatory phenotypes, and pro-inflammatory microglia and astrocytes play an important role in neuroinflammation, which in turn increases the expression of striatal NMDAR GluN1 or GluN2 subunits and activates phosphorylation pathways, thereby exacerbating LID. Anti-inflammatory microglia and astrocytes, on the other hand, release anti-inflammatory cytokines and neuroprotective factors that slow down the progression of LID, and the conversion of the two may be a new direction of research for the treatment of LID in the future. The activation of microglia and astrocytes, the interconversion of the two, and the release of neuroinflammatory factors such as TNF-α and IL-1β can directly promote dopaminergic neuronal death as well as stimulate the release of Glu. Both microglia and astrocytes contribute to NMDAR overexpression in postsynaptic neurons and exacerbate LID by mediating LTP and the MEK/ERK/MAPK phosphorylation pathways and altering synaptic neuronal plasticity.

Currently, the most common anti-LID drug is amantadine. While ketamine, simvastatin, guanfacine, and other drugs still have a certain effect on LID, there is a lack of relevant clinical evidence. The development of new drugs and clinical translational applications targeting inflammatory factors, NMDARs, and LID will benefit patients who develop AIM after clinical treatment with L-DOPA drugs, improve their quality of life, and reduce the burden on society.

Author contributions

FZ and ML contributed to the conception and design of this study and collected and reviewed the relevant literature. FZ and ML designed the article structure. FZ wrote the first draft of this manuscript. All authors contributed to the manuscript revision and read and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

References

1. Armstrong MJ, Okun MS. Diagnosis and treatment of Parkinson disease: A review. *Jama* (2020) 323:548–60. doi: 10.1001/jama.2019.22360

2. Cramb KML, Beccano-Kelly D, Cragg SJ, Wade-Martins R. Impaired dopamine release in Parkinson’s disease. *Brain* (2023) 146:3117–32. doi: 10.1093/brain/awad064

3. Channer B, Matt SM, Nickoloff-Bybel EA, Pappa V, Agarwal Y, Wickman J, et al. Dopamine, immunity, and disease. *Pharmacol Rev* (2023) 75:62–158. doi: 10.1124/pharmrev.122.000618

4. Zheng Z, Lv Y, Rong S, Sun T, Chen L. Physical frailty, genetic predisposition, and incident Parkinson disease. *JAMA Neurol* (2023) 80(5):455–61. doi: 10.1001/jamaneurol.2023.0183

5. Reich SG, Savitt JM. Parkinson’s disease. *Med Clin North Am* (2019) 103:337–50. doi: 10.1016/j.mcna.2018.10.014

6. Yan AJ, Song L, Zhang Y, Wang XJ, Liu ZG. Systemic inflammation increases the susceptibility to levodopa-induced dyskinesia in 6-OHDA lesioned rats by targeting the

NR2B-medicated PKC/MEK/ERK pathway. *Front Aging Neurosci* (2021) 12. doi: 10.3389/fnagi.2020.625166

7. Roland KP, Jakobi JM, Powell C, Jones GR. Factors related to functional independence in females with Parkinson's disease: a systematic review. *Maturitas* (2011) 69:304–11. doi: 10.1016/j.maturitas.2011.05.009

8. Gerschlager W, Münchau A, Katzenschlager R, Brown P, Rothwell JC, Quinn N, et al. Natural history and syndromic associations of orthostatic tremor: a review of 41 patients. *Mov Disord* (2004) 19:788–95. doi: 10.1002/mds.20132

9. Tran TN, Vo TNN, Frei K, Truong DD. Levodopa-induced dyskinesia: clinical features, incidence, and risk factors. *J Neural Transm (Vienna)* (2018) 125:1109–17. doi: 10.1007/s00702-018-1900-6

10. Bishop C. Neuroinflammation: fanning the fire of L-dopa-induced dyskinesia. *Movement Disord* (2019) 34:1758–60. doi: 10.1002/mds.27900

11. Boi L, Pisanu A, Greig NH, Scerba MT, Tweedie D, Mulas G, et al. Immunomodulatory drugs alleviate l-dopa-induced dyskinesia in a rat model of Parkinson's disease. *Mov Disord* (2019) 34:1818–30. doi: 10.1002/mds.27799

12. Lanza K, Perkins AE, Deak T, Bishop C. Late aging-associated increases in L-DOPA-induced dyskinesia are accompanied by heightened neuroinflammation in the hemi-parkinsonian rat. *Neurobiol Aging* (2019) 81:190–9. doi: 10.1016/j.neurobiolaging.2019.05.019

13. Zheng CQ, Fan HX, Li XX, Li JJ, Sheng S, Zhang F. Resveratrol alleviates levodopa-induced dyskinesia in rats. *Front Immunol* (2021) 12:683577. doi: 10.3389/fimmu.2021.683577

14. Carta AR, Mulas G, Bortolanza M, Duarte T, Pillai E, Fisone G, et al. l-DOPA-induced dyskinesia and neuroinflammation: do microglia and astrocytes play a role? *Eur J Neurosci* (2017) 45:73–91. doi: 10.1111/ejn.13482

15. Ferrari DP, Bortolanza M, Del Bel EA. Interferon-γ Involvement in the neuroinflammation associated with parkinson's disease and L-DOPA-induced dyskinesia. *Neurotox Res* (2021) 39:705–19. doi: 10.1007/s12640-021-00345-x

16. Yan A, Song L, Zhang Y, Wang X, Liu Z. Systemic inflammation increases the susceptibility to levodopa-induced dyskinesia in 6-OHDA lesioned rats by targeting the NR2B-medicated PKC/MEK/ERK pathway. *Front Aging Neurosci* (2020) 12:625166. doi: 10.3389/fnagi.2020.625166

17. Lindholm D, Mäkelä J, Di Liberto V, Mudò G, Belluardo N, Eriksson O, et al. Current disease modifying approaches to treat Parkinson's disease. *Cell Mol Life Sci* (2016) 73:1365–79. doi: 10.1007/s00018-015-2101-1

18. Bandopadhyay R, Mishra N, Rana R, Kaur G, Ghoneim MM, Alshehri S, et al. Molecular mechanisms and therapeutic strategies for levodopa-induced dyskinesia in Parkinson's disease: A perspective through preclinical and clinical evidence. *Front Pharmacol* (2022) 13:805388. doi: 10.3389/fphar.2022.805388

19. Chen B, Qin G, Xiao J, Deng X, Lin A, Liu H. Transient neuroinflammation following surgery contributes to long-lasting cognitive decline in elderly rats via dysfunction of synaptic NMDA receptor. *J Neuroinflamm* (2022) 19:181. doi: 10.1186/s12974-022-02528-5

20. Zhang Y, Chu JM, Wong GT. Cerebral glutamate regulation and receptor changes in perioperative neuroinflammation and cognitive dysfunction. *Biomolecules* (2022) 12(4):597. doi: 10.3390/biom12040597

21. Angelopoulou E, Paudel YN, Julian T, Shaikh MF, Piperi C. Pivotal role of Fyn kinase in Parkinson's disease and levodopa-induced dyskinesia: a novel therapeutic target? *Mol Neurobiol* (2021) 58:1372–91. doi: 10.1007/s12035-020-02201-z

22. Rahman T, Purves-Tyson T, Geddes AE, Huang XF, Newell KA, Weickert CS. N-Methyl-d-Aspartate receptor and inflammation in dorsolateral prefrontal cortex in schizophrenia. *Schizophr Res* (2022) 240:61–70. doi: 10.1016/j.schres.2021.11.045

23. Flores AJ, Bartlett MJ, Root BK, Parent KL, Heien ML, Porreca F, et al. The combination of the opioid glycopeptide MMP-2200 and a NMDA receptor antagonist reduced l-DOPA-induced dyskinesia and MMP-2200 by itself reduced dopamine receptor 2-like agonist-induced dyskinesia. *Neuropharmacology* (2018) 141:260–71. doi: 10.1016/j.neuropharm.2018.09.005

24. Dupre KB, Eskow KL, Steiniger A, Kliueva A, Negron GE, Lormand L, et al. Effects of coincident 5-HT1A receptor stimulation and NMDA receptor antagonism on L-DOPA-induced dyskinesia and rotational behaviors in the hemi-parkinsonian rat. *Psychopharmacol (Berl)* (2008) 199:99–108. doi: 10.1007/s00213-008-1135-6

25. Borgkvist A, Lieberman OJ, Sulzer D. Synaptic plasticity may underlie l-DOPA induced dyskinesia. *Curr Opin Neurobiol* (2018) 48:71–8. doi: 10.1016/j.conb.2017.10.021

26. Wang Q, Zhang W. Maladaptive synaptic plasticity in L-DOPA-induced dyskinesia. *Front Neural Circuits* (2016) 10:105. doi: 10.3389/fncir.2016.00105

27. Picconi B, Centonze D, Håkansson K, Bernardi G, Greengard P, Fisone G, et al. Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia. *Nat Neurosci* (2003) 6:501–6. doi: 10.1038/nn1040

28. Prescott IA, Dostrovsky JO, Moro E, Hodaie M, Lozano AM, Hutchison WD. Levodopa enhances synaptic plasticity in the substantia nigra pars reticulata of Parkinson's disease patients. *Brain* (2009) 132:309–18. doi: 10.1093/brain/awn322

29. Varley JA, Webb AJS, Balint B, Fung VSC, Sethi KD, Tijssen MAJ, et al. The Movement disorder associated with NMDAR antibody-encephalitis is complex and characteristic: an expert video-rating study. *J Neurol Neurosurg Psychiatry* (2019) 90:724–6. doi: 10.1136/jnnp-2018-318584

30. Culberson JW, Kopel J, Sehar U, Reddy PH. Urgent needs of caregiving in ageing populations with Alzheimer's disease and other chronic conditions: Support our loved ones. *Ageing Res Rev* (2023) 90:102001. doi: 10.1016/j.arr.2023.102001

31. Leal TP, Rao SC, French-Kwawu JN, Gouveia MH, Borda V, Bandres-Ciga S, et al. X-chromosome association study in latin american cohorts identifies new loci in Parkinson's disease. *Mov Disord* (2023) 38(9):1625–35. doi: 10.1002/mds.29508

32. Chung SJ, Yoo HS, Shin NY, Park YW, Lee HS, Hong JM, et al. Perivascular spaces in the basal ganglia and long-term motor prognosis in newly diagnosed Parkinson disease. *Neurology* (2021) 96:e2121–31. doi: 10.1212/WNL.0000000000011797

33. Scarduzio M, Hess EJ, Standaert DG, Eskow Jaunarajs KL. Striatal synaptic dysfunction in dystonia and levodopa-induced dyskinesia. *Neurobiol Dis* (2022) 166:105650. doi: 10.1016/j.nbd.2022.105650

34. Calon F, Rajput AH, Hornykiewicz O, Bédard PJ, Di Paolo T. Levodopa-induced motor complications are associated with alterations of glutamate receptors in Parkinson's disease. *Neurobiol Dis* (2003) 14:404–16. doi: 10.1016/j.nbd.2003.07.003

35. Perszyk RE, Zheng ZS, Banke TG, Zhang J, Xie LL, Mcdaniel MJ, et al. The negative allosteric modulator EU1794-4 reduces single-channel conductance and Ca2+ Permeability of GluN1/GluN2A N-methyl-D-aspartate receptors. *Mol Pharmacol* (2021) 99:399–411. doi: 10.1124/molpharm.120.000218

36. Pokkula S, Thakur SR. Icariin ameliorates partial sciatic nerve ligation induced neuropathic pain in rats: an evidence of in silico and in *vivo* studies. *J Pharm Pharmacol* (2021) 73:874–80. doi: 10.1093/jpp/rgab021

37. Loureiro CM, Shuhama R, Fachim HA, Menezes PR, Del-Ben CM, Louzada P. Low plasma concentrations of N-methyl-D-aspartate receptor subunits as a possible biomarker for psychosis. *Schizophr Res* (2018) 202:55–63. doi: 10.1016/j.schres.2018.06.037

38. Lee J, Kim C, Yeom HD, Nguyen KVA, Eom S, Lee S, et al. Subunit-specific effects of poricoic acid A on NMDA receptors. *Pharmacol Rep* (2020) 72:472–80. doi: 10.1007/s43440-019-00036-7

39. Xiong L, Sun CF, Zhang J, Gao YB, Wang LF, Zuo HY, et al. Microwave exposure impairs synaptic plasticity in the rat hippocampus and PC12 cells through over-activation of the NMDA receptor signaling pathway. *Biomed Environ Sci* (2015) 28:13–24. doi: 10.3967/bes2015.002

40. Fouad IA, Sharaf NM, Abdelghany RM, El Sayed NSE. Neuromodulatory effect of thymoquinone in attenuating glutamate-mediated neurotoxicity targeting the amyloidogenic and apoptotic pathway. *Front Neurol* (2018) 9. doi: 10.3389/fneur.2018.00236

41. Swaminathan M, Hill-Yardin EL, Bornstein JC, Foong JPP. Endogenous glutamate excites myenteric calbindin neurons by activating group I metabotropic glutamate receptors in the mouse colon. *Front Neurosci* (2019) 13. doi: 10.3389/fnins.2019.00426

42. Verma M, Lizama BN, Chu CT. Excitotoxicity, calcium and mitochondria: a triad in synaptic neurodegeneration. *Transl Neurodegener* (2022) 11:3. doi: 10.1186/s40035-021-00278-7

43. Bagetta V, Ghiglieri V, Sgobio C, Calabresi P, Picconi B. Synaptic dysfunction in Parkinson's disease. *Biochem Soc Trans* (2010) 38:493–7. doi: 10.1042/BST0380493

44. Finlay C, Duty S. Therapeutic potential of targeting glutamate receptors in Parkinson's disease. *J Neural Transm (Vienna)* (2014) 121:861–80. doi: 10.1007/s00702-014-1176-4

45. Morin N, Di Paolo T. Pharmacological treatments inhibiting levodopa-induced dyskinesias in MPTP-lesioned monkeys: brain glutamate biochemical correlates. *Front Neurol* (2014) 5:144. doi: 10.3389/fneur.2014.00144

46. Krapivinsky G, Krapivinsky L, Manasian Y, Ivanov A, Tyzio R, Pellegrino C, et al. The NMDA receptor is coupled to the ERK pathway by a direct interaction between NR2B and RasGRF1. *Neuron* (2003) 40:775–84. doi: 10.1016/S0896-6273(03)00645-7

47. Morin N, Morissette M, Grégoire L, Di Paolo T. mGlu5, dopamine D2 and adenosine A2A receptors in L-DOPA-induced dyskinesias. *Curr Neuropharmacol* (2016) 14:481–93. doi: 10.2174/1570159X14666151201185652

48. Lee H-K, Barbarosie M, Kameyama K, Bear MF, Huganir RL. Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature* (2000) 405:955–9. doi: 10.1038/35016089

49. Calabrese V, Di Maio A, Marino G, Cardinale A, Natale G, De Rosa A, et al. Rapamycin, by inhibiting mTORC1 signaling, prevents the loss of striatal bidirectional synaptic plasticity in a rat model of L-DOPA-induced dyskinesia. *Front Aging Neurosci* (2020) 12. doi: 10.3389/fnagi.2020.00230

50. Thiele SL, Chen B, Lo C, Gertler TS, Warre R, Surmeier JD, et al. Selective loss of bi-directional synaptic plasticity in the direct and indirect striatal output pathways accompanies generation of parkinsonism and l-DOPA induced dyskinesia in mouse models. *Neurobiol Dis* (2014) 71:334–44. doi: 10.1016/j.nbd.2014.08.006

51. Cerovic M, Bagetta V, Pendolino V, Ghiglieri V, Fasano S, Morella I, et al. Derangement of Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1) and extracellular signal-regulated kinase (ERK) dependent striatal plasticity in L-DOPA-induced dyskinesia. *Biol Psychiatry* (2015) 77:106–15. doi: 10.1016/j.biopsych.2014.04.002

52. Ba M, Kong M, Yang H, Ma G, Lu G, Chen S, et al. Changes in subcellular distribution and phosphorylation of GluR1 in lesioned striatum of 6-hydroxydopamine-lesioned and l-dopa-treated rats. *Neurochem Res* (2006) 31:1337–47. doi: 10.1007/s11064-006-9177-9

53. Quintana A, Melon C, Kerkerian-Le Goff L, Salin P, Savasta M, Sgambato-Faure V. Forelimb dyskinesia mediated by high-frequency stimulation of the subthalamic nucleus is linked to rapid activation of the NR2B subunit of N-methyl-D-aspartate receptors. *Eur J Neurosci* (2010) 32:423–34. doi: 10.1111/j.1460-9568.2010.07290.x

54. Quintana A, Sgambato-Faure V, Savasta M. Effects of L-DOPA and STN-HFS dyskinesiogenic treatments on NR2B regulation in basal ganglia in the rat model of Parkinson's disease. *Neurobiol Dis* (2012) 48:379–90. doi: 10.1016/j.nbd.2012.06.009

55. Jindal A, Rajagopal S, Winter L, Miller JW, Jacobsen DW, Brigman J, et al. Hyperhomocysteinemia leads to exacerbation of ischemic brain damage: Role of GluN2A NMDA receptors. *Neurobiol Dis* (2019) 127:287–302. doi: 10.1016/j.nbd.2019.03.012

56. Shen W, Plotkin JL, Francardo V, Ko WK, Xie Z, Li Q, et al. M4 muscarinic receptor signaling ameliorates striatal plasticity deficits in models of L-DOPA-induced dyskinesia. *Neuron* (2015) 88:762–73. doi: 10.1016/j.neuron.2015.10.039

57. Shen W, Plotkin JL, Francardo V, Ko WK, Xie Z, Li Q, et al. M4 muscarinic receptor signaling ameliorates striatal plasticity deficits in models of L-DOPA-induced dyskinesia. *Neuron* (2016) 90:1139. doi: 10.1016/j.neuron.2016.05.017

58. Mellone M, Zianni E, Stanic J, Campanelli F, Marino G, Ghiglieri V, et al. NMDA receptor GluN2D subunit participates to levodopa-induced dyskinesia pathophysiology. *Neurobiol Dis* (2019) 121:338–49. doi: 10.1016/j.nbd.2018.09.021

59. Amani M, Zolghadrnasab M, Salari AA. NMDA receptor in the hippocampus alters neurobehavioral phenotypes through inflammatory cytokines in rats with sporadic Alzheimer-like disease. *Physiol Behav* (2019) 202:52–61. doi: 10.1016/j.physbeh.2019.01.005

60. Porras G, Berthet A, Dehay B, Li Q, Ladepeche L, Normand E, et al. PSD-95 expression controls L-DOPA dyskinesia through dopamine D1 receptor trafficking. *J Clin Invest* (2012) 122:3977–89. doi: 10.1172/JCI59426

61. Schramm E, Waisman A. Microglia as central protagonists in the chronic stress response. *Neurol Neuroimmunol Neuroinflamm* (2022) 9(6):e200023. doi: 10.1212/NXI.0000000000200023

62. Paolicelli RC, Sierra A, Stevens B, Tremblay ME, Aguzzi A, Ajami B, et al. Microglia states and nomenclature: A field at its crossroads. *Neuron* (2022) 110:3458–83. doi: 10.1016/j.neuron.2022.10.020

63. Caffarel MM, Braza MS. Microglia and metastases to the central nervous system: victim, ravager, or something else? *J Exp Clin Cancer Res* (2022) 41:327. doi: 10.1186/s13046-022-02535-7

64. Xu J, Farsad HL, Hou Y, Barclay K, Lopez BA, Yamada S, et al. Human striatal glia differentially contribute to AD- and PD-specific neurodegeneration. *Nat Aging* (2023) 3:346–65. doi: 10.1038/s43587-023-00363-8

65. Nascimento GC, Santos BM, Pedrazzi JF, Silva-Amaral D, Bortolanza M, Harris GT, et al. Effects of hydrogen gas inhalation on L-DOPA-induced dyskinesia. *Brain Behav Immun Health* (2023) 30:100623. doi: 10.1016/j.bbih.2023.100623

66. Lecours C, St-Pierre MK, Picard K, Bordeleau M, Bourque M, Awogbindin IO, et al. Levodopa partially rescues microglial numerical, morphological, and phagolysosomal alterations in a monkey model of Parkinson's disease. *Brain Behav Immun* (2020) 90:81–96. doi: 10.1016/j.bbi.2020.07.044

67. Innes S, Pariante CM, Borsini A. Microglial-driven changes in synaptic plasticity: A possible role in major depressive disorder. *Psychoneuroendocrinology* (2019) 102:236–47. doi: 10.1016/j.psyneuen.2018.12.233

68. Vikman KS, Rycroft BK, Christie MJ. Switch to Ca2+-permeable AMPA and reduced NR2B NMDA receptor-mediated neurotransmission at dorsal horn nociceptive synapses during inflammatory pain in the rat. *J Physiol* (2008) 586:515–27. doi: 10.1113/jphysiol.2007.145581

69. Dedek A, Xu J, Kandegedara CM, Lorenzo L, Godin AG, De Koninck Y, et al. Loss of STEP61 couples disinhibition to N-methyl-d-aspartate receptor potentiation in rodent and human spinal pain processing. *Brain* (2019) 142:1535–46. doi: 10.1093/brain/awz105

70. Guo W, Wei F, Zou S, Robbins MT, Sugiyo S, Ikeda T, et al. Group I metabotropic glutamate receptor NMDA receptor coupling and signaling cascade mediate spinal dorsal horn NMDA receptor 2B tyrosine phosphorylation associated with inflammatory hyperalgesia. *J Neurosci* (2004) 24:9161–73. doi: 10.1523/JNEUROSCI.3422-04.2004

71. Bu F, Tian H, Gong S, Zhu Q, Xu GY, Tao J, et al. Phosphorylation of NR2B NMDA subunits by protein kinase C in arcuate nucleus contributes to inflammatory pain in rats. *Sci Rep* (2015) 5:15945. doi: 10.1038/srep15945

72. Litosch I. Regulating G protein activity by lipase-independent functions of phospholipase C. *Life Sci* (2015) 137:116–24. doi: 10.1016/j.lfs.2015.07.025

73. Park ES, Kim SY, Youn DH. NMDA receptor, PKC and ERK prevent fos expression induced by the activation of group I metabotropic glutamate receptors in the spinal trigeminal subnucleus oralis. *Mol Cells* (2010) 30:461–6. doi: 10.1007/s10059-010-0140-x

74. Liu Y, Zhao N, Li C, Chang Q, Liu X, Liao Y, et al. Longistyline C acts antidepressant in *vivo* and neuroprotection in *vitro* against glutamate-induced cytotoxicity by regulating NMDAR/NR2B-ERK pathway in PC12 cells. *PloS One* (2017) 12:e0183702. doi: 10.1371/journal.pone.0183702

75. Jiang S, Li X, Jin W, Duan X, Bo L, Wu J, et al. Ketamine-induced neurotoxicity blocked by N-Methyl-d-aspartate is mediated through activation of PKC/ERK pathway in developing hippocampal neurons. *Neurosci Lett* (2018) 673:122–31. doi: 10.1016/j.neulet.2018.02.051

76. Peng JM, Xu LS, Zhu Q, Gong S, Yu XM, Guo SY, et al. Enhanced NMDA receptor NR1 phosphorylation and neuronal activity in the arcuate nucleus of hypothalamus following peripheral inflammation. *Acta Pharmacol Sin* (2011) 32:160–6. doi: 10.1038/aps.2010.190

77. Kwon HS, Koh SH. Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. *Transl Neurodegener* (2020) 9:42. doi: 10.1186/s40035-020-00221-2

78. Qiu YM, Zhang CL, Chen AQ, Wang HL, Zhou YF, Li YN, et al. Immune cells in the BBB disruption after acute ischemic stroke: targets for immune therapy? *Front Immunol* (2021) 12:678744. doi: 10.3389/fimmu.2021.678744

79. Li T, Chen X, Zhang C, Zhang Y, Yao W. An update on reactive astrocytes in chronic pain. *J Neuroinflamm* (2019) 16:140. doi: 10.1186/s12974-019-1524-2

80. Fonteles AA, Neves JCS, Menezes APF, Pereira JF, Silva ATA, Cunha RA, et al. ATP signaling controlling dyskinesia through P2X7 receptors. *Front Mol Neurosci* (2020) 13:111. doi: 10.3389/fnmol.2020.00111

81. Wang Y, Li L, Wu Y, Zhang S, Ju Q, Yang Y, et al. CD44 deficiency represses neuroinflammation and rescues dopaminergic neurons in a mouse model of Parkinson's disease. *Pharmacol Res* (2022) 177:106133. doi: 10.1016/j.phrs.2022.106133

82. Pereira MD, Abreu GHD, Rocca J, Hamadat S, Raisman-Vozari R, Michel PP, et al. Contributive role of TNF-alpha to L-DOPA-induced dyskinesia in a unilateral 6-OHDA lesion model of Parkinson's disease. *Front Pharmacol* (2021) 11. doi: 10.3389/fphar.2020.617085

83. Russ K, Teku G, Bousset L, Redeker V, Piel S, Savchenko E, et al. TNF-α and α-synuclein fibrils differently regulate human astrocyte immune reactivity and impair mitochondrial respiration. *Cell Rep* (2021) 34:108895. doi: 10.1016/j.celrep.2021.108895

84. Liu Y, Liu T, Zhou Y, Li W, Wang M, Song N, et al. Impeding the combination of astrocytic ASCT2 and NLRP3 by talniflumate alleviates neuroinflammation in experimental models of Parkinson's disease. *Acta Pharm Sin B* (2023) 13(2):662–77. doi: 10.1016/j.apsb.2022.07.021

85. Lee Y, Lee S, Chang SC, Lee J. Significant roles of neuroinflammation in Parkinson's disease: therapeutic targets for PD prevention. *Arch Pharm Res* (2019) 42:416–25. doi: 10.1007/s12272-019-01133-0

86. Zhang W, Xiao D, Mao Q, Xia H. Role of neuroinflammation in neurodegeneration development. *Signal Transduct Target Ther* (2023) 8:267. doi: 10.1038/s41392-023-01486-5

87. Trudler D, Sanz-Blasco S, Eisele YS, Ghatak S, Bodhinathan K, Akhtar MW, et al. α-synuclein oligomers induce glutamate release from astrocytes and excessive extrasynaptic NMDAR activity in neurons, thus contributing to synapse loss. *J Neurosci* (2021) 41:2264–73. doi: 10.1523/JNEUROSCI.1871-20.2020

88. Baier MP, Nagaraja RY, Yarbrough HP, Owen DB, Masingale AM, Ranjit R, et al. Selective ablation of Sod2 in astrocytes induces sex-specific effects on cognitive function, d-serine availability, and astrogliosis. *J Neurosci* (2022) 42:5992–6006. doi: 10.1523/JNEUROSCI.2543-21.2022

89. Lavaur J, Le Nogue D, Lemaire M, Pype J, Farjot G, Hirsch EC, et al. The noble gas xenon provides protection and trophic stimulation to midbrain dopamine neurons. *J Neurochem* (2017) 142:14–28. doi: 10.1111/jnc.14041

90. Zhou J, Geng Y, Su T, Wang Q, Ren Y, Zhao J, et al. NMDA receptor-dependent prostaglandin-endoperoxide synthase 2 induction in neurons promotes glial proliferation during brain development and injury. *Cell Rep* (2022) 38:110557. doi: 10.1016/j.celrep.2022.110557

91. Koh W, Park M, Chun YE, Lee J, Shim HS, Park MG, et al. Astrocytes render memory flexible by releasing D-serine and regulating NMDA receptor tone in the hippocampus. *Biol Psychiatry* (2022) 91:740–52. doi: 10.1016/j.biopsych.2021.10.012

92. Park A, Croset V, Otto N, Agarwal D, Treiber CD, Meschi E, et al. Gliotransmission of D-serine promotes thirst-directed behaviors in Drosophila. *Curr Biol* (2022) 32:3952–3970.e8. doi: 10.1016/j.cub.2022.07.038

93. Bodner O, Radzishevsky I, Foltyn VN, Touitou A, Valenta AC, Rangel IF, et al. D-serine signaling and NMDAR-mediated synaptic plasticity are regulated by system A-type of glutamine/D-serine dual transporters. *J Neurosci* (2020) 40:6489–502. doi: 10.1523/JNEUROSCI.0801-20.2020

94. Lalo U, Bogdanov A, Pankratov Y. Diversity of astroglial effects on aging- and experience-related cortical metaplasticity. *Front Mol Neurosci* (2018) 11:239. doi: 10.3389/fnmol.2018.00239

95. Bortolanza M, Cavalcanti-Kiwiatkoski R, Padovan-Neto FE, Da-Silva CA, Mitkovski M, Raisman-Vozari R, et al. Glial activation is associated with l-DOPA induced dyskinesia and blocked by a nitric oxide synthase inhibitor in a rat model of Parkinson's disease. *Neurobiol Dis* (2015) 73:377–87. doi: 10.1016/j.nbd.2014.10.017

96. Ohlin KE, Francardo V, Lindgren HS, Sillivan SE, O'sullivan SS, Luksik AS, et al. Vascular endothelial growth factor is upregulated by L-dopa in the parkinsonian brain: implications for the development of dyskinesia. *Brain* (2011) 134:2339–57. doi: 10.1093/brain/awr165

97. Hanslik KL, Marino KM, Ulland TK. Modulation of glial function in health, aging, and neurodegenerative disease. *Front Cell Neurosci* (2021) 15:718324. doi: 10.3389/fncel.2021.718324

98. Stevenson R, Samokhina E, Rossetti I, Morley JW, Buskila Y. Neuromodulation of glial function during neurodegeneration. *Front Cell Neurosci* (2020) 14:278. doi: 10.3389/fncel.2020.00278

99. Yuan Y, Zhang X, Wu Y, Lian P, Cao X, Xu Y. ONO-2506 can delay levodopa-induced dyskinesia in the early stage. *Neuroscience* (2023) 523:61–79. doi: 10.1016/j.neuroscience.2023.02.004

100. Saijo K, Winner B, Carson CT, Collier JG, Boyer L, Rosenfeld MG, et al. A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death. *Cell* (2009) 137:47–59. doi: 10.1016/j.cell.2009.01.038

101. Absinta M, Maric D, Gharagozloo M, Garton T, Smith MD, Jin J, et al. A lymphocyte-microglia-astrocyte axis in chronic active multiple sclerosis. *Nature* (2021) 597:709–14. doi: 10.1038/s41586-021-03892-7

102. Keaney J, Gasser J, Gillet G, Scholz D, Kadiu I. Inhibition of bruton's tyrosine kinase modulates microglial phagocytosis: therapeutic implications for Alzheimer's disease. *J Neuroimmune Pharmacol* (2019) 14:448–61. doi: 10.1007/s11481-019-09839-0

103. Takeuchi H, Mizuno T, Zhang G, Wang J, Kawanokuchi J, Kuno R, et al. Neuritic beading induced by activated microglia is an early feature of neuronal dysfunction toward neuronal death by inhibition of mitochondrial respiration and axonal transport. *J Biol Chem* (2005) 280:10444–54. doi: 10.1074/jbc.M413863200

104. Takeuchi H, Jin S, Wang J, Zhang G, Kawanokuchi J, Kuno R, et al. Tumor necrosis factor-alpha induces neurotoxicity via glutamate release from hemichannels of activated microglia in an autocrine manner. *J Biol Chem* (2006) 281:21362–8. doi: 10.1074/jbc.M600504200

105. Choi DW. Glutamate neurotoxicity and diseases of the nervous system. *Neuron* (1988) 1:623–34. doi: 10.1016/0896-6273(88)90162-6

106. Sriram K, Matheson JM, Benkovic SA, Miller DB, Luster MI, O'callaghan JP. Mice deficient in TNF receptors are protected against dopaminergic neurotoxicity: implications for Parkinson's disease. *FASEB J* (2002) 16:1474–6. doi: 10.1096/fj.02-0216fje

107. Kaushal V, Schlichter LC. Mechanisms of microglia-mediated neurotoxicity in a new model of the stroke Penumbra. *J Neurosci* (2008) 28:2221–30. doi: 10.1523/JNEUROSCI.5643-07.2008

108. Triviño JJ, Von Bernhardi R. The effect of aged microglia on synaptic impairment and its relevance in neurodegenerative diseases. *Neurochem Int* (2021) 144:104982. doi: 10.1016/j.neuint.2021.104982

109. Wang XS, Zhang ZR, Zhang XR, Chen SY, Shao B, Xie CL. Modulation of CaMKIIa-GluN2B interaction in levodopa-induced dyskinesia in 6-OHDA-lesioned Parkinson's rats. *BioMed Pharmacother* (2018) 107:769–76. doi: 10.1016/j.biopha.2018.08.062

110. Shen W, Ren W, Zhai S, Yang B, Vanoye CG, Mitra A, et al. Striatal Kir2 K+ channel inhibition mediates the antidyskinetic effects of amantadine. *J Clin Invest* (2020) 130:2593–601. doi: 10.1172/JCI133398

111. Calvello R, Cianciulli A, Nicolardi G, De Nuccio F, Giannotti L, Salvatore R, et al. Vitamin D treatment attenuates neuroinflammation and dopaminergic neurodegeneration in an animal model of Parkinson's disease, shifting M1 to M2 microglia responses. *J Neuroimmune Pharmacol* (2017) 12:327–39. doi: 10.1007/s11481-016-9720-7

112. Lane EL, Soulet D, Vercammen L, Cenci MA, Brundin P. Neuroinflammation in the generation of post-transplantation dyskinesia in Parkinson's disease. *Neurobiol Dis* (2008) 32:220–8. doi: 10.1016/j.nbd.2008.06.011

113. Kim YS, Joh TH. Microglia, major player in the brain inflammation: their roles in the pathogenesis of Parkinson's disease. *Exp Mol Med* (2006) 38:333–47. doi: 10.1038/emm.2006.40

114. Takaki J, Fujimori K, Miura M, Suzuki T, Sekino Y, Sato K. L-glutamate released from activated microglia downregulates astrocytic L-glutamate transporter expression in neuroinflammation: the 'collusion' hypothesis for increased extracellular L-glutamate concentration in neuroinflammation. *J Neuroinflamm* (2012) 9:275. doi: 10.1186/1742-2094-9-275

115. Beppu K, Kosai Y, Kido MA, Akimoto N, Mori Y, Kojima Y, et al. Expression, subunit composition, and function of AMPA-type glutamate receptors are changed in activated microglia; possible contribution of GluA2 (GluR-B)-deficiency under pathological conditions. *Glia* (2013) 61:881–91. doi: 10.1002/glia.22481

116. Shi P, Li Z, He T, Li N, Xu X, Yu P, et al. Astrocyte-selective STAT3 knockdown rescues methamphetamine withdrawal-disrupted spatial memory in mice via restoring the astrocytic capacity of glutamate clearance in dCA1. *Glia* (2021) 69:2404–18. doi: 10.1002/glia.24046

117. Morales I, Rodriguez M. Self-induced accumulation of glutamate in striatal astrocytes and basal ganglia excitotoxicity. *Glia* (2012) 60:1481–94. doi: 10.1002/glia.22368

118. Shi H, Yang X, Zhao H, Zhang S, Zu J, Zhang W, et al. Ranitidine reduced levodopa-induced dyskinesia by remodeling neurochemical changes in hemiparkinsonian model of rats. *Neuropsychiatr Dis Treat* (2015) 11:1331–7. doi: 10.2147/NDT.S80174

119. Paz RM, Tubert C, Stahl A, Díaz AL, Etchenique R, Murer MG, et al. Inhibition of striatal cholinergic interneuron activity by the Kv7 opener retigabine and the nonsteroidal anti-inflammatory drug diclofenac. *Neuropharmacology* (2018) 137:309–21. doi: 10.1016/j.neuropharm.2018.05.010

120. Cesaroni V, Blandini F, Cerri S. Dyskinesia and Parkinson's disease: animal model, drug targets, and agents in preclinical testing. *Expert Opin Ther Targets* (2022) 26:837–51. doi: 10.1080/14728222.2022.2153036

121. Rentsch P, Stayte S, Egan T, Clark I, Vissel B. Targeting the cannabinoid receptor CB2 in a mouse model of l-dopa induced dyskinesia. *Neurobiol Dis* (2020) 134:104646. doi: 10.1016/j.nbd.2019.104646

122. Bresink I, Danysz W, Parsons CG, Mutschler E. Different binding affinities of NMDA receptor channel blockers in various brain regions–indication of NMDA receptor heterogeneity. *Neuropharmacology* (1995) 34:533–40. doi: 10.1016/0028-3908(95)00017-Z

123. Bartlett MJ, Flores AJ, Ye T, Smidt SI, Dollish HK, Stancati JA, et al. Preclinical evidence in support of repurposing sub-anesthetic ketamine as a treatment for L-DOPA-induced dyskinesia. *Exp Neurol* (2020) 333:113413. doi: 10.1016/j.expneurol.2020.113413

124. Bresink I, Danysz W, Parsons CG, Mutschler E. Different binding affinities of NMDA receptor channel blockers in various brain regions—Indication of NMDA receptor heterogeneity. *Neuropharmacology* (1995) 34:533–40. doi: 10.1016/0028-3908(95)00017-Z

125. Sherman SJ, Estevez M, Magill AB, Falk T. Case reports showing a long-term effect of subanesthetic ketamine infusion in reducing L-DOPA-induced dyskinesias. *Case Rep Neurol* (2016) 8:53–8. doi: 10.1159/000444278

126. Pottenger AE, Bartlett MJ, Sherman SJ, Falk T, Morrison HW. Evaluation of microglia in a rodent model of Parkinson's disease primed with L-DOPA after sub-anesthetic ketamine treatment. *Neurosci Lett* (2021) 765:136251. doi: 10.1016/j.neulet.2021.136251

127. Delamarre A, Tison F, Li Q, Galitzky M, Rascol O, Bezard E, et al. Assessment of plasma creatine kinase as biomarker for levodopa-induced dyskinesia in Parkinson's disease. *J Neural Transm (Vienna)* (2019) 126:789–93. doi: 10.1007/s00702-019-02015-x

128. Yan J, Xu Y, Zhu C, Zhang L, Wu A, Yang Y, et al. Simvastatin prevents dopaminergic neurodegeneration in experimental parkinsonian models: the association with anti-inflammatory responses. *PloS One* (2011) 6:e20945. doi: 10.1371/journal.pone.0020945

129. Azar YO, Badawi GA, Zaki HF, Ibrahim SM. Agmatine-mediated inhibition of NMDA receptor expression and amelioration of dyskinesia via activation of Nrf2 and suppression of HMGB1/RAGE/TLR4/MYD88/NF-κB signaling cascade in rotenone lesioned rats. *Life Sci* (2022) 311:121049. doi: 10.1016/j.lfs.2022.121049

130. Li X, Zhu J, Tian L, Ma X, Fan X, Luo L, et al. Agmatine protects against the progression of sepsis through the imidazoline I2 receptor-ribosomal S6 kinase 2-nuclear factor-κB signaling pathway. *Crit Care Med* (2020) 48:e40–7. doi: 10.1097/CCM.0000000000004065

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Potential therapeutic effect of olfactory ensheathing cells in neurological diseases: neurodegenerative diseases and peripheral nerve injuries

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Neurological diseases are destructive, mainly characterized by the failure of endogenous repair, the inability to recover tissue damage, resulting in the increasing loss of cognitive and physical function. Although some clinical drugs can alleviate the progression of these diseases, but they lack therapeutic effect in repairing tissue injury and rebuilding neurological function. More and more studies have shown that cell therapy has made good achievements in the application of nerve injury. Olfactory ensheathing cells (OECs) are a special type of glial cells, which have been proved to play an important role as an alternative therapy for neurological diseases, opening up a new way for the treatment of neurological problems. The functional mechanisms of OECs in the treatment of neurological diseases include neuroprotection, immune regulation, axon regeneration, improvement of nerve injury microenvironment and myelin regeneration, which also include secreted bioactive factors. Therefore, it is of great significance to better understand the mechanism of OECs promoting functional improvement, and to recognize the implementation of these treatments and the effective simulation of nerve injury disorders. In this review, we discuss the function of OECs and their application value in the treatment of neurological diseases, and position OECs as a potential candidate strategy for the treatment of nervous system diseases.

KEYWORDS

OECS, neurological diseases, treatment, nerve regeneration, role

1 Introduction

The ability of nerve regeneration and tissue repair is limited after the occurrence of neurological diseases, which can usually lead to permanent disability. Neurological diseases, especially neurodegenerative diseases, describe a clinical condition characterized by selective and progressive neuronal loss. Neurological diseases caused by

progressive loss of neuronal structure or function, and lead to varying degrees of paralysis and cognitive and sensory loss (1). Neurological diseases such as Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS), characterized by progressive loss of the structure, function, or number of neurons in the brain or spinal cord (2, 3). Although the clinical use of some drugs can alleviate the progression of these diseases. New drugs for the treatment of neurological diseases have become urgently needed. Clinicians, patients and their families are waiting for the development of effective drugs for neurological diseases that are basically untreatable at present. The drug development process is complex and expensive, the clinical use of these diseases is limited. Unfortunately, the treatment currently available are not sufficient to prevent neurodegeneration. Therefore, it is necessary to find and explore prospective therapeutic method to repair neurological function and improve symptoms.

With the exploration of treatment methods, researchers have found that cell therapy have developability and broad prospects, and have become the savior of many diseases. The use of cell therapy to treat neurological diseases is based on the assumption that these therapies will mimic the normal process of cell repair and development in the nervous system to eliminate the causes of the disease (4, 5). Cell therapy, also known as regenerative therapy, uses special types of active cells or their derivatives to improve the repair response of dysfunctional and damaged tissue (6, 7). OECs are special glial cells, which can survive and renew in the central nervous system, and have been widely used in nerve regeneration and tissue repair (8, 9). OECs therapy usually focuses on cell substitution or environmental enrichment. Application of OECs therapy provides a valuable and attractive choice for neurological diseases. Transplantation of OECs to the injured nerve can promote regeneration and reconstruct the nerve function (10). The function of OECs in the treatment of neurological diseases includes secreted active biomolecules, such as neurotrophic factors, VEGF, NT-3 and extracellular matrix. The production of these secretions provides a good nutritional basis for axonal regeneration and myelination (11, 12). Moreover, OECs can exert neuroprotection and immune regulation to improve the activity of immune cells in nerve injury, reduce nerve injury and nerve inflammation, and play a therapeutic role (11, 13, 14). All these reflect the potential and sustainability of OECs in the treatment of neurological diseases. Therefore, we focus on the functional role of OECs in neurological diseases and the existing problems as a cell therapy.

2 A brief introduction to the biological characteristics of OECs

OECs are glial cells of the primary olfactory nervous system, which are composed of the olfactory nerve and the outer nerve fiber layer of the olfactory bulb (Figure 1). The olfactory nerve is located between the olfactory epithelium at the top of the nasal cavity and the olfactory bulb of the anterior cranial fossa (Figure 1). The primary olfactory nervous system is unique in that it can constantly regenerate. Even after injury, as long as the deeper olfactory bulb inside the olfactory bulb remains intact, it can regenerate (15, 16). It is now possible to remove olfactory bulb tissue and olfactory mucosa (outermost layer and lamina propria, which belong to the central nervous system and peripheral nervous system, respectively), which also suggests the potential value of OECs therapy in central nervous system and peripheral nervous system diseases. OECs can be successfully cultured *in vitro*, which lays a foundation for the study of OECs in nerve regeneration and tissue repair (17). It is worth mentioning that OECs derived from olfactory mucosa and olfactory bulb express different characteristics, have heterogeneity, and play different roles in the repair of nerve injury (11, 18). OECs derived from olfactory mucosa can regulate the process of inflammation and the formation of extracellular matrix, but the ability of regeneration is poor. While OECs derived from olfactory bulb can promote functional recovery by inducing targeted axonal regeneration (19). Differentially expressed genes and proteins of OECs derived from olfactory bulb play a key role in nerve regeneration, axon regeneration and extension, transmission of nerve impulses and response to axonal injury. Differentially expressed genes and proteins of OECs derived from olfactory mucosa are mainly involved in inflammatory response, defense response, cytokine binding, cell migration and positive regulation of wound healing (20). All these reflect the functional differences of OECs derived from different sites.

During the development of the primary olfactory system, the axons are inaccurately located and inappropriately projected to the target layer or over-projected to the deeper layer of the olfactory bulb. Therefore, there is a large number of apoptosis of primary olfactory neurons during embryonic and postnatal development, and the axons of degenerated neurons need to be removed (21). Phagocytosis of axonal fragments in adult and postnatal animals is known to be essential for the regeneration of the primary olfactory nervous system (21, 22). Studies have shown that the phagocytosis of axonal fragments in postnatal animals. It has been found that macrophages often appear near OECs, but they only play a small role in clearing axonal fragments (23). It is considered that OECs are the primary phagocytes of primary olfactory nerve from the early stage of embryonic development (23, 24).

The regeneration and repair of neurological diseases is a complex process, involving many steps and factors. On the one hand, the key to the repair of nerve injury lies in the survival and function of neurons. On the other hand, the injured axon has the function of bridging the damaged site or the broken end (25, 26). Axon regeneration requires myelin regeneration and functional synaptic reconstruction, neurotrophic delivery, improvement of inflammation and regulation of immune response, which is essential for nerve injury repair (27). Different studies have confirmed that the functional role of OECs in nerve regeneration and tissue repair, and their biological characteristics have been recognized in the following aspects (Figure 2).

1. OECs can produce immune function, secrete immune regulatory molecules and exert their phagocytic activity, maintain the homeostasis of microenvironment and support neuronal survival and axonal growth (28, 29). OECs reduce the infiltration of immune cells and secondary tissue damage, protect neurons and axons in the diseased core, and help remove myelin fragments (30).

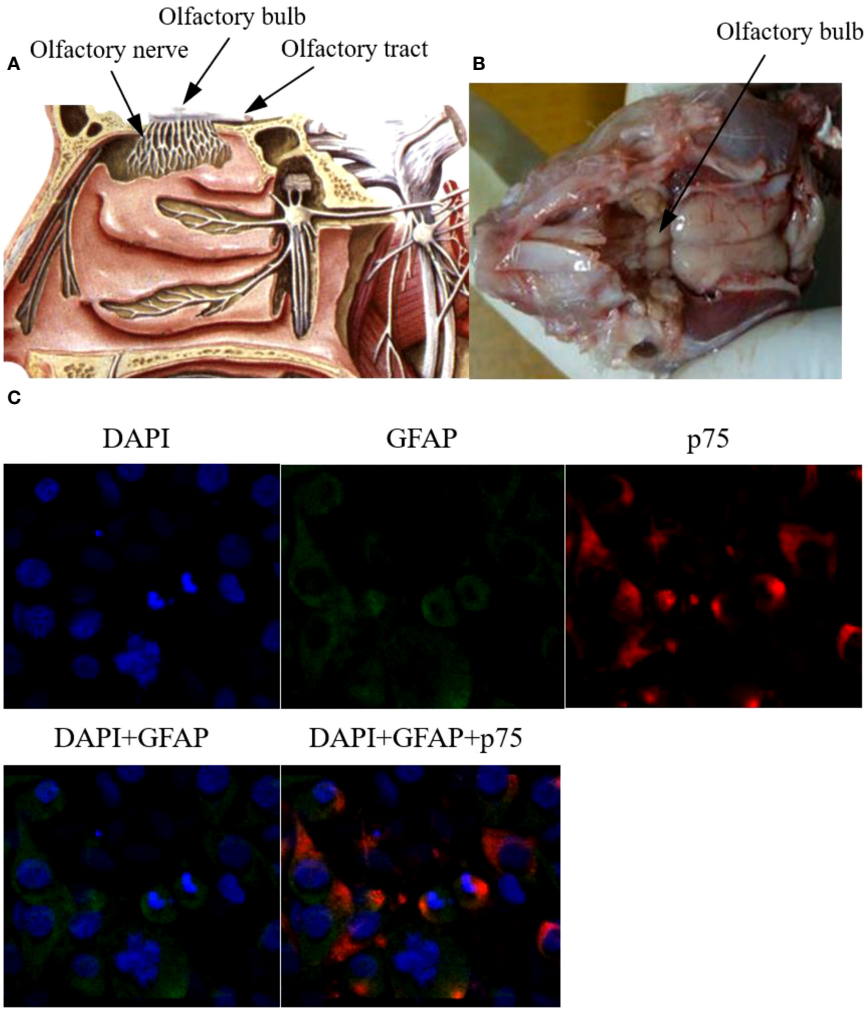

FIGURE 1
Anatomical localization of olfactory bulb and immunofluorescence staining of OECs. **(A)** Anatomical diagram of olfactory bulb tissue, source site of OECs. **(B)** Schematic diagram of olfactory bulb tissue in rats. Olfactory bulb tissues were lysed and digested *in vitro*, and OECs were obtained by culture system *in vitro*. **(C)** Immunofluorescence staining of OECs. GFAP: glial fibrillary acidic protein), p75: a marker for OECs.

Biological characteristics of OECs

- OECs have the characteristics of self-renewal and continuous survival.
- Phagocytosis of OECs: Phagocytosis of axonal fragments and microorganisms, protection of microenvironment stability.
- Promoting axonal regeneration and myelination.
- Heterogeneity of OECs: There are functional differences in different parts of the source (olfactory bulb tissue and olfactory mucosa).
- Immune regulation and neuroprotection: Reduce immune cell infiltration, protect neurons, and reduce tissue damage.
- Migration and athletic ability.
- Secretion group: Secretion of neurotrophic factors, adhesion molecules and extracellular matrix, etc., to provide a good basic environment for nerve regeneration.
- Inhibit the formation of glial scar, guide the newborn axon through the scar area and bridge with the distal axon.

FIGURE 2
OECs play the functional characteristics of promoting nerve regeneration and tissue repair.

2. OECs exert their characteristics of promoting axon growth and provide structural support by extending the thin processes enclosing the axon group as insulators (22, 31). 3. OECs have the ability of migration and movement. OECs can migrate under the physiological and pathological conditions of nervous system, such as inflammation, hypoxia and neurodegenerative diseases, which are closely related to the cytoskeleton of OECs. Analysis of OECs actin cytoskeleton revealed the stress fibers, membrane spinous process, folded membrane and layered fat deposition during cell migration, as well as the distribution and migration ability of α-actin in the membrane process (32–34). 4. Phagocytic activity of OECs. OECs can remove axonal fragments and microorganisms through phenotypic changes, enhance their cytoskeleton hypertrophy and rearrangement, transition from resting state to phagocytic activity, and protect the stability of nervous system microenvironment (35, 36). Studies have shown that OECs have higher phagocytosis and transport capacity than Schwann cells and

produce lower amounts of proinflammatory cytokines (37). 5. OECs can produce bioactive substances such as platelet-derived growth factor, glial-derived connexin, ciliary neurotrophic factor (CNTF), neurotrophic factor Y and cell adhesion molecules (such as L1-nerve cell adhesion molecule (L1-NCAM) and nerve cell adhesion molecule-1 (NCAM1)). These secreted bioactive substances provide a good basic environment for nerve regeneration and repair (10, 38, 39). The secretory group produced by OECs also includes paracrine exosomes and extracellular vesicles, which can protect neurons, reduce neurotoxicity and play a key role in the repair of nerve injury (40, 41). Studies have shown that OECs-derived exosomes can inhibit the polarization of pro-inflammatory macrophages/microglia, increase the number of anti-inflammatory cells, promote neuronal survival and functional recovery after spinal cord injury (42).

3 OECs and neurological diseases

Neurological diseases, including PD, AD, HD and ALS, are caused by disorders of protein homeostasis, characterized by the loss of specific groups of neurons and inclusion bodies consisting of insoluble and unfolded proteins. This pathogenic process leads to the gradual loss and paralysis of sensory, cognitive and motor neurons (43, 44). Although great progress has been made in the mechanisms of neurological diseases, including the identification of mutated genes that cause these diseases, but the exact mechanism of neuronal death is still unclear and there are still no effective method to slow the progression of these diseases. With the research and exploration of the treatment of neurological diseases, cell therapy has been introduced into the field of vision of researchers and has been greatly encouraged. These applied bioactive cells, including OECs, can protect neurons, reconstruct neural networks and exert therapeutic functions by producing special bioactive factors at the injured site (45–47). The goal of cell therapy in neurological diseases includes obtaining specific neuronal subtypes and rebuilding neural networks similar to those lost in the disease. Another way to treat neurological diseases is to create environmental enrichment to support host neurons by producing neurotrophic factors and removing toxic factors or by establishing auxiliary neural networks around the affected areas (48, 49). Although the application of cell therapy is still in its infancy, it has become a safe and effective new strategy for the treatment of neurological diseases.

3.1 OECs and Alzheimer's disease

AD is associated with selective damage to brain areas and neural circuits critical to cognition and memory, including neurons in the neocortex, hippocampus, amygdala, basal forebrain cholinergic system and monoaminergic nucleus of the brainstem. The pathological process of AD has two typical pathological features: β-amyloid plaque deposition and hyperphosphorylated tau nerve fiber tangles (50). The existing research theory shows that the production of long A β peptide, especially in the form of highly toxic oligomer, leads to the accumulation and deposition of A β in the brain. Aggregated A β causes to neurotoxicity, degeneration of neurofibrils, activation of microglia and ultimately leads to the loss of synapses and neurons (51, 52). Diagnosis of AD is based on compliance with clinical manifestations and other auxiliary examinations, including imaging and biomarkers. The current treatment is symptomatic treatment to alleviate the progression of the disease.

OECs may have certain effects on the potential pathophysiology of nervous system diseases, including neuroplasticity and natural β-amyloid peptide. β-amyloid peptide is the main component of senile plaque peculiar to the brain of AD. The precursor protein of amyloid β protein (A β) is cut into amyloid polypeptide. Aβ 25-35 is considered to be the functional domain of A β and is responsible for its neurotoxic properties (53). *In vitro* studies found that A β may affect the molecular conformation of OECs (54, 55). After OECs were exposed to A β (1-42) fragments, the expression level of transglutaminase Tg2 in OECs increased, and the expression patterns of their subtypes (Tg2-long and Tg2-short) were also different. A β may stimulate nerve regeneration in AD by changing the conformation of Tg2 in OECs (54). Another *in vitro* study showed that A β (1-42) or A β (25-35) increased the expression of Tg2, vimentin and caspase cleavage in OECs. Basic fibroblast growth factor or glial cell line-derived neurotrophic factor can restore the protein level in OECs and play a key role in AD (56). *In vitro* studies have shown that OECs conditioned medium can improve A β (25-35)-induced oxidative damage of cells by inhibiting mitochondrial pathway, increase cell survival rate and produce protective function (53). These studies suggest the relationship between OECs and AD, but more studies are needed to confirm the usefulness of OECs as a treatment for AD.

3.2 OECs and Parkinson's disease

PD caused by loss of dopaminergic neurons in the midbrain. Its pathological features are selective loss of dopaminergic neurons in substantia nigra pars compacta of ventral midbrain and ubiquitin deposition in residual neurons (57, 58). Although PD cannot be cured, there are a variety of pharmacological treatments that can alleviate some of the functional defects caused by dopamine depletion. Similarly, researchers have received consistent recognition through the application and transplantation of special bioactive cells (such as neural stem cells and mesenchymal stem cells) to treat and improve PD. In view of the biological characteristics of OECs, the treatment of transplanted OECs in PD has also achieved exciting results. Indeed, *in vivo* studies have shown that transplantation/injection of OECs into the body can improve the amount of dopamine in the striatum and restore dopaminergic activity, and play a therapeutic role in PD (59). *In vivo* studies have shown that combined transplantation of OECs and vascular endothelial cells results in a significant increase in [3H]-spirocyclone binding rate, dopamine and 3-dihydroxyphenylacetic acid levels in rats, which supports the activity of dopaminergic cells and contributes to PD-induced functional recovery (59). *In vivo* studies have shown that the

transplantation of OECs into the striatum can improve the survival of neurons in the dopaminergic and inhibit the immune response of the transplantation site to improve the survival rate of striatal grafts (60). In addition, OECs can restore the nigra striatal pathway in PD and play a therapeutic role. An *in vivo* study found that OECs could improve the survival rate of grafted ventral mesencephalic tissue and promote the recovery of motor function by promoting the prolongation of dopaminergic and serotonergic axons in bridged grafts (61).

OECs express a variety of factors, such as nuclear receptor related factor 1 (Nurr1), neurogenic protein 2 (Ngn2), NGF, bFGF, GDNF, and NT3, which have neuroprotective effects on PD (59, 62, 63). *in vivo* and *in vitro* studies have shown that transplantation of OECs-Nurr1-Ngn2 can improve behavioral disorder in rats with PD (62). OECs-Nurr1-Ngn2 have significant neuroprotective, antioxidant and anti-apoptotic effects on PD by up-regulating neurotrophin-TrkB pathway (62). In the study of *in vivo* model, these growth factors and adhesion molecules expressed and secreted by OECs can also improve the survival rate of graft-derived dopaminergic neurons, facilitate communication between host and graft, and promote axonal growth (64, 65). *In vivo* studies have shown that OECs not only significantly increase the survival and neurite growth of dopaminergic neurons derived from neural stem cells, but also have a protective effect on 6-OHDA neurotoxicity under co-culture conditions (65).

Although these studies have revealed the role of OECs in the treatment of PD, but OECs transplantation has been challenged. Heterotopic transplantation of cells is unlikely to integrate into normal brain circuits. Even if OECs are transplanted into the primitive sites of PD, such as substantia nigra and striatum, new dopaminergic neurons will not regenerate the axons of striatum. Another challenge is that an obstacle to PD cell replacement therapy is the lack of abundant, reliable and renewable sources of dopaminergic neurons.

3.3 Cell therapy and Huntington's disease

HD is a dominant inherited neurodegenerative disease caused by abnormal amplification of CAG repeats (36 or more) in exon 1 of the Huntington protein gene located on chromosome 4p16.3. Patients with HD mainly showed neuronal degeneration in striatum and cerebral cortex (66, 67). The most important new discovery is the highly variable nature of brain degeneration (68). The pathology of HD includes massive atrophy of the striatum and degeneration of cortical pyramidal neurons. As the development of disease, the loss of neurons becomes more comprehensive, affects many brain regions. Severe cortical loss followed, eventually leading to death (69, 70). Therefore, the use of some special graft replacement therapy to improve these striatum and neuronal degeneration can produce certain protective and therapeutic effects.

Although there are few reports on OECs in the application and treatment of HD. But some studies have found that the transplantation of functional active cells can be used as a potential treatment of HD. The therapeutic mechanisms of these transplanted cells include secreting neurotrophic factors, reducing neuroinflammation, enhancing neuronal plasticity and cell substitution. Importantly, transplanted cells (such as neural stem cells) must properly migrate to the central nervous system and integrate with host neurons to form circuits to enhance neuroplasticity (71, 72). *In vitro* studies have shown that neural stem cells/early neural progenitor cells can be cultured on a large scale and differentiated into neurons, which paves the way for the treatment of HD (73). Some *in vivo* and *in vitro* studies have found that human embryonic or fetal neural stem cells are transplanted into the striatum of HD mice or rats to survive, in some cases, improve functional status (74, 75). Despite these encouraging results, there is a lack of direct evidence and reports on OECs transplantation in the treatment of HD. Therefore, the role of OECs transplantation in HD needs more research to explore. However, cell therapy in the treatment of HD has been recognized by many studies, which is a prospective treatment strategy for the treatment of HD.

3.4 OECs and amyotrophic lateral sclerosis

ALS is a rapidly progressive and fatal neurodegenerative disease. In sporadic ALS, both upper motoneurons (in the cortex) and lower motoneurons (in the spinal cord) degenerate for unknown reasons. Its pathological feature is that it affects motor neurons in the motor cortex, brainstem and spinal cord, resulting in paralysis and death. Mutations were found in familial ALS cases, but these mutations accounted for only about 10% of all cases (76–78). At present, some studies have proposed several pathological mechanisms of ALS-induced motoneuron death, including glutamate-induced excitotoxicity, abnormal cytoskeleton, protein aggregation, oxidative stress, mitochondrial dysfunction and extracellular SOD1 toxicity. Currently, there is no effective treatment for this devastating disease. In recent years, researchers have proposed a therapeutic strategy of reconstructing neural function by transplanting special types of cells to replace these lost or degenerative motor neurons. For example, a clinical trial reported that mesenchymal stem cell transplantation can delay the progression of ALS and improve survival (79). *In vivo* studies have shown that transplantation of motoneurons derived from human umbilical cord mesenchymal stem cells with high expression of BDNF can improve the motor ability and prolong the survival time of mice with ALS (80). Although cell therapy for motor neuron replacement, support or as a carrier of neuroprotective molecules is in preliminary exploration and there are many challenges and problems, however, the beneficial effects of transplantation therapy observed in animal models of motor neuron disease have raised hopes (81).

The application and exploration of OECs transplantation in ALS has also achieved some exciting results. A clinical trial reported that OECs transplantation can reduce the amplitude of motor unit action potential, increase the number of motor unit action potential, and effectively control or reverse the physiological deterioration caused by ALS (82). *In vivo* studies have shown that transplantation of OECs can protect the survival of motor neurons, repair the injured axons with myelin sheath through pyramidal tract, and

improve motor nerve function (83). *In vivo* studies found that OECs could prolong and increase the survival of animals with ALS by giving full play to their ability of neuroprotection and myelin regeneration (84). *In vivo* studies have shown that transplantation of OECs into the brain of rats with ALS can increase the survival and prolong the survival time of motor neurons in the motor cortex and ventral horn of the spinal cord. Behavioral tests, including screen tests, hindlimb stretching, rotating sticks and gait control, have shown that OECs transplantation can improve these symptoms (85). These studies have revealed the therapeutic role of OECs in the progression of ALS and may be used as a potential candidate cell replacement therapy for ALS therapy. But, unfortunately, people soon realized that this is an extremely difficult task because new motor neurons have to project to distant axons and connect with host neurons through neural networks to reconstruct function. The white matter in the central nervous system can produce resistance to supporting axonal growth and some evidence suggests that the toxic environment in the degenerated spinal cord may not support new transplantation substitutes, which is also the key problem to be overcome.

3.5 OECs transplantation and peripheral nerve injury

The process of peripheral nerve injury regeneration is a dynamic process of multicellular regulation, which involves neurons, Schwann cells and immune cells. The interaction between these cells and the molecules regulates the process of peripheral nerve regeneration. It is understood that the peripheral nervous system maintains the same inherent lack of regeneration as the central nervous system, but the difference is that after peripheral nervous system injury, neurons (which can undergo growth phenotypic changes) can produce regenerative axons. this process involves phagocytosis, clearance, secretion of factors including growth and chemokines and myelination of Schwann cells and macrophages (47). The main obstacles to the repair and regeneration of peripheral nerve injury are axonal degeneration, nerve inflammation and demyelination. Therefore, the axons that promote the repair of peripheral nerve injury mainly leads the regeneration to form a new neural connection with the axons at the distal end of the host injury, form a new neural network. Therefore, exploring and finding effective ways to change these pathological processes and make progress in a favorable direction for the repair of nerve injury. Despite the progress of microsurgical technology and the improvement of the understanding of nerve regeneration, there are still some challenges in the repair of peripheral nerve injury (86).

More and more studies have revealed that cell transplantation has been proved to play a great role in the treatment of peripheral nerve injury, which encourages people to explore the broad application and prospect of cell transplantation therapy. As described in the previous biological function of OECs, the mechanism of OECs promoting nerve regeneration is by secreting multiple neurotrophic factors, protecting neurons, inhibiting inflammatory response, and promoting axonal regeneration (10, 87). OECs can guide the establishment of new axons through the injured microenvironment to reach both ends of nerve injury and produce bridging function (26, 88). In addition, OECs transplantation has the ability to migrate and penetrate glial scar (26, 88). *In vivo* studies have shown that the transplantation of OECs effectively promotes the axon regeneration of recurrent laryngeal nerve injury and improves the function of laryngeal muscle (17). *In vivo* studies have shown that OECs are transplanted into the injured sciatic nerve, it is found that OECs can form *Ranvier* nodules of regenerated axonal myelin sheath, increase nerve conduction velocity and improve function (89). In view of the biological characteristics of OECs and the role of repairing injured nerves. Some researchers have made some achievements by combining OECs with other functional active cells or biomaterials to improve the function of OECs in peripheral nerve injury. *In vivo* experiments, the application of PCL catheter or PLGA catheter containing OECs to the site of sciatic nerve resection promoted nerve regeneration and angiogenesis (90, 91). *In vivo* and *in vitro* studies found that OECs were co-cultured with exosomes of human umbilical cord mesenchymal stem cells, these exosomes could promote the survival and migration of OECs under hypoxia, and effectively increased the gene expression and secretion of brain-derived neurotrophic factor (92). Co-transplantation of them into the injured sciatic nerve could promote the regeneration of the injured sciatic nerve and restored the motor and sensory function (92) (Table 1).

It is understood that Schwann cells (SCs) form the myelin sheath of the peripheral nerve, protect and nourish neurons, and play an irreplaceable role in the repair of peripheral nerve injury. There is no transcriptional difference between OECs and SCs. OECs is highly similar to SCs and expresses the biomarker of SCs (94). *In vivo* studies have shown that the transplantation of OECs can regulate the changes of SCs and enhance the repair function of SCs in peripheral nerve injury, and the co-transplantation of the two can play a synergistic role in promoting nerve regeneration (93). It is worth mentioning that OECs have higher phagocytosis and transport ability and produce lower amounts of proinflammatory cytokines than SCs *in vitro*, which may be better than SCs transplantation in the damaged nervous system (37). These studies have confirmed that the functional role of OECs in peripheral nerve injury and may be used as an alternative cell therapy for peripheral nerve injury.

4 Clinical application and current challenges of OECs transplantation in neurological diseases

With the aging of the population and the participation of many factors, the disability and lethality of neurological diseases are increasing, but the clinical treatment of neurological diseases has always been a thorny problem. Therefore, delaying or stopping the progression of these diseases is a major but unmet urgent need. Unfortunately, due to the lack of a considerable amount of direct evidence to explain the pathological process of these diseases and to mine reliable biomarkers, this hinders the study of effective

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TABLE 1 Preclinical study on the application of OECs in neurological diseases.

Cell source	Type	Disease model	Animal	Therapeutic effect.	Refs
Olfactory mucosa or olfactory bulb	*In vivo*	Recurrent laryngeal nerve injury model	Fischer rats	OECs transplantation can effectively promote axonal regeneration of recurrent laryngeal nerve injury and improve laryngeal muscle function.	17
Olfactory bulbs	*In vivo* and *in vitro*	6-hydroxydopamine (6-OHDA)-lesioned rat model of PD	Rats	OECs can maintain dopaminergic activity in striatum, restore D-amphetamine-induced rotational behavior and spontaneous activity, and promote functional recovery in rats.	59
Olfactory bulbs	*In vivo*	PD rat model	Rats	OECs reduced the immune response (mediated by CD3+T cells and OX-6+ microglia) and increased the survival rate of dopaminergic neurons in the striatum.	60
Olfactory bulbs	*In vivo*	PD rat model	Rats	OECs can improve the survival rate of transplanted ventral mesencephalic tissue and promote the recovery of motor function. OECs can promote the prolongation of dopaminergic and serotonergic axons in bridged grafts.	61
Olfactory bulbs	*In vivo* and *in vitro*	PD rat model	SD rats	*In vitro*, it was found that OECs-Nurr1-Ngn2 increased the vitality of PC12 cells and inhibited oxidative stress and apoptosis. Transplantation of OECs-Nurr1-Ngn2/VMCs into vivo can improve the behavioral disorder of PD rats, and has significant neuroprotective, antioxidant and anti-apoptotic effects on PD.	62
Olfactory bulb tissue of SD rats	*In vivo* and *in vitro*	6-hydroxydopamine lesion rat model of PD	SD rats	OECs and transplanted fetal ventral mesencephalic tissue can enhance the functional recovery of dopamine cell survival, striatal reinnervation and rotation induced by amphetamine and apomorphine. Only in the case of combined transplantation of OECs can dopamine neurons extend to astonishingly long processes and reach the surrounding striatal septum.	64
Olfactory bulb tissue of adult rats	*In vivo* and *in vitro*	6-OHDA lesioned PD rats	Rats	OECs significantly increased the survival, neurite growth and dopamine release of dopamine neurons derived from neural stem cells, and had a protective effect on 6-OHDA neurotoxicity under co-culture conditions, promoted the recovery of motor function and neurochemistry.	65
Mouse olfactory bulb	*In vivo*	ALS mice model	Mice	The neural progenitor cells derived from the transplanted olfactory ensheathing tissue-neurospheres can differentiate into choline acetyltransferase positive large spinal cord neurons, intermediate neurons and glial cells, reduce the loss of motor neurons and promote the recovery of motor function in ALS mice.	83
olfactory bulb of neonatal “green” rats	*In vivo*	ALS rat model	SD rats	OECs transplantation can reduce the loss and collapse of motor neurons in the anterior horn of spinal cord, promote myelin regeneration and change the microenvironment of ALS.	84
olfactory bulb of approximately 7-day-old neonatal “green” male rats	*In vivo*	Adult SOD1 mutant rats with ALS	SD rats	OECs transplantation increased the survival of motor neurons in the motor cortex and ventral horn of spinal cord, prolonged survival time and behavioral tests (including screen test, hindlimb extension, rotating stick and gait control) in rats.	85
Olfactory bulbs of transgenic SD rats	*In vivo*	Rat model of sciatic nerve injury by transection	SD rats	Footprint analysis showed that OECs could survive and integrate into the repaired nerve. OECs can transform the regenerated axonal myelin sheath into *Ranvier* nodules, increase the sciatic nerve conduction velocity and improve the function of rats.	89
Allogenic neonatal rats	*In vivo*	Rat model of sciatic nerve injury by transection	F344 inbred rats	The transplantation of PCL catheter containing OECs increased the sciatic nerve conduction velocity, wet muscle weight and nerve density, and promoted the regeneration of sciatic nerve injury.	90
2 to 7 day old neonatal Wistal rats	*In vivo*	Rat model of sciatic nerve injury by transection	Wistal rats	OECs can migrate along the nerve axis after transplantation. The transplantation of PLGA catheter filled with OECs increased the nerve conduction velocity and the amplitude of compound muscle activity potential. However, 12 weeks after operation, the sciatic nerve function index was not improved due to the injury model.	91
Olfactory bulb tissue of SD rats	*In vivo* and *in vitro*	Rat model of sciatic nerve injury by transection	SD rats	Exosomes derived from umbilical cord mesenchymal stem cells can promote the vitality and proliferation of OECs and promote the motor and sensory function of injured sciatic nerve. Co-transplantation of OECs and exosomes can better promote nerve regeneration and functional recovery.	92
Olfactory bulb tissue of neonatal SD rats	*In vivo*	Rat model of sciatic nerve injury by transection	SD rats	Compared to OECs transplantation, co transplantation of SCs and OECs can increase axonal regeneration by 28%. The gastrocnemius muscle has significantly recovered and has a synergistic effect on the repair of the sciatic nerve.	93

treatment and brings great challenges to clinical treatment (95). Although some drugs or other physiotherapy are used to delay or improve the progression of neurological diseases, the results are still not satisfactory or even failed. Drug therapy cannot change these pathological changes and reconstruct the neural network, which leads to the limitation of long-term clinical application.

The expected effect of OECs transplantation was confirmed in the disease model, and these studies revealed the value of OECs in neurological diseases. On the basis of the results of animal model research, some researchers have risen to the observation of clinical trials and made some progress, but most studies have focused on OECs in ALS clinical trials (Table 2), while other neurological diseases have not been reported. These studies suggest that OECs transplantation is safe and feasible, has therapeutic value for neurological diseases, and can improve neurological function and/or reduce progressive deterioration in patients (98, 101). OECs transplantation may play a role in the treatment of patients with ALS. A clinical trial showed that fetal OECs were transplanted into the bilateral corona radiata of the pyramidal tract of the frontal lobe in 15 patients with ALS. After 4 months of follow-up, the functional deterioration of the patients was significantly slower and the total score of ALS was increased, and the neurological function was

TABLE 2 Study on OECs in ALS clinical trial.

OECs origin	Transplant method and location	Transplant dose	follow-up time	Treatment results	Side effect	Refs
Olfactory bulb tissue of human fetus	Injected into the bilateral radiation corona of the frontal pyramidal tract	Two million cells.	4 months	A total of 15 patients received OECs transplantation, the total score of ALS-FRS increased and the deterioration of neurological symptoms slowed down. Improvement of neurological function in 7 patients	No obvious side effects and complications were found, and no death after OECs transplantation was found.	96
Human embryonic OECs	Spinal cord. Radiation corona of spinal cord and bilateral frontal lobe. Double frontal lobe radiation crown	50 μl ($1x10^6$) OECs was transplanted into the spinal cord, and 50 μl OECs ($2 x10^6$) was injected into the radiation corona of both sides of the brain,	4 weeks	A total of 327 patients received OECs transplantation, neurological function was improved in 252 cases. In 261 cases, the spontaneous potential decreased or disappeared, the EMG amplitude decreased during contraction, and the potential density increased.	Postoperative adverse reactions occurred in 16 cases, including headache, short-term fever, major seizures, central nervous system infection, pneumonia, respiratory failure, urinary tract infection, heart failure and pulmonary embolism, of which 4 cases died.	82
Fetal OECs	Cerebral injection	2000000 of cells (100μl)	12-24 months	There are obvious glial and inflammatory reactions around the transplanted cells. There was no evidence of axonal regeneration, neuronal differentiation and myelin formation. Transplantation of OECs did not change the neuropathological changes in patients with ALS.	There was no clinical adverse reaction after operation, but the patient died of respiratory insufficiency in the later stage.	97
Fetal OECs	Corona radiata	100μl (about 2 million)	An average of 47.2 months	OECs transplantation could not prevent the progression of disease in patients with ALS.	No obvious adverse events were observed.	98
Olfactory ensheathing tissue of female fetus born at 12-16 weeks	Brain parenchyma	100μl (about 2000000)	2 weeks	Seven patients with ALS were treated with OECs transplantation, of which 2 patients' ALSFRS score increased and their neurological symptoms and EMG were improved. OECs improved the symptoms of the patients in a short time. and the other 5 patients remained stable.	No obvious adverse reactions were reported.	99
Embryonic olfactory bulb tissue	Unreported		1 year	Symptoms of 7 patients were not improved objectively after receiving OECs treatment. OECs had no beneficial effect on the treatment of patients with ALS.	Two patients developed venous thrombosis and respiratory insufficiency	100
Embryonic olfactory bulb tissue	Spinal cord and brain	Unreported	2-8 weeks	Transplantation of OECs into the brain and spinal cord is feasible and safe. They have good therapeutic value for central nervous system diseases such as chronic spinal cord injury, ALS, cerebral palsy and stroke sequelae, and can improve patients' neurological function and/or reduce progressive deterioration.	Among the 1255 patients with ALS, 4 patients died of heart failure, pulmonary embolism, respiratory failure and severe pneumonia.	101

improved in 7 cases (96). The clinical condition of 2 patients was stable, and the ALS-FRS score of the other 5 patients decreased by 4.4 points on average (96). This means that OECs transplantation seems to slow down the clinical progress of ALS. OECs were transplanted into the spinal cord and/or bilateral radiation coronary lesion areas of 327 patients with ALS and were evaluated by using the ALS functional rating scale (ALSFRS). The results showed that the spontaneous potential decreased or disappeared, the amplitude of motor unit action potential decreased, and the number of motor unit action potential increased significantly (82). Among them, 16 cases had various complications including headache, short-term fever, seizures, central nervous system infection, pneumonia, respiratory failure, urinary tract infection, heart failure and possible pulmonary embolism, and 4 cases died (82). This study suggests that OECs transplantation may effectively control or reverse the physiological deterioration caused by ALS, but it also means that there are some side effects and security risks in OECs transplantation. In other clinical trials, 7 patients with ALS received OECs transplantation, of which 2 cases showed significant improvement in short term after OECs transplantation (99).

However, OECs transplantation is not very satisfactory in human trials of neurological diseases, and it has always been controversial. Some studies have found that OECs transplantation is not beneficial to patients with ALS (100). A case report showed that female patients with ALS who received frontal lobe injection of OECs, found that ALS progressed faster after surgery and suffered crippling side effects (102). Although this case shows that OECs transplantation has achieved poor results in the treatment of ALS, this is only a case and does not represent the feasibility of OECs transplantation treatment. More case data are needed to support this result. Another clinical trial found no evidence of axonal regeneration, neuronal differentiation and myelin formation after transplantation of fetal OECs (97). It was further found that the transplanted cells did not change the neuropathological changes in patients with ALS (97). There are many factors leading to these results, which may be related to the differences in symptoms and signs of patients, time of nerve injury, operation, time and dose of OECs transplantation and evaluation index.

Although animal experiments have recognized the value of OECs transplantation in the treatment of neurological diseases, the inherent variability and diversity of human patients have been ignored. Therefore, it is not clear whether these transplanted cells will produce similar results when used in patient populations, and there is a lack of more direct data support in clinical trials. At this stage, it is not feasible to extrapolate the results of these *in vivo* studies directly to human patients. Therefore, the results and functional effects of transplanted OECs in animal experiments need to be verified in clinical trials. But at present, the application of OECs in clinical trials is greatly restricted, this may be due to some problems that need to be solved to hinder its development and application in clinical trials.

The first problem is the stability and safety of transplanted OECs. Studies have shown that transplantation of OECs into the body can produce some side effects, such as headache, short-term fever, seizures and even aggravate the progression of the disease (96, 102). Due to the differences between animals and humans, the active therapeutic effects of OECs in animals and produce favorable results, but these may not be significant in humans or even the opposite results. A major obstacle to the progress of OECs therapy is to understand how OECs work in the body and how they integrate with the target tissue/organ to overcome tissue and environment-specific barriers. Therefore, the extensive use of OECs in clinical trials requires more animal research to fully understand the characteristics and safety of OECs therapy and reduce the risk of additional injury.

The second major problem is that OECs can be derived from olfactory mucosa or olfactory bulb tissue. however, it takes a long time to prepare OECs, and the purification system of OECs *in vitro* is still not perfect, which may miss the key treatment window. Porcine OECs changed systematically with time *in vitro*, resulting in decreased expression of p75, decreased proliferation and decreased myelin regeneration ability (103). This means that it takes a relatively short time for the expanded culture and purification of cells *in vitro* to enhance the viability and proliferation of cultured cells. Therefore, it is very important to obtain safe and efficient cells in a short time. However, the technology and instruments currently used are difficult to achieve, although the task is arduous, but with the continuous efforts of researchers will eventually be solved.

The third problem is that transplantation of autologous or allogeneic OECs into the body will produce a certain degree of immune rejection, which can reduce the success rate and survival rate of cell transplantation, reduce the therapeutic effect and even cause immune injury. The microencapsulated cell technology has good biocompatibility, can exert immune barrier and anti-inflammatory effects, increase the survival rate of transplanted cells, and contribute to the therapeutic effect of transplanted cells in the field of treatment (104–106). We previously explored the effect of transplantation of microencapsulated OECs into sciatic nerve injury in pain relief. The results showed that compared with OECs alone, microencapsulated OECs could bitter promote the myelination of sciatic nerve and relieve pain (107, 108). Although this technique has been recognized by researchers and feasible in the basic field, but clinical trials based on cell encapsulation have not produced any approved treatments. Progress in this area has been slow, in part because of the complexity of technology and possible uncertain risk factors such as biodegradability, toxicity and metabolism. Therefore, exploring a safe and feasible method to reduce the immune response and inflammatory response of OECs transplantation into the host, and improve the survival rate and survival time may improve the therapeutic effect.

The fourth problem is that the methods of OECs transplantation into the body including intracranial injection, intramedullary injection and intravenous injection. Different transplantation methods will produce different therapeutic effects. At present, there is no tracking method to determine whether the transplanted cells can accurately locate and gather in the injured site, especially for vein transplantation or subarachnoid transplantation, the cells will disperse and move in the blood or brain effusion. It is possible that only a few transplanted cells will be colonized in the trauma site, which will significantly reduce the therapeutic effect and increase other additional potential risks. We

believe that using image guidance, puncture or minimally invasive surgery to accurately implant cells into the injured site or the site that needs treatment may be the best solution at present. In addition, different doses and time of transplantation of OECs have different effects. At present, there is no unified application standard for the best time and quantity of cell transplantation.

The fifth challenge is that for peripheral neurodegenerative diseases, OECs transplantation can protect injured neurons, promote axonal regeneration and repair injured nerves by secreting neurotrophic factors. However, these factors such as BDNF and NGF can also be used as pain-related molecules (11, 47). It may lead to pathological pain in the process of repairing injured nerve. Therefore, it is necessary to fully understand the characteristics of transplanted cells and the specific mechanism by which transplanted cells mediate secretory factors to regulate nerve injury.

Although cell transplantation is a promising choice in current and future clinical applications, the natural limitations of cell source and *in vitro* expansion are still the main challenges to the development of autologous cell transplantation. This difficulty may be overcome by immortalizing primary cells, allowing unlimited expansion and reprogramming of somatic cells to produce a large number of induced pluripotent stem cells. Although cell transplantation therapy is in the primary stage of exploration, there are many uncertain factors and many problems that need to be solved, but these will not prevent researchers from carrying out in-depth exploration and intensified work.

5 Conclusion

The pathological mechanism of neurological diseases is complex, and treatment has always been a thorny problem at present, but the clinical effect of traditional medicine is not good, and even has devastating sequelae. The development of a cell-based therapy for the treatment of neurological diseases is a prospective exploratory method. As many studies have shown, considerable progress has been made in the direction of cell therapy. OECs are characterized by their unique biological characteristics, lifelong survival and continuous renewal, and play a favorable role in nerve regeneration and tissue repair. The possible mechanisms of OECs in the treatment of neurological diseases include the secretion of neurotrophic factors, immune regulation, axonal regeneration, myelination and neuroprotection, thus improving and delaying the progress of neurological diseases. However, the results obtained in clinical trials are not very satisfactory, and the effectiveness of these cell-based therapies remains to be proved by more studies. Before OECs may be used in clinical trials to treat neurological diseases, many existing problems need to be solved, and more in-depth studies on the practical application of cell therapy strategies are needed. Despite these unresolved issues, with the continuous efforts of researchers, the cell transplantation strategy is expected to be successfully applied to clinical treatment in the future.

Author contributions

L-PZ: Data curation, Formal analysis, Methodology, Writing – review & editing. J-XL: Data curation, Formal analysis, Writing – review & editing. Y-YL: Investigation, Methodology, Software, Writing – review & editing. H-LL: Supervision, Writing – review & editing. W-JZ: Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Dutta S, Singh G, Sreejith S, Mamidi MK, Husin JM, Datta I, et al. Cell therapy: the final frontier for treatment of neurological diseases. *CNS Neurosci Ther* (2013) 19 (1):5–11. doi: 10.1111/cns.12027

2. Alessandrini M, Preynat-Seauve O, De Bruin K, Pepper MS. Stem cell therapy for neurological disorders. *S Afr Med J* (2019) 109(8b):70–7. doi: 10.7196/SAMJ.2019.v109i8b.14009

3. Misra MK, Damotte V, Hollenbach JA. The immunogenetics of neurological disease. *Immunology.* (2018) 153(4):399–414. doi: 10.1111/imm.12869

4. Peterson DA. Stem cell therapy for neurological disease and injury. *Panminerva Med* (2004) 46(1):75–80. doi: 10.7196/SAMJ.2019.v109i8b.14009

5. Yasuhara T, Kameda M, Sasaki T, Tajiri N, Date I. Cell therapy for parkinson's disease. *Cell Transplant.* (2017) 26(9):1551–9. doi: 10.1177/0963689717735411

6. Zhang WJ, Luo HL, Zhu JF, Hu CG, Zhu ZM. Transplantation of olfactory ensheathing cells combined with chitosan down-regulates the expression of P2X7 receptor in the spinal cord and inhibits neuropathic pain. *Brain Res* (2020) 1748:147058. doi: 10.1016/j.brainres.2020.147058

7. Zeng YS, Ding Y, Xu HY, Zeng X, Lai BQ, Li G, et al. Electro-acupuncture and its combination with adult stem cell transplantation for spinal cord injury treatment: A summary of current laboratory findings and a review of literature. *CNS Neurosci Ther* (2022) 28(5):635–47. doi: 10.1111/cns.13813

8. Li M, Zhu Q, Liu J. Olfactory ensheathing cells in facial nerve regeneration. *Braz J Otorhinolaryngol* (2020) 86(5):525–33. doi: 10.1016/j.bjorl.2018.07.006

9. Ekberg JA, Amaya D, Mackay-Sim A, St John JA. The migration of olfactory ensheathing cells during development and regeneration. *Neurosignals.* (2012) 20 (3):147–58. doi: 10.1159/000330895

10. Delarue Q, Guérout N. Transplantation of Olfactory Ensheathing Cells: Properties and Therapeutic Effects after Transplantation into the Lesioned Nervous System. *Neuroglia* (2022) 3:1–22. doi: 10.3390/neuroglia3010001

11. Liu JP, Wang JL, Hu BE, Zou FL, Wu CL, Shen J, et al. Olfactory ensheathing cells and neuropathic pain. *Front Cell Dev Biol* (2023) 11:1147242. doi: 10.3389/fcell.2023.1147242

12. Ruitenberg MJ, Vukovic J, Sarich J, Busfield SJ, Plant GW. Olfactory ensheathing cells: characteristics, genetic engineering, and therapeutic potential. *J Neurotrauma.* (2006) 23(3-4):468–78. doi: 10.1089/neu.2006.23.468

13. López-Vales R, García-Alías G, Forés J, Vela JM, Navarro X, Verdú E. Transplanted olfactory ensheathing cells modulate the inflammatory response in the injured spinal cord. *Neuron Glia Biol* (2004) 1(3):201–9. doi: 10.1017/S1740925X05000037

14. Wang Y, Gong M, He X, Wu Y, Li Y, Li J, et al. Transplantation of olfactory ensheathing cells decreases local and serological monocyte chemoattractant protein 1 level during the acute phase of rat spinal cord injury. *Neuroreport.* (2022) 33(17):729–41. doi: 10.1097/WNR.0000000000001839

15. Graziadei PP, Graziadei GA. Neurogenesis and neuron regeneration in the olfactory system of mammals. I. Morphological aspects of differentiation and structural organization of the olfactory sensory neurons. *J Neurocytol.* (1979) 8(1):1–18. doi: 10.1007/BF01206454

16. Graziadei PP, Monti Graziadei GA. Neurogenesis and plasticity of the olfactory sensory neurons. *Ann N Y Acad Sci* (1985) 457:127–42. doi: 10.1111/j.1749-6632.1985.tb20802.x

17. Guerout N, Paviot A, Bon-Mardion N, Honoré A, Obongo R, Duclos C, et al. Transplantation of olfactory ensheathing cells to evaluate functional recovery after peripheral nerve injury. *J Vis Exp* (2014) 84):e50590. doi: 10.3791/50590

18. Guérout N, Derambure C, Drouot L, Bon-Mardion N, Duclos C, Boyer O, et al. Comparative gene expression profiling of olfactory ensheathing cells from olfactory bulb and olfactory mucosa. *Glia.* (2010) 58(13):1570–80. doi: 10.1002/glia.21030

19. Paviot A, Guérout N, Bon-Mardion N, Duclos C, Jean L, Boyer O, et al. Efficiency of laryngeal motor nerve repair is greater with bulbar than with mucosal olfactory ensheathing cells. *Neurobiol Dis* (2011) 41(3):688–94. doi: 10.1016/j.nbd.2010.12.004

20. Lan YX, Yang P, Zeng Z, Yadav N, Zhang LJ, Wang LB, et al. Gene and protein expression profiles of olfactory ensheathing cells from olfactory bulb *versus* olfactory mucosa. *Neural Regener Res* (2022) 17(2):440–9. doi: 10.4103/1673-5374.317986

21. Reshamwala R, Shah M, St John J, Ekberg J. Survival and integration of transplanted olfactory ensheathing cells are crucial for spinal cord injury repair: insights from the last 10 years of animal model studies. *Cell Transplant.* (2019) 28 (1_suppl):132S–59S. doi: 10.1177/0963689719883823

22. Barton MJ, John JS, Clarke M, Wright A, Ekberg J. The glia response after peripheral nerve injury: A comparison between schwann cells and olfactory ensheathing cells and their uses for neural regenerative therapies. *Int J Mol Sci* (2017) 18(2):287. doi: 10.3390/ijms18020287

23. Nazareth L, Lineburg KE, Chuah MI, Tello Velasquez J, Chehrehasa F, St John JA, et al. Olfactory ensheathing cells are the main phagocytic cells that remove axon debris during early development of the olfactory system. *J Comp Neurol* (2015) 523 (3):479–94. doi: 10.1002/cne.23694

24. Amaya DA, Wegner M, Stolt CC, Chehrehasa F, Ekberg JA, St John JA. Radial glia phagocytose axonal debris from degenerating overextending axons in the developing olfactory bulb. *J Comp Neurol* (2015) 523(2):183–96. doi: 10.1002/cne.23665

25. Min Q, Parkinson DB, Dun XP. Migrating Schwann cells direct axon regeneration within the peripheral nerve bridge. *Glia.* (2021) 69(2):235–54. doi: 10.1002/glia.23892

26. Radtke C, Kocsis JD. Peripheral nerve injuries and transplantation of olfactory ensheathing cells for axonal regeneration and remyelination: fact or fiction? *Int J Mol Sci* (2012) 13(10):12911–24. doi: 10.3390/ijms131012911

27. Chen JF, Liu K, Hu B, Li RR, Xin W, Chen H, et al. Enhancing myelin renewal reverses cognitive dysfunction in a murine model of Alzheimer's disease. *Neuron.* (2021) 109(14):2292–2307.e5. doi: 10.1016/j.neuron.2021.05.012

28. Su Z, Chen J, Qiu Y, Yuan Y, Zhu F, Zhu Y, et al. Olfactory ensheathing cells: the primary innate immunocytes in the olfactory pathway to engulf apoptotic olfactory nerve debris. *Glia.* (2013) 61(4):490–503. doi: 10.1002/glia.22450

29. He BR, Xie ST, Wu MM, Hao DJ, Yang H. Phagocytic removal of neuronal debris by olfactory ensheathing cells enhances neuronal survival and neurite outgrowth via p38MAPK activity. *Mol Neurobiol* (2014) 49(3):1501–12. doi: 10.1007/s12035-013-8588-2

30. Khankan RR, Griffis KG, Haggerty-Skeans JR, Zhong H, Roy RR, Edgerton VR, et al. Olfactory ensheathing cell transplantation after a complete spinal cord transection mediates neuroprotective and immunomodulatory mechanisms to facilitate regeneration. *J Neurosci* (2016) 36(23):6269–86. doi: 10.1523/JNEUROSCI.0085-16.2016

31. Roet KC, Verhaagen J. Understanding the neural repair-promoting properties of olfactory ensheathing cells. *Exp Neurol* (2014) 261:594–609. doi: 10.1016/j.expneurol.2014.05.007

32. Grassi S, Giussani P, Mauri L, Prioni S, Sonnino S, Prinetti A. Lipid rafts and neurodegeneration: structural and functional roles in physiologic aging and neurodegenerative diseases. *J Lipid Res* (2020) 61(5):636–54. doi: 10.1194/jlr.TR119000427

33. Campos FSO, Piña-Rodrigues FM, Reis A, Atella GC, Mermelstein CS, Allodi S, et al. Lipid rafts from olfactory ensheathing cells: molecular composition and possible roles. *Cell Mol Neurobiol* (2021) 41(3):525–36. doi: 10.1007/s10571-020-00869-4

34. Santos-Silva A, Piña-Rodrigues FM, Mermelstein CDS, Allodi S, Barradas PC, Cavalcante LA. A role for gangliosides and β1-integrin in the motility of olfactory ensheathing glia. *J Anat.* (2019) 235(5):977–83. doi: 10.1111/joa.13057

35. Li Y, Huo S, Fang Y, Zou T, Gu X, Tao Q, et al. ROCK inhibitor Y27632 induced morphological shift and enhanced neurite outgrowth-promoting property of olfactory ensheathing cells via YAP-dependent up-regulation of L1-CAM. *Front Cell Neurosci* (2018) 12:489. doi: 10.3389/fncel.2018.00489

36. Hao DJ, Liu C, Zhang L, Chen B, Zhang Q, Zhang R, et al. Lipopolysaccharide and curcumin co-stimulation potentiates olfactory ensheathing cell phagocytosis via enhancing their activation. *Neurotherapeutics.* (2017) 14(2):502–18. doi: 10.1007/s13311-016-0485-8

37. Nazareth L, Shelper TB, Chacko A, Basu S, Delbaz A, Lee JYP, et al. Key differences between olfactory ensheathing cells and Schwann cells regarding phagocytosis of necrotic cells: implications for transplantation therapies. *Sci Rep* (2020) 10(1):18936. doi: 10.1038/s41598-020-75850-8

38. Witheford M, Westendorf K, Roskams AJ. Olfactory ensheathing cells promote corticospinal axonal outgrowth by a L1 CAM-dependent mechanism. *Glia.* (2013) 61 (11):1873–89. doi: 10.1002/glia.22564

39. Gu M, Gao Z, Li X, Guo L, Lu T, Li Y, et al. Conditioned medium of olfactory ensheathing cells promotes the functional recovery and axonal regeneration after contusive spinal cord injury. *Brain Res* (2017) 1654(Pt A):43–54. doi: 10.1016/j.brainres.2016.10.023

40. Tu YK, Hsueh YH. Extracellular vesicles isolated from human olfactory ensheathing cells enhance the viability of neural progenitor cells. *Neurol Res* (2020) 42(11):959–67. doi: 10.1080/01616412.2020.1794371

41. Chen Z, Fan H, Chen ZY, Jiang C, Feng MZ, Guo XY, et al. OECs prevented neuronal cells from apoptosis partially through exosome-derived BDNF. *J Mol Neurosci* (2022) 72(12):2497–506. doi: 10.1007/s12031-022-02097-5

42. Fan H, Chen Z, Tang HB, Shan LQ, Chen ZY, Wang XH, et al. Exosomes derived from olfactory ensheathing cells provided neuroprotection for spinal cord injury by switching the phenotype of macrophages/microglia. *Bioeng Transl Med* (2021) 7(2): e10287. doi: 10.1002/btm2.10287

43. Liao YZ, Ma J, Dou JZ. The role of TDP-43 in neurodegenerative disease. *Mol Neurobiol* (2022) 59(7):4223–41. doi: 10.1007/s12035-022-02847-x

44. Fayazi N, Sheykhhasan M, Soleimani Asl S, Najafi R. Stem cell-derived exosomes: a new strategy of neurodegenerative disease treatment. *Mol Neurobiol* (2021) 58(7):3494–514. doi: 10.1007/s12035-021-02324-x

45. Assinck P, Duncan GJ, Hilton BJ, Plemel JR, Tetzlaff W. Cell transplantation therapy for spinal cord injury. *Nat Neurosci* (2017) 20(5):637–47. doi: 10.1038/nn.4541

46. Gilmour AD, Reshamwala R, Wright AA, Ekberg JAK, St John JA. Optimizing olfactory ensheathing cell transplantation for spinal cord injury repair. *J Neurotrauma.* (2020) 37(5):817–29. doi: 10.1089/neu.2019.6939

47. Zhang WJ, Wu CL, Liu JP. Schwann cells as a target cell for the treatment of cancer pain. *Glia.* (2023) 71(10):2309–22. doi: 10.1002/glia.24391

48. Lunn JS, Sakowski SA, Hur J, Feldman EL. Stem cell technology for neurodegenerative diseases. *Ann Neurol* (2011) 70(3):353–61. doi: 10.1002/ana.22487

49. Dantuma E, Merchant S, Sugaya K. Stem cells for the treatment of neurodegenerative diseases. *Stem Cell Res Ther* (2010) 1(5):37. doi: 10.1186/scrt37

50. Weller J, Budson A. Current understanding of Alzheimer's disease diagnosis and treatment. *F1000Res* (2018) 7:F1000Faculty Rev–1161. doi: 10.12688/f1000research.14506.1

51. Peng J, Zeng X. The role of induced pluripotent stem cells in regenerative medicine: neurodegenerative diseases. *Stem Cell Res Ther* (2011) 2(4):32. doi: 10.1186/scrt73

52. Bertram L, Tanzi RE. Thirty years of Alzheimer's disease genetics: the implications of systematic meta-analyses. *Nat Rev Neurosci* (2008) 9(10):768–78. doi: 10.1038/nrn2494

53. Fu QQ, Wei L, Sierra J, Cheng JZ, Moreno-Flores MT, You H, et al. Olfactory ensheathing cell-conditioned medium reverts $A\beta_{25-35}$-induced oxidative damage in SH-SY5Y cells by modulating the mitochondria-mediated apoptotic pathway. *Cell Mol Neurobiol* (2017) 37(6):1043–54. doi: 10.1007/s10571-016-0437-1

54. Chiu SC, Hung HS, Lin SZ, Chiang E, Liu DD. Therapeutic potential of olfactory ensheathing cells in neurodegenerative diseases. *J Mol Med (Berl).* (2009) 87(12):1179–89. doi: 10.1007/s00109-009-0528-2

55. Campisi A, Sposito G, Grasso R, Bisicchia J, Spatuzza M, Raciti G, et al. Effect of astaxanthin on tissue transglutaminase and cytoskeletal protein expression in amyloid-beta stressed olfactory ensheathing cells: molecular and delayed luminescence studies. *Antioxidants (Basel).* (2023) 12(3):750. doi: 10.3390/antiox12030750

56. Pellitteri R, Bonfanti R, Spatuzza M, Cambria MT, Ferrara M, Raciti G, et al. Effect of some growth factors on tissue transglutaminase overexpression induced by β-amyloid in olfactory ensheathing cells. *Mol Neurobiol* (2017) 54(9):6785–94. doi: 10.1007/s12035-016-0152-4

57. Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. *Neuron.* (2003) 39(6):889–909. doi: 10.1016/S0896-6273(03)00568-3

58. Raza C, Anjum R, Shakeel NUA. Parkinson's disease: Mechanisms, translational models and management strategies. *Life Sci* (2019) 226:77–90. doi: 10.1016/j.lfs.2019.03.057

59. Agrawal AK, Shukla S, Chaturvedi RK, Seth K, Srivastava N, Ahmad A, et al. Olfactory ensheathing cell transplantation restores functional deficits in rat model of Parkinson's disease: a cotransplantation approach with fetal ventral mesencephalic cells. *Neurobiol Dis* (2004) 16(3):516–26. doi: 10.1016/j.nbd.2004.04.014

60. Weng SJ, Chen CF, Huang YS, Chiu CH, Wu SC, Lin CY, et al. Olfactory ensheathing cells improve the survival of porcine neural xenografts in a Parkinsonian rat model. *Xenotransplantation.* (2020) 27(2):e12569. doi: 10.1111/xen.12569

61. Weng SJ, Li IH, Huang YS, Chueh SH, Chou TK, Huang SY, et al. KA-bridged transplantation of mesencephalic tissue and olfactory ensheathing cells in a Parkinsonian rat model. *J Tissue Eng Regener Med* (2017) 11(7):2024–33. doi: 10.1002/term.2098

62. Liu Q, Qin Q, Sun H, Zhong D, An R, Tian Y, et al. Neuroprotective effect of olfactory ensheathing cells co-transfected with Nurr1 and Ngn2 in both in *vitro* and in *vivo* models of Parkinson's disease. *Life Sci* (2018) 194:168–76. doi: 10.1016/j.lfs.2017.12.038

63. Shukla A, Mohapatra TM, Parmar D, Seth K. Neuroprotective potentials of neurotrophin rich olfactory ensheathing cell's conditioned media against 6OHDA-induced oxidative damage. *Free Radic Res* (2014) 48(5):560–71. doi: 10.3109/10715762.2014.894636

64. Johansson S, Lee IH, Olson L, Spenger C. Olfactory ensheathing glial co-grafts improve functional recovery in rats with 6-OHDA lesions. *Brain* (2005) 128(Pt 12):2961–76. doi: 10.1093/brain/awh644

65. Shukla S, Chaturvedi RK, Seth K, Roy NS, Agrawal AK. Enhanced survival and function of neural stem cells-derived dopaminergic neurons under influence of olfactory ensheathing cells in parkinsonian rats. *J Neurochem* (2009) 109(2):436–51. doi: 10.1111/j.1471-4159.2009.05983.x

66. Barnat M, Capizzi M, Aparicio E, Boluda S, Wennagel D, Kacher R, et al. Huntington's disease alters human neurodevelopment. *Science.* (2020) 369(6505):787–93. doi: 10.1126/science.aax3338

67. Virlogeux A, Scaramuzzino C, Lenoir S, Carpentier R, Louessard M, Genoux A, et al. Increasing brain palmitoylation rescues behavior and neuropathology in Huntington disease mice. *Sci Adv* (2021) 7(14):eabb0799. doi: 10.1126/sciadv.abb0799

68. Waldvogel HJ, Kim EH, Tippett LJ, Vonsattel JP, Faull RL. The neuropathology of huntington's disease. *Curr Top Behav Neurosci* (2015) 22:33–80. doi: 10.1007/7854_2014_354

69. Ross CA, Tabrizi SJ. Huntington's disease: from molecular pathogenesis to clinical treatment. *Lancet Neurol* (2011) 10(1):83–98. doi: 10.1016/S1474-4422(10)70245-3

70. Hong EP, MacDonald ME, Wheeler VC, Jones L, Holmans P, Orth M, et al. Huntington's disease pathogenesis: two sequential components. *J Huntingtons Dis* (2021) 10(1):35–51. doi: 10.3233/JHD-200427

71. De Gioia R, Biella F, Citterio G, Rizzo F, Abati E, Nizzardo M, et al. Neural stem cell transplantation for neurodegenerative diseases. *Int J Mol Sci* (2020) 21(9):3103. doi: 10.3390/ijms21093103

72. Kim SU, Lee HJ, Kim YB. Neural stem cell-based treatment for neurodegenerative diseases. *Neuropathology.* (2013) 33(5):491–504. doi: 10.1111/neup.12020

73. D'Aiuto L, Zhi Y, Kumar Das D, Wilcox MR, Johnson JW, McClain L, et al. Large-scale generation of human iPSC-derived neural stem cells/early neural progenitor cells and their neuronal differentiation. *Organogenesis.* (2014) 10(4):365–77. doi: 10.1080/15476278.2015.1011921

74. Aubry L, Bugi A, Lefort N, Rousseau F, Peschanski M, Perrier AL. Striatal progenitors derived from human ES cells mature into DARPP32 neurons in *vitro* and in quinolinic acid-lesioned rats. *Proc Natl Acad Sci U S A.* (2008) 105(43):16707–12. doi: 10.1073/pnas.0808488105

75. Cho IK, Hunter CE, Ye S, Pongos AL, Chan AWS. Combination of stem cell and gene therapy ameliorates symptoms in Huntington's disease mice. *NPJ Regener Med* (2019) 4:7. doi: 10.1038/s41536-019-0066-7

76. Pasinelli P, Brown RH. Molecular biology of amyotrophic lateral sclerosis: insights from genetics. *Nat Rev Neurosci* (2006) 7(9):710–23. doi: 10.1038/nrn1971

77. Norris SP, Likanje MN, Andrews JA. Amyotrophic lateral sclerosis: update on clinical management. *Curr Opin Neurol* (2020) 33(5):641–8. doi: 10.1097/WCO.0000000000000864

78. Iguchi Y, Katsuno M, Ikenaka K, Ishigaki S, Sobue G. Amyotrophic lateral sclerosis: an update on recent genetic insights. *J Neurol* (2013) 260(11):2917–27. doi: 10.1007/s00415-013-7112-y

79. De Marchi F, Mareschi K, Ferrero I, Cantello R, Fagioli F, Mazzini L. Effect of mesenchymal stromal cell transplantation on long-term survival in amyotrophic lateral sclerosis. *Cytotherapy.* (2023) 25(8):798–802. doi: 10.1016/j.jcyt.2023.02.005

80. Wang J, Hu W, Feng Z, Feng M. BDNF-overexpressing human umbilical cord mesenchymal stem cell-derived motor neurons improve motor function and prolong survival in amyotrophic lateral sclerosis mice. *Neurol Res* (2021) 43(3):199–209. doi: 10.1080/01616412.2020.1834775

81. Gowing G, Svendsen CN. Stem cell transplantation for motor neuron disease: current approaches and future perspectives. *Neurotherapeutics.* (2011) 8(4):591–606. doi: 10.1007/s13311-011-0068-7

82. Chen L, Huang H, Zhang J, Zhang F, Liu Y, Xi H, et al. Short-term outcome of olfactory ensheathing cells transplantation for treatment of amyotrophic lateral sclerosis. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi.* (2007) 21(9):961–6.

83. Martin LJ, Liu Z. Adult olfactory bulb neural precursor cell grafts provide temporary protection from motor neuron degeneration, improve motor function, and extend survival in amyotrophic lateral sclerosis mice. *J Neuropathol Exp Neurol* (2007) 66(11):1002–18. doi: 10.1097/nen.0b013e318158822b

84. Li Y, Bao J, Khatibi NH, Chen L, Wang H, Duan Y, et al. Olfactory ensheathing cell transplantation into spinal cord prolongs the survival of mutant SOD1(G93A) ALS rats through neuroprotection and remyelination. *Anat Rec (Hoboken).* (2011) 294(5):847–57. doi: 10.1002/ar.21362

85. Li Y, Chen L, Zhao Y, Bao J, Xiao J, Liu J, et al. Intracranial transplant of olfactory ensheathing cells can protect both upper and lower motor neurons in amyotrophic lateral sclerosis. *Cell Transplant.* (2013) 22 Suppl 1:S51–65. doi: 10.3727/096368913X672208

86. Kubiak CA, Grochmal J, Kung TA, Cederna PS, Midha R, Kemp SWP. Stem-cell-based therapies to enhance peripheral nerve regeneration. *Muscle Nerve.* (2020) 61(4):449–59. doi: 10.1002/mus.26760

87. Yang H, He BR, Hao DJ. Biological roles of olfactory ensheathing cells in facilitating neural regeneration: a systematic review. *Mol Neurobiol* (2015) 51(1):168–79. doi: 10.1007/s12035-014-8664-2

88. Radtke C, Wewetzer K, Reimers K, Vogt PM. Transplantation of olfactory ensheathing cells as adjunct cell therapy for peripheral nerve injury. *Cell Transplant.* (2011) 20(2):145–52. doi: 10.3727/096368910X522081

89. Radtke C, Aizer AA, Agulian SK, Lankford KL, Vogt PM, Kocsis JD. Transplantation of olfactory ensheathing cells enhances peripheral nerve regeneration after microsurgical nerve repair. *Brain Res* (2009) 1254:10–7. doi: 10.1016/j.brainres.2008.11.036

90. Lee JY, Kim YH, Kim BY, Jang DH, Choi SW, Joen SH, et al. Peripheral nerve regeneration using a nerve conduit with olfactory ensheathing cells in a rat model. *Tissue Eng Regener Med* (2021) 18(3):453–65. doi: 10.1007/s13770-020-00326-9

91. Li BC, Jiao SS, Xu C, You H, Chen JM. PLGA conduit seeded with olfactory ensheathing cells for bridging sciatic nerve defect of rats. *J BioMed Mater Res A.* (2010) 94(3):769–80. doi: 10.1002/jbm.a.32727

92. Zhang Y, Wang WT, Gong CR, Li C, Shi M. Combination of olfactory ensheathing cells and human umbilical cord mesenchymal stem cell-derived exosomes promotes sciatic nerve regeneration. *Neural Regener Res* (2020) 15(10):1903–11. doi: 10.4103/1673-5374.280330

93. You H, Wei L, Liu Y, Oudega M, Jiao SS, Feng SN, et al. Olfactory ensheathing cells enhance Schwann cell-mediated anatomical and functional repair after sciatic nerve injury in adult rats. *Exp Neurol* (2011) 229(1):158–67. doi: 10.1016/j.expneurol.2010.08.034

94. Ulrich R, Imbschweiler I, Kalkuhl A, Lehmbecker A, Ziege S, Kegler K, et al. Transcriptional profiling predicts overwhelming homology of Schwann cells, olfactory ensheathing cells, and Schwann cell-like glia. *Glia.* (2014) 62(10):1559–81. doi: 10.1002/glia.22700

95. Stanzione P, Tropepi D. Drugs and clinical trials in neurodegenerative diseases. *Ann Ist Super Sanita.* (2011) 47(1):49–54. doi: 10.4415/ANN_11_01_11

96. Huang H, Chen L, Xi H, Wang H, Zhang J, Zhang F, et al. Fetal olfactory ensheathing cells transplantation in amyotrophic lateral sclerosis patients: a controlled pilot study. *Clin Transplant.* (2008) 22(6):710–8. doi: 10.1111/j.1399-0012.2008.00865.x

97. Giordana MT, Grifoni S, Votta B, Magistrello M, Vercellino M, Pellerino A, et al. Neuropathology of olfactory ensheathing cell transplantation into the brain of two amyotrophic lateral sclerosis (ALS) patients. *Brain Pathol* (2010) 20(4):730–7. doi: 10.1111/j.1750-3639.2009.00353.x

98. Gamez J, Carmona F, Raguer N, Ferrer-Sancho J, Martín-Henao GA, Martí-Beltrán S, et al. Cellular transplants in amyotrophic lateral sclerosis patients: an observational study. *Cytotherapy.* (2010) 12(5):669–77. doi: 10.3109/14653241003774037

99. Huang H, Tan K, Chen L, Xue Y, Wang H, Xi H, et al. MR spectroscopy evaluation and short-term outcome of olfactory ensheathing cells transplantation in amyotrophic lateral sclerosis patients. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi.* (2007) 21(1):52–7.

100. Piepers S, van den Berg LH. No benefits from experimental treatment with olfactory ensheathing cells in patients with ALS. *Amyotroph Lateral Scler.* (2010) 11(3):328–30. doi: 10.3109/17482961003663555

101. Huang H, Chen L, Xi H, Wang Q, Zhang J, Liu Y, et al. [Olfactory ensheathing cells transplantation for central nervous system diseases in 1,255 patients]. *Zhongguo*

Xiu Fu Chong Jian Wai Ke Za Zhi. (2009) 23(1):14–20. doi: 10.1080/17482960701419315

102. Chew S, Khandji AG, Montes J, Mitsumoto H, Gordon PH. Olfactory ensheathing glia injections in Beijing: misleading patients with ALS. *Amyotroph Lateral Scler*. (2007) 8(5):314–6. doi: 10.1080/17482960701419315

103. Radtke C, Lankford KL, Wewetzer K, Imaizumi T, Fodor WL, Kocsis JD. Impaired spinal cord remyelination by long-term cultured adult porcine olfactory ensheathing cells correlates with altered in *vitro* phenotypic properties. *Xenotransplantation*. (2010) 17(1):71–80. doi: 10.1111/j.1399-3089.2009.00562.x

104. Zhang YL, Chen DJ, Yang BL, Liu TT, Li JJ, Wang XQ, et al. Microencapsulated Schwann cell transplantation inhibits P2X3 receptor expression in dorsal root ganglia and neuropathic pain. *Neural Regener Res* (2018) 13(11):1961–7. doi: 10.4103/1673-5374.238715

105. Zhao Y, Shi Y, Yang H, Liu M, Shen L, Zhang S, et al. Stem cell microencapsulation maintains stemness in inflammatory microenvironment. *Int J Oral Sci* (2022) 14(1):48. doi: 10.1038/s41368-022-00198-w

106. Ashimova A, Yegorov S, Negmetzhanov B, Hortelano G. Cell encapsulation within alginate microcapsules: immunological challenges and outlook. *Front Bioeng Biotechnol* (2019) 7:380. doi: 10.3389/fbioe.2019.00380

107. Zhang WJ, Luo C, Huang C, Liu SC, Luo HL. Microencapsulated neural stem cells inhibit sciatic nerve injury-induced pain by reducing P2 × 4 receptor expression. *Front Cell Dev Biol* (2021) 9:656780. doi: 10.3389/fcell.2021.656780

108. Zhang W, Liu Y, Sun Y, Liu Z. Microencapsulated olfactory ensheathing cell transplantation reduces P2X4 receptor overexpression and inhibits neuropathic pain in rats. *Brain Res* (2019) 1724:146465. doi: 10.1016/j.brainres.2019.146465

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