DENDRITIC EPIDERMAL T CELLS IN WOUND HEALING AND CANCER

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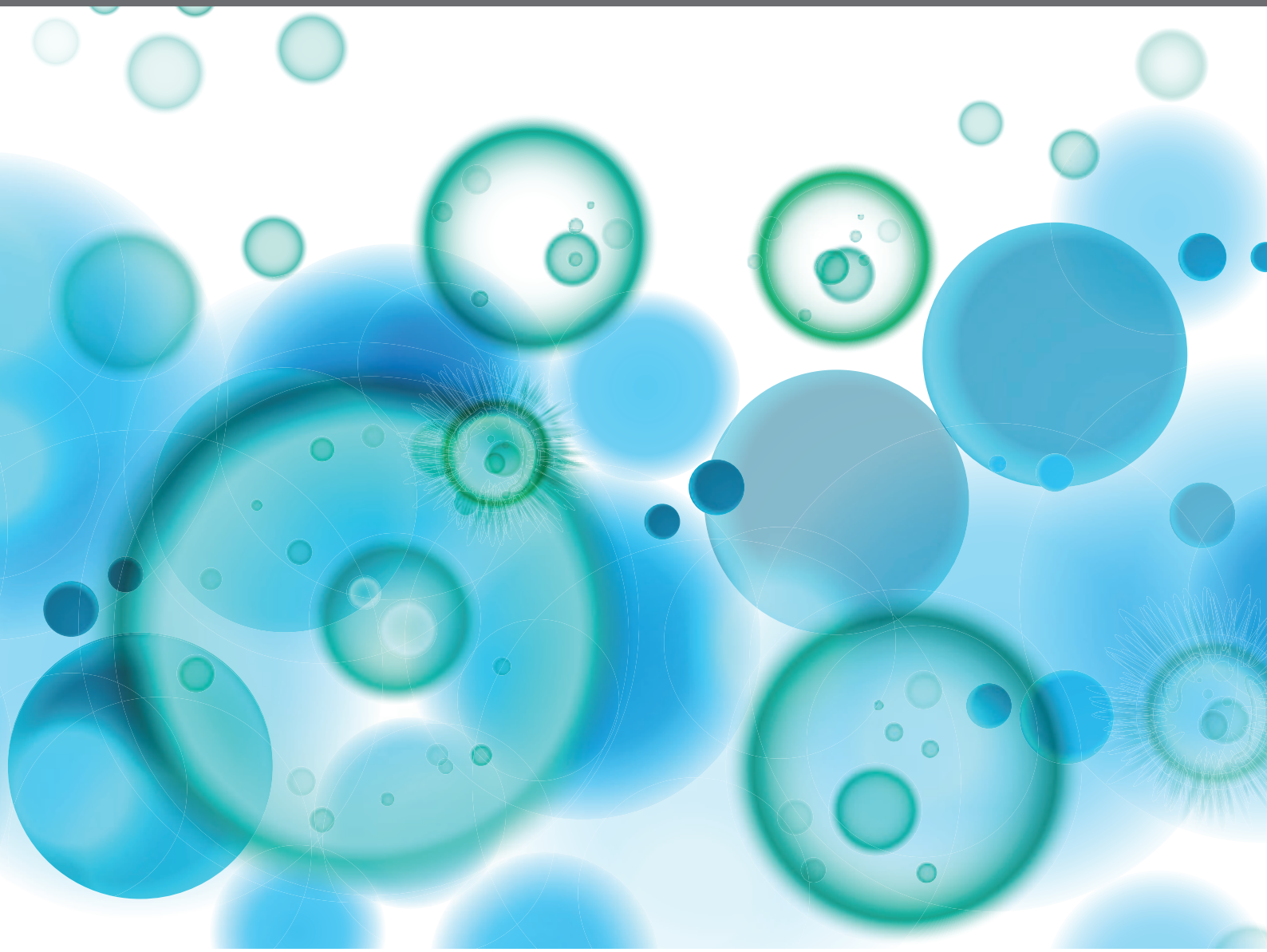

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DENDRITIC EPIDERMAL T CELLS IN WOUND HEALING AND CANCER

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ORIGINAL RESEARCH
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Bibliometric Analysis of Dendritic Epidermal T Cell (DETC) Research From 1983 to 2019

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Dendritic epidermal T cells (DETC) are a group of immune cells expressing canonical γδ TCR in the murine epidermis. Similar to γδ T cells in the human epidermis, DETC serve an important barrier cell in the skin and participate in skin immune surveillance, immune regulation, skin homeostasis, tissue protection, and other activities. Since its discovery in 1983, research on DETC has grown rapidly and unevenly. To evaluate DETC research trends and map the DETC knowledge structure, we have applied bibliometric methods and techniques. A total of 384 DETC-related articles obtained from the Scopus database published between 1983 and 2019 were analyzed using indicators of publication and citation metrics, country and international cooperation, author and co-authorship, and keyword co-occurrence cluster. The present research status, the emerging global trends and the future development direction are also visualized and discussed. In summary, this study provides novel and useful data for the DETC research scientific community, and will help researchers explore DETC more intuitively and effectively.

Keywords: DETC, gamma delta T cells, bibliometrics, Scopus, VOSviewer

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INTRODUCTION

Dendritic epidermal T cells (DETC or DETCs), which are restricted to the murine epidermis and bear a canonical γδ TCR, are the first T cells to develop in the embryonic thymus (1). As one of the constituent cells that act as an important barrier in the skin, DETC are involved in skin homeostasis, immune surveillance, immune regulation, protection of tissues, and other physiological and pathological activities, and also play an important role in pathogen invasion resistance, wound healing, malignancy, and autoimmune diseases (2, 3).

Research on DETC has been ongoing for nearly four decades, with the important milestones for the field shown in **Figure 1**. Since Tschachler et al., and Bergstresser et al., simultaneously first encountered previously unrecognized Thy-1$^+$ dendritic epidermal cells within murine epidermis, named Thy-1$^+$ EC or Thy-1$^+$ DEC (4, 5) in 1983, an increasing number of studies have been performed to better understand the characteristics and functions of Thy-1$^+$ dendritic epidermal cells, which were renamed DETCs in 1988 (6). In 1987, Stingl et al., Konging et al., and Kuziel et al., found that Thy-1$^+$ DEC express a CD3-associated γδ T cell receptor (7–9). Subsequently, additional research has led to a more clear understanding of DETC, including on expressing a canonical Vγ3$^+$ Vδ1$^+$ TCR [Garman nomenclature (10); alternative Heilig and Tonegawa nomenclature, Vγ5$^+$ Vδ1$^+$ (11)] (12, 13); important cell surface markers (6); DETC development, maturation, migration, and participating related receptors and ligands (1, 14–19); and the connections between DETC and epidermal homeostasis (20–24), inflammation (25–27), microbial infection (28–31),

FIGURE 1 | Selected milestones in DETC research.

contact hypersensitivity (32–35), transplantation (36–38), tumor surveillance (39–41), and wound healing (42, 43). Hence, DETC are also considered to be a potentially ideal model for studying skin-resident γδ T cells in humans and other mammals.

To date, DETC have attracted considerable attention from various research fields, and in turn, publications on DETC have increased rapidly. However, few studies have focused on understanding DETC research in a comprehensive manner through longitudinal and global perspectives. Therefore, it is necessary and essential to apply a scientific and advanced information visualization method to reveal the progress and trends in DETC research.

Bibliometric analysis, a novel and scientific information visualization method used worldwide to evaluate the knowledge

structure, development, and trends within a topic both quantitatively and qualitatively, is now widely used in a variety of fields (44, 45). The use of bibliometric analysis is still in the early stage in the immunology field and not many articles using it have been published outside of the tumor immunotherapy, microbiome, sepsis, and vaccine fields (46–49). Here, we apply a bibliometric analysis of DETC research publications (prior to September 17 2019) in terms of numbers of publications and citations, journal, country, and international cooperation, author and co-authorship and keyword co-occurrence cluster. Our aims are to help more scientists in related areas gain a better understanding of the past, present, and future of DETC, leading to more efficient and effective exploration of the frontiers, hotspots, and trends of DETC research.

METHODS

Source Database

The publication search was performed using Scopus (https://www.scopus.com/), one of the most comprehensive databases of scientific peer-reviewed literature that includes different types of documents together with sufficient relevant information essential to our study (45).

Search Design

We decided to search for documents related to DETC research of all types by their titles, abstracts, and keywords. In order to cover as many results that met our requirements as possible, the search query string was designed as described below.

After studying all our references, we found that DETC was first discovered as dendritic cells expressing Thy-1 antigen in murine epidermis (4, 5). After that, as further research was conducted, these cells were also commonly referred to by their cellular classification as $V\gamma3^+$ $V\delta1^+$ T cells (Garman nomenclature) or $V\gamma5^+$ $V\delta1^+$ T cells (Heilig and Tonegawa nomenclature) that exist in the skin of mice and rats (10, 11), or even γδ T cells located in murine epidermal in many articles.

Considering various wording and writing formats, we also studied the rules that govern the Scopus search system. All words in a term automatically include both their plurals and spelling variants (if any) at the same time and return the results altogether. Furthermore, lowercase and uppercase letters are blended when searching, and a Greek alphabet is recognized the same as its English spelling form (only when it appears alone, uncombined with other letters or words). All punctuation, hyphens, and more than one blank in a term are ignored (as one blank) in both terms and results, which means the use of alternative forms such as "Vγ3-Vδ1", "Vγ3/Vδ1", "Vγ3 Vδ1", and "$V\gamma3^+$ $V\delta1^+$" will not make a difference, but the combination caused by deletion between words like "Vγ3Vδ1" and the insertion within a word like "Vγ-3 Vδ-1" will yield different results. We found that the harmonization does not deal with a Greek letter or its English spelling form combined with other letters or words (Vγ3 and Vgamma3 will not be harmonized as the same, for example), and plurals of an abbreviation (such as DETCs) are not automatically included. Thus, we had to list all these possible situations as exhaustively as we could think of in the search formula.

TABLE 1 | Collocation of qualifiers and subjects in the search formula.

Qualifier[a]	Subject[b]
(Not applicable)	**Dendritic epidermal T cell** ("dendritic epidermal T cell" OR "dendritic epidermal T lymphocyte" OR DETC OR DETCs OR "epidermal dendritic T cell" OR "epidermal dendritic T lymphocyte")
epiderm OR **skin** OR **cutaneous**	**Vγ3** OR **Vγ5** OR **Vγ3Vδ1** OR **Vγ5Vδ1** (Vγ3 OR Vgamma3 OR "V γ 3" OR "Vγ 3" OR "Vgamma 3" OR Vγ5 OR Vgamma5 OR "V γ 5" OR "Vγ 5" OR "Vgamma 5" OR Vγ3Vδ1 OR Vgamma3Vdelta1 OR "Vγ3 Vδ1" OR "Vgamma3 Vdelta1" OR "V γ 3 V δ 1" OR "Vγ 3 Vδ 1" OR "Vgamma 3 Vdelta 1" OR "V γ 3V δ 1" OR "Vγ 3Vδ 1" OR "Vgamma 3Vdelta 1" OR Vγ5Vδ1 OR Vgamma5Vdelta1 OR "Vγ5 Vδ1" OR "Vgamma5 Vdelta1" OR "V γ 5 V δ 1" OR "Vγ 5 Vδ 1" OR "Vgamma 5 Vdelta 1" OR "V γ 5V δ 1" OR "Vγ 5Vδ 1" OR "Vgamma 5Vdelta 1")
epiderm OR (**skin** AND ***epitheli****) OR (**cutaneous** AND ***epitheli****)	**γδ T cell** ("γδ T cell" OR "γδ T lymphocyte" OR "gammadelta T cell" OR "gammadelta T lymphocyte" OR "γ δ T cell" OR "γ δ T lymphocyte")
Thy 1 OR **Thy1**	(***epiderm*** AND **dendritic**) OR (**skin** AND **dendritic**) OR (**cutaneous** AND **dendritic**) OR **DEC** OR **DECs**
(Not applicable)	**Skint1** OR **Skint 1**

[a] *Subjects with no qualifier to collocate with are tagged as Not applicable.*
[b] *For some subjects, contents enclosed in brackets are directly extracted from the search formula to list all the possible wording and writing formats used in it.*

According to Scopus rules, phrases enclosed in double quotes are marked as a whole (ex, "dendritic epidermal T cell") in which words must appear together in the exact order while Boolean operators AND and OR are used to combine separate words depending on certain logical relationship. Moreover, operators (enclosed in brackets) are processed following the precedence order of OR (must contain at least one of the terms) prior to AND (both terms must be contained whenever they appear separately or together) when searching. Wildcard (*), like when used in *epiderm* for example, will return words such as epidermal, epidermis, intraepidermal, etc.

Search terms about $V\gamma3^+$ $V\delta1^+$ T cell, $V\gamma5^+$ $V\delta1^+$ T cell, γδ T cell, and Thy-1^+ dendritic cell need to be confined to work in the skin or epidermis of mice or rats. To do this, qualifiers were used, with more information provided in **Table 1**. In addition, Skint1, a gene recently identified as essential for DETC development, was added as a search term for results related to the selection and upkeep of the cell (14, 50).

Ultimately, the search formula used was as follows: TITLE-ABS-KEY ("dendritic epidermal T cell" OR "dendritic epidermal T lymphocyte" OR DETC OR DETCs OR "epidermal dendritic T cell" OR "epidermal dendritic T lymphocyte") OR TITLE-ABS-KEY (*epiderm* OR skin OR cutaneous AND Vγ3 OR Vgamma3 OR "V γ 3" OR "Vγ 3" OR "Vgamma 3" OR Vγ5 OR Vgamma5 OR "V γ 5" OR "Vγ 5" OR "Vgamma 5" OR Vγ3Vδ1 OR Vgamma3Vdelta1 OR "Vγ3 Vδ1" OR "Vgamma3 Vdelta1" OR "V γ 3 V δ 1" OR "Vγ 3 Vδ 1" OR "Vgamma

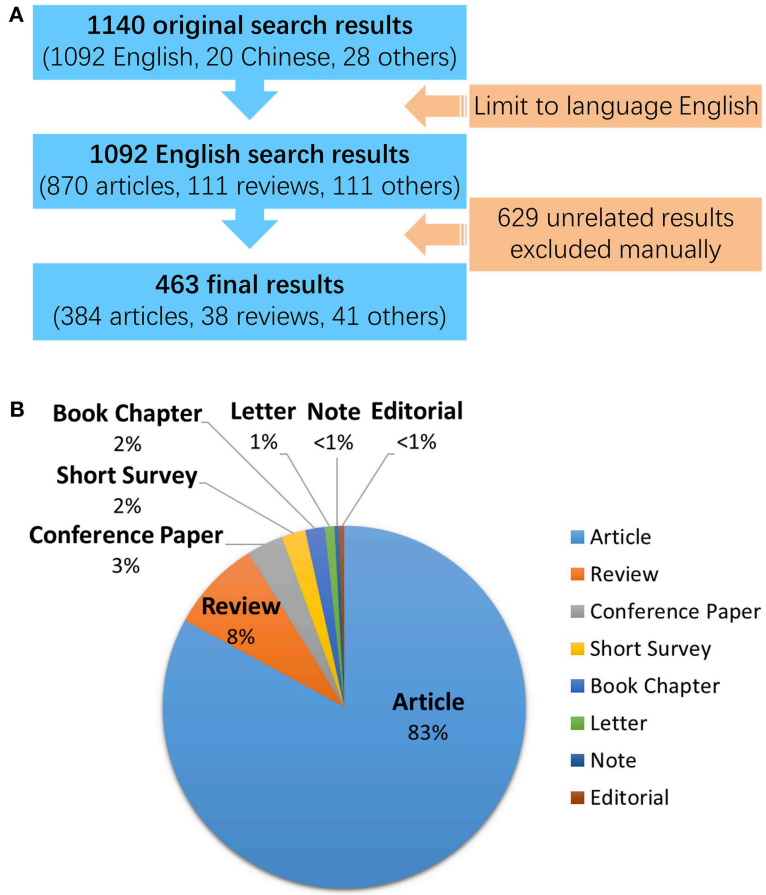

FIGURE 2 | Data filtration processing and results. A total of 1,140 results of all document types and languages were found on Scopus using the original search formula given in this article. **(A)** Details of the data filtration process. A language filter was applied to the original search on Scopus so that only English results were exported regardless of their document types. Then, a manual review was performed on the exported results to filter out documents with definite relevance to DETC research. **(B)** Document types of all 463 English results identified for DETC. The pie chart illustrates the proportion of document number of each type.

3 Vdelta 1" OR "V γ 3V δ 1" OR "Vγ 3Vδ 1" OR "Vgamma 3Vdelta 1" OR Vγ5Vδ1 OR Vgamma5Vdelta1 OR "Vγ5 Vδ1" OR "Vgamma5 Vdelta1" OR "V γ 5V δ 1" OR "Vγ 5 Vδ 1" OR "Vgamma 5 Vdelta 1" OR "V γ 5V δ 1" OR "Vγ 5Vδ 1" OR "Vgamma 5Vdelta 1") OR TITLE-ABS-KEY (*epiderm* OR (skin AND *epitheli*) OR (cutaneous AND *epitheli*) AND "γδ T cell" OR "γδ T lymphocyte" OR "gammadelta T cell" OR "gammadelta T lymphocyte" OR "γ δ T cell" OR "γ δ T lymphocyte") OR TITLE-ABS-KEY (Thy1 OR "Thy 1" AND (*epiderm* OR skin OR cutaneous AND dendritic) OR DEC OR DECs) OR TITLE-ABS-KEY ("skint1" OR "skint 1") AND (LIMIT-TO (LANGUAGE, "English")). The limitation, (LIMIT-TO (LANGUAGE, "English")), at the end of the formula was added in the data filtration process (explained in greater detail in section Data Filtration).

Data Collection

All results were searched on Scopus using the formula above and exported together with as much relevant information as possible in CSV format. The search was performed all within 1 day, September 17, 2019, in case of changes brought about by update of the database.

Data Filtration

Only results of documents published in English were exported. This was accomplished by directly limiting the results to English only when searching. Then, manual filtration was performed by viewing each title, abstract, keywords, and full text, to judge whether there was a clear correlation with DETC in this content. A total of 629 unrelated results were excluded among the 1,092 exported results, most of which were on γδ T cells but not in murine or rat epidermis, while others are articles that came up in the results due to multiple meanings that are sometimes ascribed to acronyms, such DETC(s), in the search terms. In total, 463 results were identified as valid after comparing and discussing both outcomes of filtration made by two authors independently (**Figure 2A**, **Supplementary Table 1**).

As shown in the pie chart in **Figure 2B**, most of the publications are articles, making up 83% (384) of the total; this is followed by reviews, which, at 8%, make up almost half of

the remaining portion. Other types of documents, in order of quantity, were conference papers (15, 3%), short surveys (10, 2%), book chapters (8, 2%), letters (4), notes (2), and editorials (2), each of which made up only a small amount of the total, with no more than 4% individually. The following analysis was restricted to articles only (**Supplementary Table 2**).

Data Analysis

The filtered database file was imported into Microsoft Excel 2016 for analysis, and included the following information: authors, title, year of publications, source journals, affiliations, citations, DOI, and keywords. GraphPad Prism 8 was also applied to create charts.

In addition to many of the self-explanatory indicators used in this paper, we employed four specialized metrics (extracted from Scopus): CiteScore, SJR, SNIP, and h-index. Each metric is explained below.

CiteScore is a new metric of journal citation impact powered by Scopus. CiteScore is the number of citations received by a journal in 1 year to documents published in the 3 previous years, divided by the number of documents indexed in Scopus published in those same 3 years (45).

Calculated by SCImago Lab and developed from Scopus data, SJR (SCImago Journal Rank) measures weighted citations received by a serial. The value of a citation is based on the subject field, quality, and reputation of the citing serial (51).

SNIP (Source Normalized Impact per Paper) was created by Professor Henk Moed at the Centre for Science and Technology Studies (CTWS), University of Leiden. It measures contextual citation impact by weighting citations based on the total number of citations in a subject field. The effect of a single citation is given a higher value for subject areas where citations are less likely and vice versa. Unlike the well-known journal impact factor, SNIP corrects for differences in citation practices between scientific fields, thereby allowing for more accurate inter-field comparisons of citation impact. CWTS Journal Indicators also provide stability intervals that indicate the reliability of the SNIP value of a journal (52).

H-index, proposed by Hirsch (53), attempts to measure both the productivity and impact of the published work of a scientist, journal, or country. The value of the index is h if a measured object has h papers that have received at least h citations each, while the rest of the papers have no more than h citations each.

Visualization Maps

Visualization tools, like VOSviewer, Citespace, Bicomb, and BibExcel, enable researchers to create knowledge maps, evaluate the latest research progress, and identify hotspots in a research field (54). Co-authorship, co-citation, and co-occurrence analyses are the most frequently used methods. In our research, we use country co-authorship, author co-authorship, author co-citation, and author keyword co-occurrence analyses. Country co-authorship analysis provides information about collaborative relationships between authors in various countries. Cooperation preferences of authors from various countries can be used to improve cooperation with foreign authors. Author co-authorship analysis reveals collaborative relationships between authors, which can help researchers understand the relationships between researchers in a field and identify potential collaborators. For example, if some authors appear together often, they may have a closer relationship than others. Author co-citation analysis looks for a co-citation relationship between two papers, both of which are cited by a third article. Citations can provide important insight into what is already known; thus, this relationship can help identify outstanding authors. This can help new researchers to better understand the basics and progress within the field, and identify research hotspots, and other bibliometric information. Author keyword co-occurrence analysis helps researchers identify central issues and developments in a subject. Frequent co-occurrence of two keywords in an article normally suggests a closer relationship between them than other keywords, which may inspire a new research idea. In addition, it can prompt trending author keywords of each year (45, 55).

Bibliographic database files were imported into VOSviewer 1.6.12 to build network visualization maps of scientific publications, scientific journals, researchers, research organizations, countries, keywords, or terminology. Items in these networks can be connected by co-authors, co-occurrences, citations, bibliographic couplings, or co-citation links. Finally, co-authorship, co-citation, and co-occurrence analyses are presented in the form of network visualization maps.

Research Ethics

The study was conducted as a bibliometric analysis. All data sources were available on the Internet; thus, no animal or human subject was involved, eliminating the need for permission from an ethics committee.

RESULTS

Analysis of the Number of Publications and Citations

The number of articles published annually and the summed total citations of annual articles related to DETC research are shown in **Figure 3**. Similar trends can be seen from both sets of data, although the one for annual publications is more regular and can be easily divided into three stages (Stage 1, from 1983 to 1996; Stage 2, from 1997 to 2009; Stage 3, after 2010). It is interesting to note that the line for summed citations changes approximately in synchrony with the annual publication number.

Stage 1 lasts for approximately 14 years, during which the amount of annual publications roughly increases over the years, forming a positively sloped line. Since its discovery in 1983 (4, 5), the number of articles published on DETC increased rapidly, and quickly reached 15 publications in 1988, implying that researchers were quite sensitive to the emergence of a new field. Moreover, the corresponding citation line in this stage shows two peaks, with the highest one (1,745 citations) in 1995, which was slightly delayed compared to the publication peak in 1993 (25 articles).

After the explosion of Stage 1, however, both metrics showed a sudden and significant fall in 1997. Since then, the annual publication number has remained below 10 throughout the

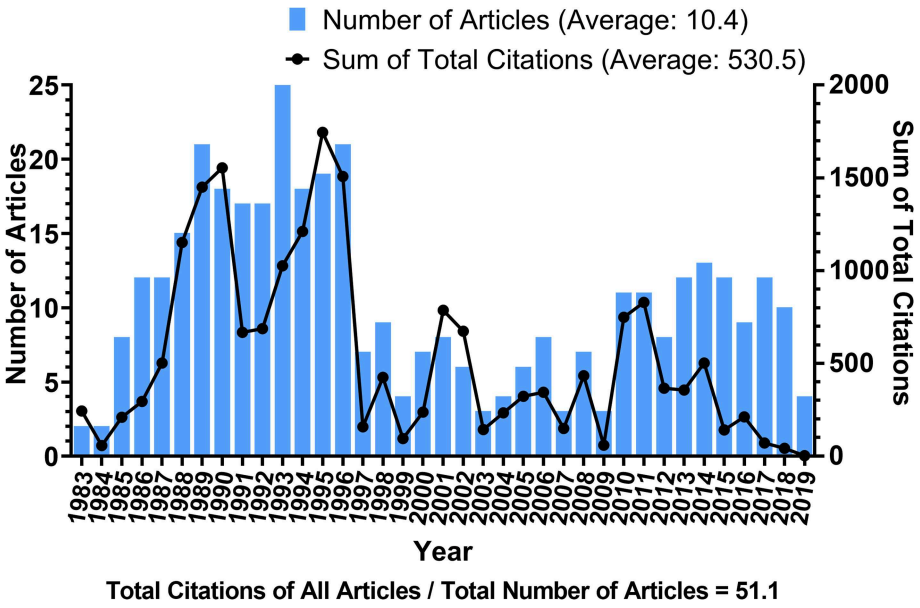

FIGURE 3 | Number of articles published each year and the sum of total citations of annual articles related to DETC research. Blue bars indicate the number of articles published each year; the black line indicates the sum of total citations that annual articles have received.

entirety of Stage 2. During this stage, the average annual publication (5.8 articles) was less than a half of it was in Stage 1 (14.8 articles).

In Stage 3, there was a slight uptick in the number of annual publication, fluctuating right above 10 for the majority of the period. Total citation of these articles is declining, which we hypothesize to be mainly a matter of time, as these articles are newly published. It should be noted that the statistics for 2019 are incomplete, but we predict that it will still follow this pattern of Stage 3 for years to come. However, there still exists the possibility for a new phase of significant development, like what was observed in Stage 1. It is likely that, with the accumulation of steady progress and technological advances, this field is likely to take another leap forward in the near future.

By dividing the total citations of all articles by the total publication number, we found that each article was cited an average of 51.1 times. **Figure 4** shows a histogram depicting the distribution of citations across DETC articles. The frequency declines so sharply as the number of citations increases that a severely differentiated distribution can be readily observed. One hundred seventeen articles (30.5%) are cited fewer than 10 times each. Among them, 68 articles have been cited fewer than five times each, including 12 articles that have gone uncited. Eighteen articles (4.7%) have been cited more than 200 times each, spanning a wide range of citation counts from 211 to 759. This histogram indicates that there could be many underutilized sources of information; thus, their potential value needs to be sought out.

The top 15 most highly cited articles are shown in **Table 2**. Twelve articles were published before 2000 (including 9 in the 1990s and 3 in the 1980s), while the remaining three were published later. Further analysis revealed that all 15 articles were published in only eight journals, all of which are in the top 20 journals for publishing the highest number of DETC articles (**Table 3**), and some of the authors can also be found among the list of the most productive authors (**Table 5**). Most of the articles in this table focused on the development, migration, regulation, or surface markers of DETC. Since these are the most frequently cited articles, we can conclude that these subjects attract a significant amount of attention within this field. Unfortunately, as we discussed above, quite a few publications have not been fully utilized, including articles on less popular subjects. We believe that this underutilization will decrease with the integration of knowledge processing, as the whole field advances in size and scope.

Again, it is important to be aware that citation does not perfectly reflect the quality of an article (particularly for new publications or publications in areas that are less popular during particular time period), nor is it the only measure. Regardless, we suggest an increased amount of multidisciplinary work and the discovery of new applications for DETC will allow each article to be fully recognized for its achievements.

Analysis of Journal

The top 20 journals, as ranked by number of articles related to DETC research, are listed in **Table 3**. We used this metric instead of using the total citations of these articles for the reasons mentioned above, though this information is shown in **Table 3**, along with the CiteScore and SJR from Scopus as an indicator of impact. For authors, articles related to DETC research are more likely to be accepted by these journals, as they have previously shown significant interest in publishing DETC-related work. Some of the listed journals, like *Frontiers in Immunology*, *Nature*

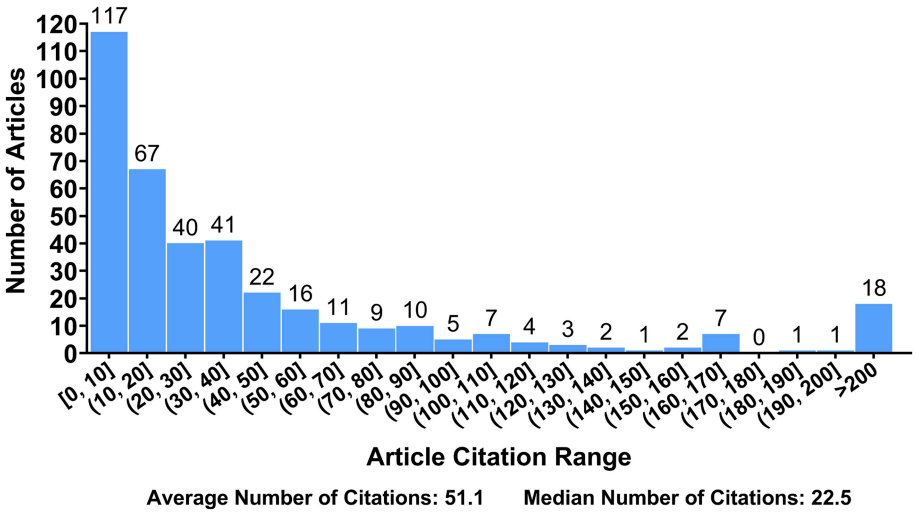

FIGURE 4 | Distribution of article citation numbers. The range of citation numbers (from 0 to 759) was divided into intervals of equal width, with the exception of those articles receiving over 200 citations. The number of articles in each interval was counted.

Immunology, and *PLoS ONE*, are newly founded, and have shown passionate interest in DETC since their establishment.

Although not shown in the table, we found that *Journal of Investigative Dermatology*, which ranks at the top of the list, is almost the only journal that published DETC-related articles prior to 1986 and published the first articles declaring the discovery of DETC (4, 5). While the 20 journals listed cover 76% of DETC articles overall, this journal alone contributes 20% of the publications.

Analysis of Country and International Cooperation

Articles analyzed were produced by 25 countries from around the world (articles co-authored by more than one country are counted repeatedly by VOSviewer). However, most are concentrated in the United States, which contributed 205 articles, exceeding the average of all 25 countries (19.3) by a significant amount. Unlike the journal analysis (**Table 3**) discussed above, citations per article did not vary much between countries, with an average of 49.6 for all 25 countries, although gaps in publications and total citations are still significant between some countries (**Table 4**). This may have something to do with the scientific and economic environment in each country, as well as the access to international resources.

We cannot ignore the fact that the ranking has been performed using articles only published in English, which means there are quite a few articles in other languages that have not been counted (shown in **Figure 2A**). If added, they might make a significant difference in the ranking; as China (20 search results in Chinese) and Russia (14 search results in Russian) in particular could have the potential to rank higher. Therefore, it seems that more work should be done on document translation to promote the sharing of resources and information, which will provide many benefits to researchers.

We used VOSviewer to analyze the co-authorship between different countries and produced two international co-authorship (international cooperation) visualization maps of publications for countries that have published more than one document. Country co-authorship maps can help researchers to understand existing partnerships and identify potential collaborators. The largest set of connected countries consists of 25 countries in nine clusters. **Figure 5A** illustrates the country co-authorship network. Clusters are grouped by the frequency of shared co-occurrence terms that represent each country. Closely related terms are grouped into the same cluster with the same color. The more publications a country has produced, the larger the size of its circle will be; the larger the scale of the cooperation is, the thicker the connecting line will be. For example, the link strength (a measure of collaboration) between United States and United Kingdom is 13, and they are connected by a thick line. The United States has the highest degree of cooperation with other countries, with a total link strength of 58. Researchers from the United Kingdom and Japan collaborate the most with American researchers. **Figure 5B** depicts the country co-authorship overlay. The color of a country is based on its average publication year. The United States, for example, with an average publication year of 1997.8 for its 205 articles, is shown in cyan. This does not mean that the United States issued the most documents in 1997 though; in fact, the United States published documents every year for the period from 1983 to 2019, making its average publication year 1997.8. As can be seen from the figure, Austria is blue (with an average publication year of 1992.8), indicating that Austrian researchers were more active at the start of DETC research; China, Singapore, and Denmark are yellow (with average publication years of 2013.7, 2015.3, and 2015.7, respectively), indicating that they are newly active in this field. The differences in the average publication years for DETC between countries and regions reflect their uneven scientific levels. National development strategies and initiatives in these

TABLE 2 | Top 15 DETC-related articles with the most citations.

Rank[a]	Title	Total Citations	Authors[b]	Source	Year	References
1	Defective lymphoid development in mice lacking expression of the common cytokine receptor γ chain	759	Cao X., Shores E.W. et al.	*Immunity*. Volume 2, Issue 3, Pages 223–38.	1995	(56)
2	Regulation of cutaneous malignancy by γδ T cells	673	Girardi M., Oppenheim D.E. et al.	*Science*. Volume 294, Issue 5542, Pages 605–9.	2001	(39)
3	Migration and maturation of langerhans cells in skin transplants and explants	541	Larsen C.P., Steinman R.M. et al.	*Journal of Experimental Medicine*. Volume 172, Issue 5, Pages 1483–93.	1990	(57)
4	Modulation of epithelial cell growth by intraepithelial γδT cells	530	Boismenu R., Havran W.L.	*Science*. Volume 266, Issue 5188, Pages 1253–5.	1994	(42)
5	Junctional sequences of T cell receptor γδ genes: Implications for γδ T cell lineages and for a novel intermediate of V-(D)-J joining	452	Lafaille J.J., DeCloux A. et al.	*Cell*. Volume 59, Issue 5, Pages 859–70.	1989	(58)
6	A role for skin γδ T cells in wound repair	405	Jameson J., Ugarte K. et al.	*Science*. Volume 296, Issue 5568, Pages 747–9.	2002	(43)
7	Homing of a γδ thymocyte subset with homogeneous T-cell receptors to mucosal epithelia	405	Itohara S., Farr A.G. et al.	*Nature*. Volume 343, Issue 6260, Pages 754–7.	1990	(59)
8	Developmental defects of lymphoid cells in Jak3 kinase-deficient mice	404	Park S.Y., Saijo K. et al.	*Immunity*. Volume 3, Issue 6, Pages 771–82.	1995	(27)
9	Limited diversity of γδ antigen receptor genes of thy-1$^+$ dendritic epidermal cells	400	Asarnow D.M., Kuziel W.A. et al.	*Cell*. Volume 55, Issue 5, Pages 837–47.	1988	(12)
10	A role for endogenous transforming growth factor β1 in Langerhans cell biology: The skin of transforming growth factor β1 null mice is devoid of epidermal Langerhans cells	397	Borkowski T.A., Letterio J.J. et al.	*Journal of Experimental Medicine*. Volume 184, Issue 6, Pages 2417–22.	1996	(60)
11	Developmentally ordered appearance of thymocytes expressing different T-cell antigen receptors	392	Havran W.L., Allison J.P.	*Nature*. Volume 335, Issue 6189, Pages 443–5.	1988	(1)
12	IL-17 is essential for host defense against cutaneous *Staphylococcus aureus* infection in mice	343	Cho J.S., Pietras E.M. et al.	*Journal of Clinical Investigation*. Volume 120, Issue 5, Pages 1762–73.	2010	(28)
13	Recognition of self-antigens by skin-derived T cells with invariant γδ antigen receptors	302	Havran W.L., Chien Y.-H., Allison J.P.	*Science*. Volume 252, Issue 5011, Pages 1430–2.	1991	(21)
14	Identification and induction of keratinocyte-derived IL-10	287	Enk A.H., Katz S.I.	*Journal of Immunology*. Volume 149, Issue 1, Pages 92–95.	1992	(61)
15	Interleukin 7 receptor-deficient mice lack γδ T cells	258	Maki K., Sunaga S. et al.	*Proceedings of the National Academy of Sciences of the United States of America*. Volume 93, Issue 14, Pages 7172–7.	1996	(62)

[a] *Ranked by total citations.*
[b] *Names of the first two authors (on Scopus) were provided.*

countries in science and technology are enabling a strengthening of their scientific practices.

Analysis of Author and Co-authorship

A total of 1,394 authors are involved in the articles analyzed here. The 21 most productive authors are listed in **Table 5** (with two authors tied for 20th). Articles drafted by multiple authors are counted multiple times by VOSviewer. The author affiliations listed in this table are the latest one shown on Scopus and are only used to distinguish different people.

Notably, the author with the highest h-index was awarded the Nobel Prize in Physiology or Medicine along with Tasuku Honjo in 2018, for their discovery of cancer therapy via the inhibition of negative immune regulation. Because of the hard work of the researchers in this field, and the fascinating discoveries they made, DETC has certainly become a more attractive field that many talented researchers are eager to join.

For co-authorship analysis (**Figure 6A**), authors in a cluster represent a closely connected group and are labeled in the same color. The size of a circle represents the number of publications of an author, and the thickness of a line represents the scale of collaboration between authors. After analyzing the articles from each cluster, we manually and subjectively defined the main research area of each cluster. There still exist several independent clusters in the same area, however. As can be seen from **Figure 6A**, these clusters are related to "TCR" (9), "Wound healing" (43), "Cancer" (39), "Development" (63), etc. These publications are listed in our references and they

TABLE 3 | Top 20 journals with the most DETC-related articles published.

Rank[a]	Journal	Articles	Total citations[b]	Citations per article	CiteScore 2018[c]	SJR 2018[c]	SNIP 2018[c]	Publisher
1	*Journal of Investigative Dermatology*	78	2,406	30.8	3.47	1.893	1.287	Elsevier
2	*Journal of Immunology*	63	3,010	47.8	4.41	2.521	1.040	American Association of Immunologists
3	*European Journal of Immunology*	22	641	29.1	3.83	2.046	0.992	Wiley-Blackwell
4	*Journal of Experimental Medicine*	15	1,846	123.1	9.83	7.941	2.143	Rockefeller University Press
5	*Proceedings of the National Academy of Sciences of the United States of America*	15	1,205	80.3	8.58	5.601	2.539	National Academy of Sciences
6	*International Immunology*	10	179	17.9	4.25	2.082	1.191	Oxford University Press
8	*Immunity*	8	1,834	229.3	14.69	11.299	3.851	Elsevier
7	*Nature*	8	1,662	207.8	15.21	16.345	9.199	Springer Nature
9	*Immunology*	8	277	34.6	3.99	1.607	0.986	Wiley-Blackwell
10	*Science*	7	2,280	325.7	15.21	13.251	7.311	American Association for the Advancement of Science
11	*PLoS ONE*	7	148	21.1	3.02	1.100	1.123	Public Library of Science
12	*Journal of Dermatological Science*	7	61	8.7	2.63	1.235	1.178	Elsevier
13	*Frontiers in Immunology*	7	57	8.1	4.71	2.021	1.092	Frontiers Media S.A.
14	*British Journal of Dermatology*	6	129	21.5	2.45	1.984	1.714	Wiley-Blackwell
15	*Journal of Leukocyte Biology*	6	73	12.2	3.69	1.929	1.003	Wiley-Blackwell
16	*Cell*	5	1,004	200.8	24.38	25.976	6.570	Elsevier
17	*Nature Immunology*	5	624	124.8	14.71	13.300	4.302	Springer Nature
18	*Carcinogenesis*	5	77	15.4	4.33	1.820	1.064	Oxford University Press
19	*Cellular Immunology*	5	75	15.0	3.13	1.275	0.808	Elsevier
20	*Journal of Clinical Investigation*	4	451	112.8	10.49	7.001	2.462	American Society for Clinical Investigation

[a] *Ranked by article number. Journals with the same number of articles were then ranked by total citations.*
[b] *Total citations refer to the sum of citations that articles related to DETC research published in each journal have totally received.*
[c] *CiteScore, SJR (SCImago Journal Rank), AND SNIP (Source Normalized Impact per Paper) are metrics extracted from Scopus, introduced in detail in the Data Analysis section above.*

TABLE 4 | Top 20 countries with the most DETC-related articles.

Rank[a]	Country	Articles	Total citations[b]	Citations per article	Rank[a]	Country	Articles	Total citations[b]	Citations per article
1	United States	205	13,156	64.2	11	France	7	306	43.7
2	Japan	75	2,469	32.9	12	Netherlands	7	265	37.9
3	United Kingdom	37	3,087	83.4	14	Sweden	5	143	28.6
4	Germany	27	755	28.0	13	Singapore	4	178	44.5
5	Austria	22	878	39.9	15	Poland	4	22	5.5
6	Switzerland	18	983	54.6	16	Israel	3	112	37.3
7	Australia	18	651	36.2	17	Denmark	3	50	16.7
8	Canada	13	313	24.1	18	Finland	2	62	31.0
9	China	12	131	10.9	19	Italy	2	42	21.0
10	Belgium	10	225	22.5	20	India	2	24	12.0

[a] *Ranked by article number. Countries with the same number of articles were then ranked by total citations.*
[b] *Total citations refer to the sum of citations that the articles have totally received.*

have made a significant contribution to each of these research areas. **Figure 6B** depicts the co-authorship overlay, and can provide valuable information. Authors' colors are based on their average publication year. Havran and Hayday are shown in aquamarine on the map, indicating that they have been working on DETC since the initiation of this field. In many countries,

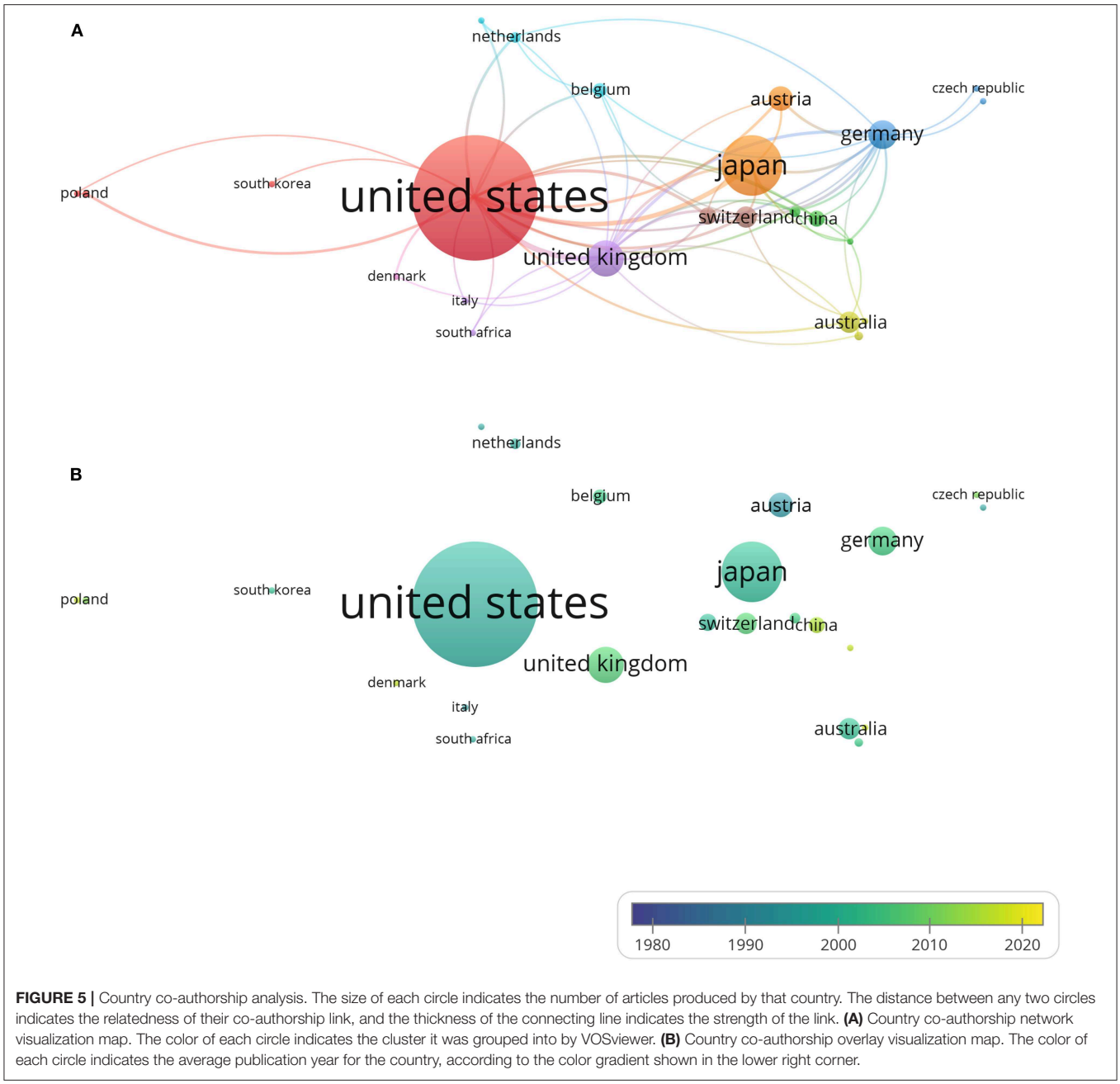

FIGURE 5 | Country co-authorship analysis. The size of each circle indicates the number of articles produced by that country. The distance between any two circles indicates the relatedness of their co-authorship link, and the thickness of the connecting line indicates the strength of the link. **(A)** Country co-authorship network visualization map. The color of each circle indicates the cluster it was grouped into by VOSviewer. **(B)** Country co-authorship overlay visualization map. The color of each circle indicates the average publication year for the country, according to the color gradient shown in the lower right corner.

as they become more developed economically, they also began to dedicate more attention and funding to scientific research to stay competitive or even lead the frontiers nowadays. This has led to the active participation of more scientists from a wider range of countries in recent years, as can be seen in the DETC field. These newer researchers are shown in yellow. He, the pioneer of DETC research in mainland China who has made many important contributions, is among them. It can be concluded from the figure that Chinese researchers, shown in yellow to indicate their newness, are enthusiastic and strongly connected with each other, but they are lacking the connections to researchers from other countries. We believe that the trend of increasing globalization will benefit researchers significantly and multinational cooperation will bring about unexpected changes to DETC research.

In the author co-citation analysis (**Figure 7**), the relevance of authors is determined by the number of times that their articles are referenced by the same article. Each node represents an author with at least seven citations. The analysis includes 17,260 authors, all of whom appear in the references lists of the 384 articles that were analyzed for this paper, and 1,106 of them have published articles no fewer than seven times. The largest set of authors with the highest total link strength consisted of 1,106 people in six clusters. In this case, the same color represents

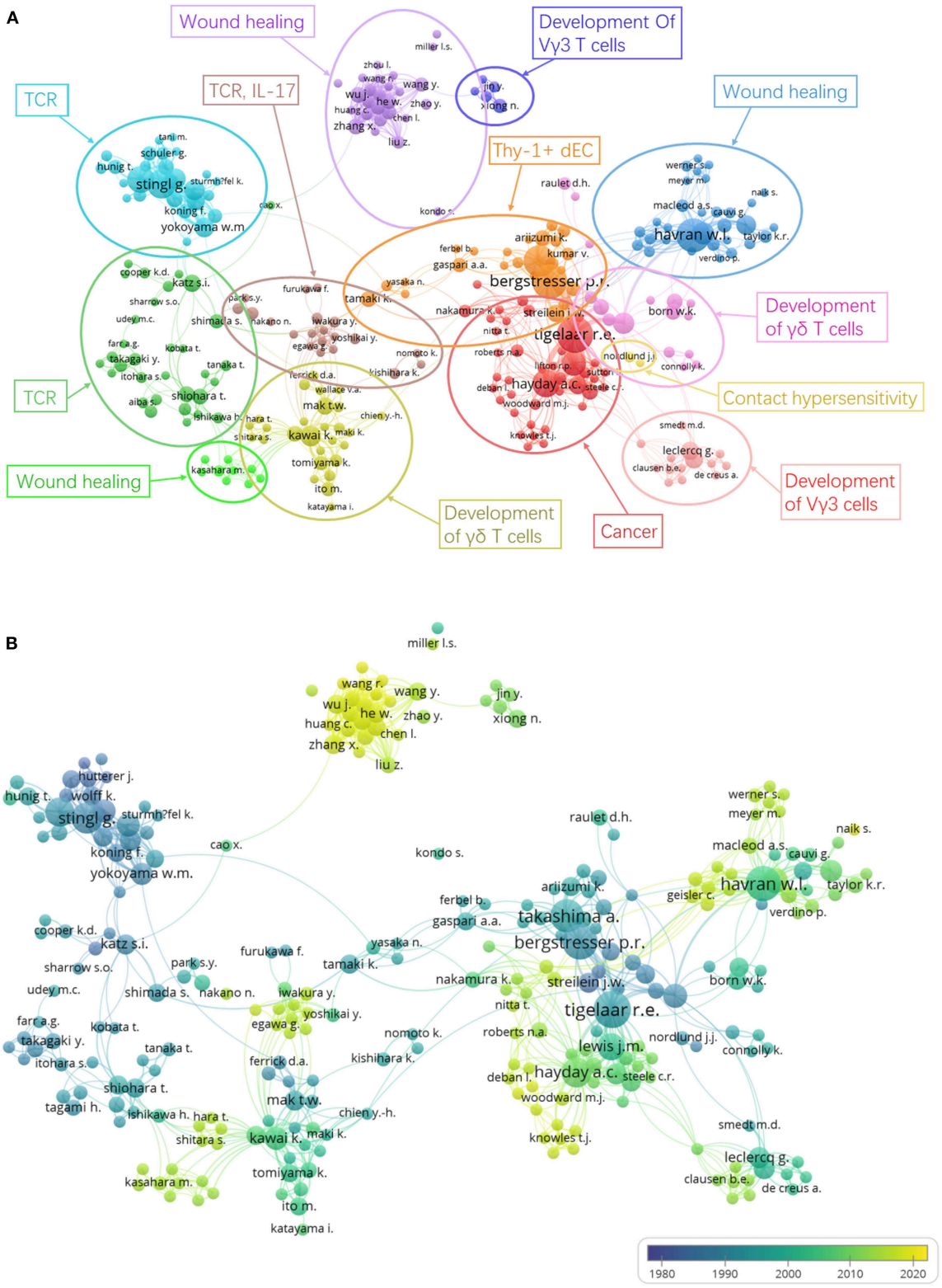

FIGURE 6 | Author co-authorship analysis. Last names of authors are listed. The size of each circle indicates the number of articles produced by the author. The distance between any two circles indicates the relatedness of their co-authorship link, and the thickness of the connecting line indicates the strength of the link. **(A)** Author co-authorship network visualization map. The color of each circle indicates the cluster it was grouped into by VOSviewer. Authors are grouped manually according to the similarity of their research areas. **(B)** Author co-authorship overlay visualization map. The color of each circle indicates the average publication year for the author, according to the color gradient in the lower right corner.

TABLE 5 | Top 20 authors with the most DETC-related articles.

Rank[a]	Author	Articles	Total citations[b]	Citations per article	Affiliation[c]	H-index[d]
1	Tigelaar Robert E.	29	2,735	94.3	Yale Skin Diseases Research Core Center, London, United Kingdom	47
2	Bergstresser Paul R.	27	1,148	42.5	UT Southwestern Medical School, Dallas, United States	55
3	Havran Wendy L.	26	3,095	119.0	Scripps Research Institute, San Diego, United States	41
4	Takashima Akira	23	1,020	44.3	University of Toledo College of Medicine, Toledo, United States	49
5	Stingl Georg	20	860	43.0	Medizinische Universitat Wien, Vienna, Austria	79
6	Hayday Adrian C.	18	1,986	110.3	The Francis Crick Institute, London, United Kingdom	73
7	Elbe Adelheid	17	494	29.1	Medizinische Universitat Wien, Vienna, Austria	17
8	Kripke Margaret L.	14	381	27.2	University of Texas MD Anderson Cancer Center, Houston, United States	61
9	Allison James P.	11	1,721	156.5	University of Texas MD Anderson Cancer Center, Houston, United States	114
10	Halliday Gary Mark	11	257	23.4	Royal Prince Alfred Hospital, Sydney, Australia	50
11	Lewis Julia M.	10	1,424	142.4	Yale University School of Medicine, New Haven, United States	22
12	Tschachler Erwin	10	595	59.5	Medizinische Universitat Wien, Vienna, Austria	60
13	Shevach Ethan M.	10	455	45.5	National Institute of Allergy and Infectious Diseases, Bethesda, United States	97
14	Kawai Kazuhiro	10	218	21.8	Kido Hospital, Niigata, Japan	24
15	Yokoyama Wayne	9	527	58.6	Washington University School of Medicine in St. Louis, St Louis, United States	79
16	Witherden Deborah A.	9	474	52.7	University of California, San Diego, San Diego, United States	21
17	Girardi Michael	8	1,359	169.9	Yale University School of Medicine, New Haven, United States	35
18	Jameson Julie Marie	8	484	60.5	California State University San Marcos, San Marcos, United States	22
19	Coligan John E.	8	445	55.6	National Institute of Allergy and Infectious Diseases, Bethesda, United States	68
20	Plum Jean R.	8	211	26.4	Universiteit Gent, Ghent, Belgium	40
21[e]	Leclercq Georges	8	211	26.4	Universiteit Gent, Ghent, Belgium	33

[a] *Ranked by article number. Authors with the same number of articles were then ranked by total citations and h-index.*
[b] *Total citations refer to the sum of citations that each author's articles related to DETC research have totally received.*
[c] *Affiliation for each author is the latest one shown on Scopus, in order to distinguish different people only.*
[d] *H-Index, is extracted from Scopus, introduced in detail in the Data Analysis section.*
[e] *Two authors tied for the 20th with the same number of articles and citations; thus, the actual number of authors in the list is 21.*

authors that are within the same topic of interest, while authors in the same cluster represent a closely connected group based on co-citation relationships. Authors who are closely related often work in the same field and have contributed to the establishment and development of the field. In addition, the size of each circle represents the number of citations of the author, with more citations generating a larger circle. This is different from the perspective provided in **Table 5**, as this figure provides a very intuitive picture of the academic status and influence of scientists in the DETC field. Because the number of citations is related to the length of the research years and the study of certain research hotspots, young scientists who are currently in a small circle may become big players in this field in the future.

Analysis of Keyword Co-occurrence Cluster

Keyword co-occurrence analysis details the topics covered in a DETC study with author keywords assigned to each article. Keywords are standardized texts or terms selected from the title and the text to express the subject matter of a paper, so the information can be more easily archived. Keywords provide a reasonable description of research hotspots and are very effective in bibliometric analysis when studying knowledge structures in scientific field. In the VOSviewer keyword co-occurrence visualization map, author keywords are marked in different colors according to their average publication years. For example, "Migration" (1995) and "Keratinocytes" (1995) were mainly found in the early years, in comparison with keywords such as "IL-17" (2014) and "Wound healing" (2013), which show up in more recent years (**Figure 8**). Keywords such as "Development," "TCR," "Trafficking receptor," and "Wound healing" are yellow-green, indicating that these fields have become popular in recent years and may become hot topics in the future. In our analysis, we found that Nielsen et al. mentioned that the ligands recognized by the DETC-associated TCR are still unknown, with information such as how the DETC-Skint interaction induces and regulates the activation of DETC and how it affects wound healing still unclear. Issues like these may become popular topics for future research (64). In the DETC field, some problems remain unsettled or have only recently been undertaken, including understanding the specific molecular mechanisms of wound healing, skin immunology, transplant rejection, and microbiological interactions. However, progress is being made in these areas. High-quality articles, like the one published in 2016 by the Fuchs lab entitled "Impaired Epidermal to Dendritic T Cell Signaling Slows Wound Repair in Aged Skin," come out every year (65). We expect there to be more breakthroughs in wound healing, skin immunology, and cell receptor function. As shown in **Figure 8**, research has yet to elucidate the connection between DETC and many

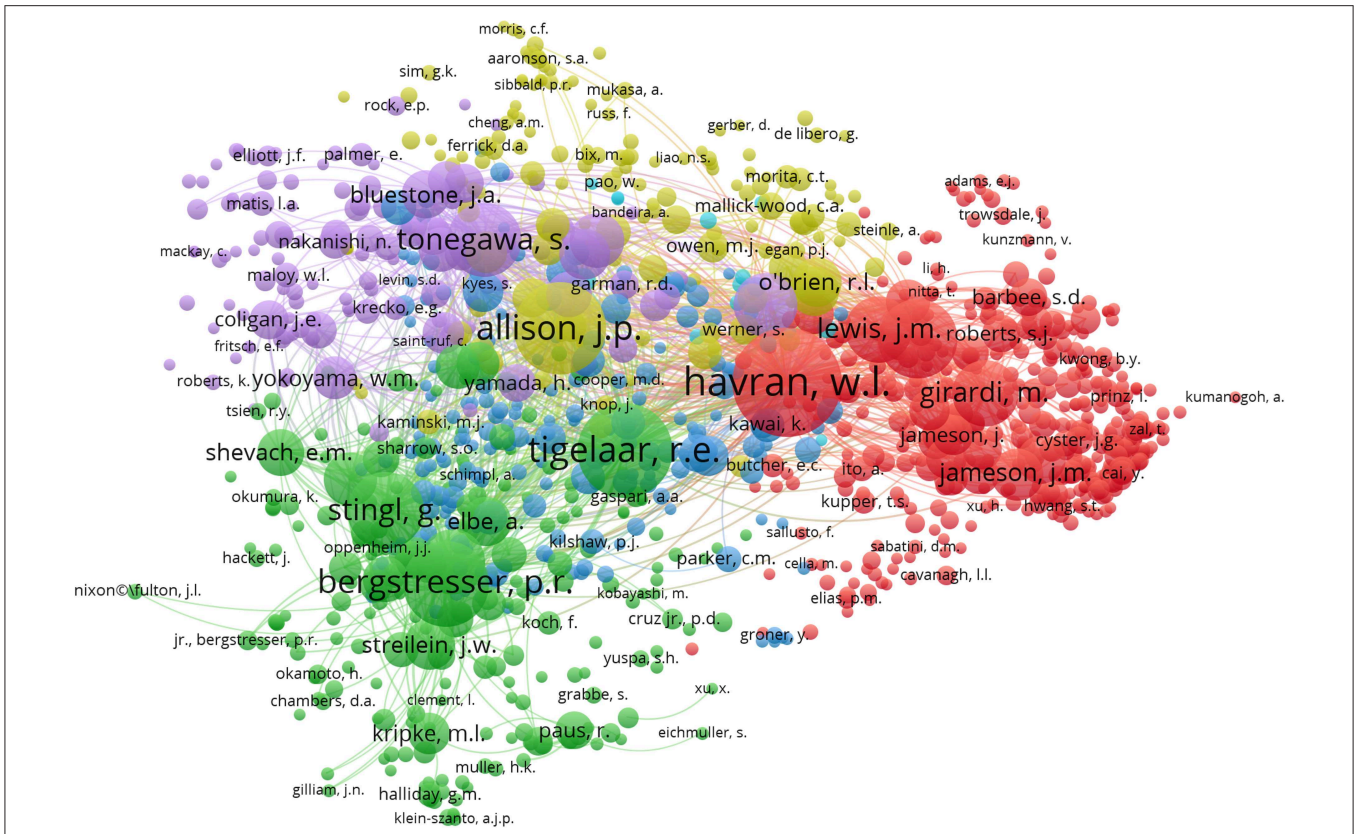

FIGURE 7 | Author co-citation network visualization map. Last names of authors are listed. The size of each circle indicates the total number of citations of the author's DETC-related articles and the color indicates the cluster it was grouped into by VOSviewer. The distance between any two circles indicates the relatedness of their co-authorship link, and the thickness of the connecting line indicates the strength of the link.

other immune cells (NK cell, NKT cell, Th cell, CTL, ILC, B cell, myeloid cells, etc.), and therefore, we can expect to see future work dissecting these relationships, leading to many more important discoveries.

DISCUSSIONS

During this present information explosion, scientific researchers are often concerned with how to manage their knowledge. Our paper seeks to provide an innovative way to manage knowledge and visualize knowledge structures. Our study provides the most up-to-date analysis of global scientific publications related to DETC research from 1983 to 2019. This is the first report to use bibliometric indicators, visualization information tools, and techniques to reveal the knowledge map of the development, evolution, and major trends of DETC research in an intuitive manner.

The number of articles in a research area can reflect the topic's productivity and development over the years (66). The development of DETC research over the past 37 years was an uneven process, as determined in our study. The number of annual publications and citations indicate that, from 1983 to 1996, there was an upsurge period. However, later in 1997, the number of publications suddenly dropped and related progress appeared to slow. This was likely because much of the interest in immunology shifted to T cells and antiviral immunity. Despite this shift, DETC was back in the spotlight in 2010 when more in-depth studies on the pathological role that DETC plays in wound healing, cancer, infection, and inflammation were carried out. Since then, annual publication has remained at a substantial level (**Figure 3**). We believe the changes driven by the interplay between immunology and newer disciplines such as epigenetics and proteomics in 2010, as well as the functional characteristics of T cell subsets and hot topics like microRNA, have helped to move forward theoretical and practical research on DETC.

The number of times an article is cited reflects the extent of its dissemination and influence, and thus may partly reflect its quality as well (67, 68). According to the citation analysis (**Figure 4**), 30.5% of the DETC articles we analyzed have been cited no more than 10 times, indicating that there are plenty of information sources that may not be utilized to the fullest extent. On one hand, the rareness of high-impact articles may be due to the limitation of work, funds, and time for a given author; on the other hand, the lack of citations may also result from a lack of attention to some subjects within the DETC field. Regardless, such underutilization is not expected; thus, we suggest more work be done on the integration of knowledge, particularly as the

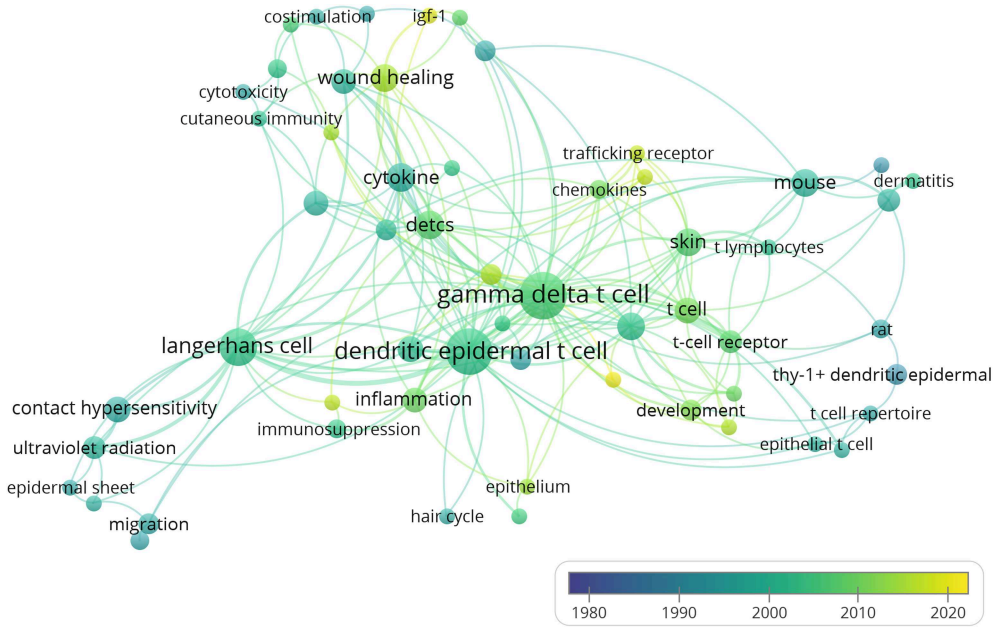

FIGURE 8 | Author keyword co-occurrence analysis. Author keywords are labeled. The color of each circle indicates the average year when the keyword appeared in articles, according to the color gradient color in the lower right corner. The size of each circle indicates the frequency of occurrence of the author keyword. The distance between any two circles is indicative of their co-occurrence link, and the thickness of the connecting line indicates the strength of the link.

DETC field continues to grow. The most cited article has acquired 759 citations thus far, and the top 9 most cited articles (cited no fewer than 400 times) were published in *Immunity* (27, 56), *Science* (39, 42, 43), *Journal of Experimental Medicine* (57), *Cell* (12, 58), and *Nature* (59), respectively (**Table 2**).

Popular journals and other trends in a research area during a certain period can be easily identified and can provide a reliable reference for researchers (69). Furthermore, core journals provide a significant amount of information, which is helpful, especially when searching for documents or submitting research achievements (70, 71). Among the journals in which DETC-related articles were published, the *Journal of Investigative Dermatology* has published the most articles, while articles in the *Journal of Immunology* have obtained the most total citations, and *Science* ranks first by citations per article (**Table 3**). Newly founded journals listed in the table, such as *Frontiers in Immunology*, *Nature Immunology*, and *PLoS ONE*, also deserve the attention of researchers. Their active performance indicates their potential to improve their rank with the publishing of more high-quality DETC-related articles in the future.

A number of national studies on research productivity have been conducted in recent years, primarily in order to help judge the science policy of a country and thereby adjust their science funding (72). Visualization tools like VOSviewer, Citespace, Bicomb, and BibExcel enable researchers to create knowledge maps (54). With the help of the co-authorship visualization map made by VOSviewer, a co-authorship analysis of countries was performed (45, 55, 73, 74). The map showed that the US, Japan, and the UK are the most involved in DETC research, with the US far ahead of the others, as it has been the most productive DETC research contributor since 1983. Still, with the improvement of academic standards and research funding in recent years, DETC-related studies are launching in countries including China, Singapore, and Denmark. There has been widespread international cooperation among DETC researchers, mainly led by countries like the US, Japan, and the UK (**Figure 5**). These developed countries have invested significant money, manpower, and material resources in scientific research. Thus, not surprisingly, they have emerged as worldwide leaders in DETC research. Meanwhile, in developing countries, especially some Asian countries like China, policies and circumstances have shifted, leading to increased support for scientific research, suggesting that these countries will become more significant players in the DETC field in the future.

In order to determine the most productive authors, we ranked authors based on their total number of DETC-related articles and performed the analysis together with other indicators to provide a more comprehensive view (75, 76). Based on the data extracted from Scopus, we found that, of the top 20 most productive authors, 12 are from the US, 3 are from Austria, 2 are from the UK, 2 are from Belgium, and the other 2 authors come from Japan and Australia (**Table 5**). In addition, the author co-authorship network visualization map produced by VOSviewer can also be applied to the analysis of author productivity and active period (77) (**Figure 6**). Notably, researchers Wendy L. Havran (3rd in **Table 5**) and Adrian C. Hayday (6th in **Table 5**) have been committed to this field from its initiation. Meanwhile, the Nobel Prize winner James

P. Allison has published just 11 DETC-related articles so far, though his citation rate per article has already reached 156.5, indicating his strong influence in this area. Regardless of which aspect is evaluated (the academic level, contribution, or influence of researchers in the DETC field), the information shown in **Figures 6**, **7** and **Table 5** all overlap to some extent, without being completely identical. This reflects dynamic changes related to the time period and hotspots in DETC research. Because of the undeniable contributions of the academic pioneers and emerging young researchers highlighted in these figures, the DETC field has been able to attract a broad group of talented researchers to join in.

In recent studies, some researchers have tried using keyword co-occurrence networks for knowledge mapping (54, 78–83). According to the VOSviewer keyword co-occurrence analysis, the early stage of DETC research was focused on the development, migration, and surface marker of these cells; then, it turned to topics such as contact hypersensitivity, ultraviolet radiation, skin cancer, immunosuppression, inflammation, cytokines, and chemokines. It appears that topics such as wound healing, skin immunology, and cell receptor function are likely to attract more attention in the future (**Figure 8**). Some areas of DETC biology remain unsolved or have made minimal progress, including elucidation of the specific molecular mechanisms underlying wound healing, skin immunology, transplant rejection, and microbiological interactions, as well as the interactions between DETC and other immune cells. However, our analysis reveals that scientists are still actively exploring the uncharted territory of DETC. High-quality articles published by the Hayday (84) and Fuchs labs (65) have gained significant attention and recognition in recent years. We expect to see more breakthroughs in skin immunology, immune metabolism, wound healing, and cell receptor function in the future.

Currently, there are fewer researchers involved in DETC studies than in other immune cell studies worldwide, and there still exist plenty of unresolved questions regarding DETC. We hope this bibliometric analysis provides a beneficial reference on the main points, as well as the future trends, of DETC research, not only for researchers who have already been working in this field, but also for new researchers preparing to become active members of this field.

DATA AVAILABILITY STATEMENT

Datasets generated for this study will be made available by the authors, to any qualified researcher on request.

AUTHOR CONTRIBUTIONS

TW designed the study. TW, ZD, and HW performed the search and wrote the paper. TW, ZD, HW, and ZC analyzed the data.

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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.2020.00259/full#supplementary-material

REFERENCES

1. Havran WL, Allison JP. Developmentally ordered appearance of thymocytes expressing different T-cell antigen receptors. *Nature.* (1988) 335:443–5. doi: 10.1038/335443a0
2. Macleod AS, Havran WL. Functions of skin-resident γδ T cells. *Cell Mol Life Sci.* (2011) 68:2399–408. doi: 10.1007/s00018-011-0702-x
3. Ramirez K, Witherden DA, Havran WL. All hands on DE(T)C: Epithelial-resident γδ T cells respond to tissue injury. *Cell Immunol.* (2015) 296:57–61. doi: 10.1016/j.cellimm.2015.04.003
4. Bergstresser PR, Tigelaar RE, Dees JH, Streilein JW. Thy-1 antigen-bearing dendritic cells populate murine epidermis. *J Invest Dermatol.* (1983) 81:286–8. doi: 10.1111/1523-1747.ep12518332
5. Tschachler E, Schuler G, Hutterer J, Leibl H, Wolff K, Stingl G. Expression of Thy-1 antigen by murine epidermal cells. *J Invest Dermatol.* (1983) 81:282–5. doi: 10.1111/1523-1747.ep12518326
6. Steiner G, Koning F, Elbe A, Tschachler E, Yokoyama WM, Shevach EM, et al. Characterization of T cell receptors on resident murine dendritic epidermal T cells. *Eur J Immunol.* (1988) 18:1323–8. doi: 10.1002/eji.1830180904
7. Koning F, Stingl G, Yokoyama WM, Yamada H, Maloy WL, Tschachler E, et al. Identification of a T3-associated gamma delta T cell receptor on Thy-1^+ dendritic epidermal cell lines. *Science.* (1987) 236:834–7. doi: 10.1126/science.2883729
8. Kuziel WA, Takashima A, Bonyhadi M, Bergstresser PR, Allison JP, Tigelaar RE, et al. Regulation of T-cell receptor γ-chain RNA expression in murine Thy-1^+ dendritic epidermal cells. *Nature.* (1987) 328:263–6. doi: 10.1038/328263a0
9. Stingl G, Koning F, Yamada H, Yokoyama WM, Tschachler E, Bluestone JA, et al. Thy-1^+ dendritic epidermal cells express T3 antigen and the T-cell receptor gamma chain. *Proc Natl Acad Sci USA.* (1987) 84:4586–90. doi: 10.1073/pnas.84.13.4586
10. Garman RD, Doherty PJ, Raulet DH. Diversity, rearrangement, and expression of murine T cell gamma genes. *Cell.* (1986) 45:733–42. doi: 10.1016/0092-8674(86)90787-7
11. Heilig JS, Tonegawa S. Diversity of murine gamma genes and expression in fetal and adult T lymphocytes. *Nature.* (1986) 322:836–40. doi: 10.1038/322836a0
12. Asarnow DM, Kuziel WA, Bonyhadi M, Tigelaar RE, Tucker PW, Allison JP. Limited diversity of γδ antigen receptor genes of Thy-1^+ dendritic epidermal cells. *Cell.* (1988) 55:837–47. doi: 10.1016/0092-8674(88)90139-0
13. Havran WL, Grell S, Duwe G, Kimura J, Wilson A, Kruisbeek AM, et al. Limited diversity of T-cell receptor gamma-chain expression of murine Thy-1+ dendritic epidermal cells revealed by V gamma 3-specific monoclonal antibody. *Proc Natl Acad Sci USA.* (1989) 86:4185–9. doi: 10.1073/pnas.86.11.4185
14. Boyden LM, Lewis JM, Barbee SD, Bas A, Girardi M, Hayday AC, et al. Skint1, the prototype of a newly identified immunoglobulin superfamily gene cluster, positively selects epidermal γδ T cells. *Nat Genet.* (2008) 40:656–62. doi: 10.1038/ng.108

15. Havran WL, Allison JP. Origin of Thy-1+ dendritic epidermal cells of adult mice from fetal thymic precursors. *Nature.* (1990) 344:68–70. doi: 10.1038/344068a0
16. Jiang X, Campbell JJ, Kupper TS. Embryonic trafficking of γδ T cells to skin is dependent on E/P selectin ligands and CCR4. *Proc Natl Acad Sci USA.* (2010) 107:7443–8. doi: 10.1073/pnas.0912943107
17. Jin Y, Xia M, Sun A, Saylor CM, Xiong N. CCR10 is important for the development of skin-specific γδ T cells by regulating their migration and location. *J Immunol.* (2010) 185:5723–31. doi: 10.4049/jimmunol.1001612
18. Kawai K, Suzuki H, Tomiyama K, Minagawa M, Mak TW, Ohashi PS. Requirement of the IL-2 receptor β chain for the development of Vγ3 dendritic epidermal T cells. *J Invest Dermatol.* (1998) 110:961–5. doi: 10.1046/j.1523-1747.1998.00214.x
19. Ye SK, Maki K, Lee HC, Ito A, Kawai K, Suzuki H, et al. Differential roles of cytokine receptors in the development of epidermal γδ T cells. *J Immunol.* (2001) 167:1929–34. doi: 10.4049/jimmunol.167.4.1929
20. Edmondson SR, Thumiger SP, Werther GA, Wraight CJ. Epidermal homeostasis: the role of the growth hormone and insulin-like growth factor systems. *Endocr Rev.* (2003) 24:737–64. doi: 10.1210/er.2002-0021
21. Havran WL, Chien YH, Allison JP. Recognition of self antigens by skin-derived T cells with invariant gamma delta antigen receptors. *Science.* (1991) 252:1430–2. doi: 10.1126/science.1828619
22. Lodolce JP, Boone DL, Chai S, Swain RE, Dassopoulos T, Trettin S, et al. IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity.* (1998) 9:669–76. doi: 10.1016/S1074-7613(00)80664-0
23. Sharp LL, Jameson JM, Cauvi G, Havran WL. Dendritic epidermal T cells regulate skin homeostasis through local production of insulin-like growth factor 1. *Nat Immunol.* (2005) 6:73–9. doi: 10.1038/ni1152
24. Su HY, Cheng WT, Chen SC, Lin CT, Lien YY, Liu HJ, et al. Mouse keratinocytes express c98, a novel gene homologous to bcl-2, that is stimulated by insulin-like growth factor 1 and prevents dexamethasone-induced apoptosis. *Biochim Biophys Acta.* (2004) 1676:127–37. doi: 10.1016/j.bbaexp.2003.11.006
25. Boismenu R, Feng L, Xia YY, Chang JC, Havran WL. Chemokine expression by intraepithelial gamma delta T cells. Implications for the recruitment of inflammatory cells to damaged epithelia. *J Immunol.* (1996) 157:985–92.
26. Matsue H, Cruz PD Jr, Bergstresser PR, Takashima A. Profiles of cytokine mRNA expressed by dendritic epidermal T cells in mice. *J Invest Dermatol.* (1993) 101:537–42. doi: 10.1111/1523-1747.ep12365917
27. Park SY, Saijo K, Takahashi T, Osawa M, Arase H, Hirayama N, et al. Developmental defects of lymphoid cells in Jak3 kinase-deficient mice. *Immunity.* (1995) 3:771–82. doi: 10.1016/1074-7613(95)90066-7
28. Cho JS, Pietras EM, Garcia NC, Ramos RI, Farzam DM, Monroe HR, et al. IL-17 is essential for host defense against cutaneous *Staphylococcus aureus* infection in mice. *J Clin Invest.* (2010) 120:1762–73. doi: 10.1172/JCI40891
29. Leclercq G, Plum J. Stimulation of TCR V gamma 3 cells by gram-negative bacteria. *J Immunol.* (1995) 154:5313–9.
30. Molne L, Corthay A, Holmdahl R, Tarkowski A. Role of gamma/delta T cell receptor-expressing lymphocytes in cutaneous infection caused by Staphylococcus aureus. *Clin Exp Immunol.* (2003) 132:209–15. doi: 10.1046/j.1365-2249.2003.02151.x
31. Shimura H, Nitahara A, Ito A, Tomiyama K, Ito M, Kawai K. Up-regulation of cell surface Toll-like receptor 4-MD2 expression on dendritic epidermal T cells after the emigration from epidermis during cutaneous inflammation. *J Dermatol Sci.* (2005) 37:101–10. doi: 10.1016/j.jdermsci.2004.11.006
32. Dieli F, Asherson GL, Sireci G, Dominici R, Gervasi F, Vendetti S, et al. γδ cells involved in contact sensitivity preferentially rearrange the Vγ3 region and require interleukin-7. *Eur J Immunol.* (1997) 27:206–14. doi: 10.1002/eji.1830270131
33. Dieli F, Ptak W, Sireci G, Romano GC, Potestio M, Salerno A, et al. Cross-talk between V β 8+ and γδ+ T lymphocytes in contact sensitivity. *Immunology.* (1998) 93:469–77. doi: 10.1046/j.1365-2567.1998.00435.x
34. Ptak W, Askenase PW. Gamma delta T cells assist alpha beta T cells in adoptive transfer of contact sensitivity. *J Immunol.* (1992) 149:3503–8.
35. Sullivan S, Bergstresser PR, Tigelaar RE, Streilein JW. Induction and regulation of contact hypersensitivity by resident, bone marrow-derived, dendritic epidermal cells: langerhans cells and Thy-1$^+$ epidermal cells. *J Immunol.* (1986) 137:2460–7.
36. Gorczynski RM, Chen Z, Hoang Y, Rossi-Bergman B. A subset of γδ T-cell receptor-positive cells produce T-helper type-2 cytokines and regulate mouse skin graft rejection following portal venous pretransplant preimmunization. *Immunology.* (1996) 87:381–9. doi: 10.1046/j.1365-2567.1996.481554.x
37. Shiohara T, Moriya N, Gotoh C, Hayakawa J, Ishikawa H, Saizawa K, et al. Loss of epidermal integrity by T cell-mediated attack induces long term local resistance to subsequent attack. II. Thymus dependency in the induction of the resistance. *J Immunol.* (1990) 145:2482–8.
38. Shiohara T., Moriya N., Hayakawa J., Itohara S., Ishikawa H. Resistance to cutaneous graft-vs.-host disease is not induced in T cell receptor delta gene-mutant mice. *J Exp Med.* (1996) 183:1483–9. doi: 10.1084/jem.183.4.1483
39. Girardi M, Oppenheim DE, Steele CR, Lewis JM, Glusac E, Filler R, et al. Regulation of cutaneous malignancy by γδ T cells. *Science.* (2001) 294:605–9. doi: 10.1126/science.1063916
40. Kaminski MJ, Cruz PDJr, Bergstresser PR, Takashima A. Killing of skin-derived tumor cells by mouse dendritic epidermal T-cells. *Cancer Res.* (1993) 53:4014–9.
41. Krahenbuhl O, Gattesco S, Tschopp J. Murine Thy-1$^+$ dendritic epidermal T cell lines express granule-associated perforin and a family of granzyme molecules. *Immunobiology.* (1992) 184:392–401. doi: 10.1016/S0171-2985(11)80596-6
42. Boismenu R, Havran WL. Modulation of epithelial cell growth by intraepithelial gamma delta T cells. *Science.* (1994) 266:1253–5. doi: 10.1126/science.7973709
43. Jameson J, Ugarte K, Chen N, Yachi P, Fuchs E, Boismenu R, et al. A role for skin γδ T cells in wound repair. *Science.* (2002) 296:747–9. doi: 10.1126/science.1069639
44. Aggarwal A, Lewison G, Idir S, Peters M, Aldige C, Boerckel W, et al. The state of lung cancer research: a global analysis. *J Thorac Oncol.* (2016) 11:1040–50. doi: 10.1016/j.jtho.2016.03.010
45. Romero L, Portillo-Salido E. Trends in sigma-1 receptor research: a 25-year bibliometric analysis. *Front Pharmacol.* (2019) 10:564. doi: 10.3389/fphar.2019.00564
46. Fernandes S, Jit M, Bozzani F, Griffiths UK, Scott JAG, Burchett HED. A bibliometric analysis of systematic reviews on vaccines and immunisation. *Vaccine.* (2018) 36:2254–61. doi: 10.1016/j.vaccine.2018.02.049
47. Lu K, Yu S, Yu M, Sun D, Huang Z, Xing H, et al. Bibliometric analysis of tumor immunotherapy studies. *Med Sci Monit.* (2018) 24:3405–14. doi: 10.12659/MSM.910724
48. Wan R, Li L, Xing C, Peng R, Gao L. Worldwide scientific productions with immunotherapy of sepsis: a bibliometric analysis. *PeerJ.* (2019) 7:e7116. doi: 10.7717/peerj.7116
49. Zyoud SH, Smale S, Waring WS, Sweileh WM, Al-Jabi SW. Global research trends in microbiome-gut-brain axis during 2009-2018: a bibliometric and visualized study. *BMC Gastroenterol.* (2019) 19:158. doi: 10.1186/s12876-019-1076-z
50. Sutoh Y, Mohamed RH, Kasahara M. Origin and evolution of dendritic epidermal T cells. *Front Immunol.* (2018) 9:1059. doi: 10.3389/fimmu.2018.01059
51. Falagas ME, Kouranos VD, Arencibia-Jorge R, Karageorgopoulos DE. Comparison of SCImago journal rank indicator with journal impact factor. *FASEB J.* (2008) 22:2623–8. doi: 10.1096/fj.08-107938
52. Villasenor-Almaraz M, Islas-Serrano J, Murata C, Roldan-Valadez E. Impact factor correlations with Scimago Journal Rank, Source Normalized Impact per Paper, Eigenfactor Score, and the CiteScore in Radiology, Nuclear Medicine & Medical Imaging journals. *Radiol Med.* (2019) 124:495–504. doi: 10.1007/s11547-019-00996-z
53. Hirsch JE. An index to quantify an individual's scientific research output. *Proc Natl Acad Sci USA.* (2005) 102:16569–72. doi: 10.1073/pnas.0507655102
54. Chen C. Searching for intellectual turning points: progressive knowledge domain visualization. *Proc Natl Acad Sci USA.* (2004) 101:5303–10. doi: 10.1073/pnas.0307513100
55. Chinchilla-Rodríguez Z, Zacca-González G, Vargas-Quesada B, Moya-Anegón F. Latin American scientific output in Public Health: combined

analysis using bibliometric, socioeconomic and health indicators. *Scientometrics.* (2015) 102:609–28. doi: 10.1007/s11192-014-1349-9
56. Cao X, Shores EW, Hu-Li J, Anver MR, Kelsall BL, Russell SM, et al. Defective lymphoid development in mice lacking expression of the common cytokine receptor γ chain. *Immunity.* (1995) 2:223–38. doi: 10.1016/1074-7613(95)90047-0
57. Larsen CP, Steinman RM, Witmer-Pack M, Hankins DF, Morris PJ, Austyn JM. Migration and maturation of Langerhans cells in skin transplants and explants. *J Exp Med.* (1990) 172:1483–93. doi: 10.1084/jem.172.5.1483
58. Lafaille JJ, DeCloux A, Bonneville M, Takagaki Y, Tonegawa S. Junctional sequences of T cell receptor γδ genes: implications for γδ T cell lineages and for a novel intermediate of V-(D)-J joining. *Cell.* (1989) 59:859–70. doi: 10.1016/0092-8674(89)90609-0
59. Itohara S, Farr AG, Lafaille JJ, Bonneville M, Takagaki Y, Haas W, et al. Homing of a γδ thymocyte subset with homogeneous T-cell receptors to mucosal epithelia. *Nature.* (1990) 343:754–7. doi: 10.1038/343754a0
60. Borkowski TA, Letterio JJ, Farr AG, Udey MC. A role for endogenous transforming growth factor β1 in langerhans cell biology: the skin of transforming growth factor β1 null mice is devoid of epidermal langerhans cells. *J Exp Med.* (1996) 184:2417–22. doi: 10.1084/jem.184.6.2417
61. Enk AH, Katz SI. Identification and induction of keratinocyte-derived IL-10. *J Immunol.* (1992) 149:92–5.
62. Maki K, Sunaga S, Komagata Y, Kodaira Y, Mabuchi A, Karasuyama H, et al. Interleukin 7 receptor-deficient mice lack gammadelta T cells. *Proc Natl Acad Sci USA.* (1996) 93:7172–7. doi: 10.1073/pnas.93.14.7172
63. De Creus A, Van Beneden K, Stevenaert F, Debacker V, Plum J, Leclercq G. Developmental and functional defects of thymic and epidermal V γ 3 cells in IL-15-deficient and IFN regulatory factor-1-deficient mice. *J Immunol.* (2002) 168:6486–93. doi: 10.4049/jimmunol.168.12.6486
64. Nielsen MM, Witherden DA, Havran WL. γδ T cells in homeostasis and host defence of epithelial barrier tissues. *Nat Rev Immunol.* (2017) 17:733–45. doi: 10.1038/nri.2017.101
65. Keyes BE, Liu S, Asare A, Naik S, Levorse J, Polak L, et al. Impaired epidermal to dendritic T cell signaling slows wound repair in aged skin. *Cell.* (2016) 167:1323–38.e14. doi: 10.1016/j.cell.2016.10.052
66. Durieux V, Gevenois PA. Bibliometric indicators: quality measurements of scientific publication. *Radiology.* (2010) 255:342–51. doi: 10.1148/radiol.09090626
67. Muniz FWMG, Celeste RK, Oballe HJR, Rosing CK. Citation analysis and trends in review articles in dentistry. *J Evid Based Dent Pract.* (2018) 18:110–8. doi: 10.1016/j.jebdp.2017.08.003
68. Seglen PO. Citations and journal impact factors: questionable indicators of research quality. *Allergy.* (1997) 52:1050–6. doi: 10.1111/j.1398-9995.1997.tb00175.x
69. Zhuang Y, Jiang N, Wu Z, Li Q, Chiu DKW, Hu H. Efficient and robust large medical image retrieval in mobile cloud computing environment. *Inf Sci.* (2014) 263:60–86. doi: 10.1016/j.ins.2013.10.013
70. Smith DR. Citation analysis and impact factor trends of 5 core journals in occupational medicine, 1985-2006. *Arch Environ Occup Health.* (2008) 63:114–22. doi: 10.3200/AEOH.63.3.114-122
71. Wang X, Guo J, Gu D, Yang Y, Yang X, Zhu K. Tracking knowledge evolution, hotspots and future directions of emerging technologies in cancers research: a bibliometrics review. *J Cancer.* (2019) 10:2643–53. doi: 10.7150/jca.32739
72. Fiala D. Bibliometric analysis of CiteSeer data for countries. *Inf Process Manag.* (2012) 48:242–53. doi: 10.1016/j.ipm.2011.10.001
73. Guo YM, Huang ZL, Guo J, Li H, Guo XR, Nkeli MJ. Bibliometric analysis on smart cities research. *Sustainability.* (2019) 11:3606. doi: 10.3390/su11133606
74. Tabatabaei-Malazy O, Ramezani A, Atlasi R, Larijani B, Abdollahi M. Scientometric study of academic publications on antioxidative herbal medicines in type 2 diabetes mellitus. *J Diabetes Metab Disord.* (2016) 15:48. doi: 10.1186/s40200-016-0273-3
75. Merigó JM, Yang JB. A bibliometric analysis of operations research and management science. *Omega.* (2017) 73:37–48. doi: 10.1016/j.omega.2016.12.004
76. Podsakoff PM, MacKenzie SB, Podsakoff NP, Bachrach DG. Scholarly influence in the field of management: a bibliometric analysis of the determinants of university and author impact in the management literature in the past quarter century. *J Manag.* (2008) 34:641–720. doi: 10.1177/0149206308319533
77. Kamdem JP, Duarte AE, Lima KRR, Rocha JBT, Hassan W, Barros LM, et al. Research trends in food chemistry: a bibliometric review of its 40 years anniversary (1976-2016). *Food Chem.* (2019) 294:448–57. doi: 10.1016/j.foodchem.2019.05.021
78. Chen C. CiteSpace II: detecting and visualizing emerging trends and transient patterns in scientific literature. *J Am Soc Inf Sci Technol.* (2006) 57:359–77. doi: 10.1002/asi.20317
79. Liang L, Xie C. Investigation of China's nanotechnology study based on frequency analysis of key words. *Stud Sci Sci.* (2003) 21:138–42. doi: 10.16192/j.cnki.1003-2053.2003.02.006
80. Radhakrishnan S, Erbis S, Isaacs JA, Kamarthi S. Novel keyword co-occurrence network-based methods to foster systematic reviews of scientific literature. *PLoS ONE.* (2017) 12:e0172778. doi: 10.1371/journal.pone.0172778
81. Spence DP, Owens KC. Lexical co-occurrence and association strength. *J Psycholinguist Res.* (1990) 19:317–30. doi: 10.1007/BF01074363
82. Watad A, Bragazzi NL, Adawi M, Amital H, Kivity S, Mahroum N, et al. Is autoimmunology a discipline of its own? A big data-based bibliometric and scientometric analyses. *Autoimmunity.* (2017) 50:269–74. doi: 10.1080/08916934.2017.1305361
83. Zhang J, Xie J, Hou W, Tu X, Xu J, Song F, et al. Mapping the knowledge structure of research on patient adherence: knowledge domain visualization based co-word analysis and social network analysis. *PLoS ONE.* (2012) 7:e34497. doi: 10.1371/journal.pone.0034497
84. Di Marco Barros R, Roberts NA, Dart RJ, Vantourout P, Jandke A, Nussbaumer O, et al. Epithelia Use Butyrophilin-like molecules to shape organ-specific γδ T cell compartments. *Cell.* (2016) 167:203–218.e17. doi: 10.1016/j.cell.2016.08.030

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REVIEW
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Dendritic Epidermal T Cells in Allergic Contact Dermatitis

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Allergic contact dermatitis (ACD) is a common inflammatory skin disease with a prevalence of approximately 20% in the European population. ACD is caused by contact allergens that are reactive chemicals able to modify non-immunogenic self-proteins to become immunogenic proteins. The most frequent contact allergens are metals, fragrances, and preservatives. ACD clinically manifests as pruritic eczematous lesions, erythema, local papules, and oedema. ACD is a T cell-mediated disease, involving both $CD4^+$ and $CD8^+$ T cells. In addition, γδ T cells appear to play an important role in the immune response to contact allergens. However, it is debated whether γδ T cells act in a pro- or anti-inflammatory manner. A special subset of γδ T cells, named dendritic epidermal T cells (DETC), is found in the epidermis of mice and it plays an important role in immunosurveillance of the skin. DETC are essential in sensing the contact allergen-induced stressed environment. Thus, allergen-induced activation of DETC is partly mediated by numerous allergen-induced stress proteins expressed on the keratinocytes (KC). Several stress proteins, like mouse UL-16-binding protein-like transcript 1 (Mult-1), histocompatibility 60 (H60) and retinoic acid early inducible-1 (Rae-1) α-ε family in mice and major histocompatibility complex (MHC) class I—chain-related A (MICA) in humans, are upregulated on allergen-exposed KC. Allergen-induced stress proteins expressed on the KC are consequently recognized by NKG2D receptor on DETC. This review focuses on the role of γδ T cells in ACD, with DETC in the spotlight, and on the role of stress proteins in contact allergen-induced activation of DETC.

Keywords: allergic contact dermatitis, skin, inflammatory disease, contact allergens, dendritic epidermal T cells, γδ T cells, stress proteins

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ALLERGIC CONTACT DERMATITIS

Allergic contact dermatitis (ACD), an inflammatory dermatosis caused by contact of the skin with substances from the environment, is known to humankind since ancient times (1). One of the first cases come from the first century A.D., where patients experienced pruritic eczema upon cutting pine trees (2).

ACD is a type IV hypersensitivity mainly orchestrated by allergen-specific T cells (3), and it is one of the most frequent forms of inflammatory skin diseases. ACD clinically manifests as pruritic eczematous lesions, erythema, local papules and oedema (4–6). In most cases, the dermatitis is localized to the site of contact with the contact allergen; however, systemic reactions can also occur (7, 8). In chronic lesions, the skin is scaly and thicker with erythema and often vesicles (9).

Allergy to contact allergens is diagnosed by patch testing. The most recent systematic review and meta-analysis conducted by *Alinaghi et al.* states that 20.1% of patch tested individuals from the general population suffer from contact allergy with twice as high prevalence in women than in men (10). The most common contact allergens are nickel, cobalt, fragrance allergens, chromium, *p*-phenylenediamine, methylchloroisothiazolinone/methylisothializanone, and colophonium (10). Treatment of ACD is still only symptomatic often including anti-inflammatory corticosteroids (11). A deeper understanding of the immune cells and signaling pathways involved in the response to contact allergens is central for the development of more specific treatments for ACD.

IMMUNE RESPONSE TO CONTACT ALLERGENS

Much of our knowledge on the immune response to contact allergens comes from studies using mouse models. Mouse models of ACD are often described as contact hypersensitivity (CHS) models. As ACD is a result of CHS, the abbreviations ACD and CHS will be used interchangeably in this review. ACD is mainly driven by T cells and its pathophysiology is divided into two phases, namely the sensitization and the elicitation/challenge phase (**Figure 1**). After penetrating the skin, contact allergens modify self-proteins into immunogenic proteins. The stability of the immunogenic proteins is crucial for a proper induction of an acquired immune response (12). Skin inflammation is rapidly induced upon exposure of the skin to contact allergens with interleukin (IL) IL-1β and IL-18 being essential in the response (13–17). Blocking IL-1β with neutralizing antibodies prior to sensitization with TNCB resulted in decreased ear swelling after challenge to TNCB, indicating that IL-1β plays an important role in the induction of ACD in mice (18). IL-1β-deficient mice showed no footpad swelling following sensitization with low concentration of TNCB and required high concentrations of TNCB to develop significant footpad swelling (19). Treatment of mice with the IL-1 receptor (IL-1R) antagonist anakinra suppressed the ear swelling in response to DNFB (20), and a similar effect was seen in IL-1R-deficient mice in response to TNCB (15). In brief, IL-1β clearly seems to be an important mediator in both the sensitization and elicitation phases (15, 18–20).

The inflammatory response leads to activation and migration of antigen-presenting cells (APC) from the skin via afferent lymphatic vessels to the draining lymph nodes (dLN), where the priming and activation of naïve T cells occur (21–23). Following activation, T cells proliferate and differentiate into effector and memory T cells. By the generation of memory T cells an individual has become sensitized to the allergen and subsequent exposures to the same allergen will induce a challenge/elicitation response.

Both $CD4^+$ and $CD8^+$ T cells are important mediators of the immune response to contact allergens. However, whereas $CD8^+$ T cells are involved in pro-inflammatory responses, $CD4^+$ T cells can mediate both pro- and anti-inflammatory responses (24, 25). $CD4^+$ and $CD8^+$ T cells mediate inflammation via production of IFNγ and IL-17A (25–31). Different subsets of memory T cells, including circulating central and effector memory T cells and tissue-resident memory T (T_{RM}) cells develop during the sensitization phase (32). A specific subset of $CD8^+$ T_{RM} cells is generated locally in the epidermis following exposure of the skin to contact allergens (32–34). Interestingly, a faster and stronger inflammatory response is induced in allergen-experienced skin compared to allergen-unexperienced skin. The increased response correlates with the production of IFNγ and IL-17A by T_{RM} cells (33).

ROLE OF γδ T CELLS IN ACD

γδ T cells are unconventional T cells (35, 36) and represent only a minor fraction of the T cells in the blood. However, γδ T cells populate non-lymphoid tissues, like the skin at a high frequency (37–42). A specific subset of γδ T cells is found in mouse epidermis, namely the dendritic epidermal T cells (DETC) that play an important role in skin homeostasis and repair (42–44). The DETC have a highly restricted TCR repertoire, expressing the invariant Vγ3Vδ1 TCR (35, 36, 45). An alternative nomenclature for γδ TCR exists in which DETC are Vγ5Vδ1 (46). Thus, DETC are able to recognize only a limited pool of antigens (35, 36, 47). The antigens recognized by the DETC are yet to be discovered (42, 44, 48–52). However, DETC possess other receptors that recognize a variety of stress-induced molecules and heat shock proteins in a MHC-independent manner (53, 54). This characteristic makes DETC efficient responders to various environmental triggers (**Figure 2**).

The role of γδ T cells, including DETC, in ACD is debated (please see **Table 1** for an overview). Some studies have suggested that γδ T cells play a pro-inflammatory role (20, 55–59, 67), whereas other studies have suggested an anti-inflammatory role of γδ T cells in ACD (63–66). A role of γδ T cells in the response to contact allergens was first shown by adoptive transfer of T cells isolated from the dLN of sensitized mice into allergen-naïve mice (55–57). It was shown that γδ T cells assist αβ T cells in the transfer of CHS from sensitized to naïve mice (55–57), as adoptive transfer of only αβ T cells was not sufficient to transfer CHS. Both αβ and γδ T cells were required to transfer CHS (55, 57). It can be speculated, that the γδ and αβ T cells rely on each other with the production of different cytokines and chemokines required for the migration to allergen-exposed sites. Interestingly, transfer of αβ T cells from mice sensitized with TNP-Cl together with γδ T cells from mice sensitized with oxazolone (OXA) to allergen-naive mice, which were subsequently challenged with TNP-Cl, resulted in increased CHS, indicating that γδ T cells assist αβ T cells in an allergen-unspecific manner (55).

The inflammatory role of γδ T cells in response to contact allergens was further underlined in studies using TCRδ-deficient mice (20, 58). Lack of γδ T cells resulted in more than a 50% reduction in the response to DNFB as measured by changes in

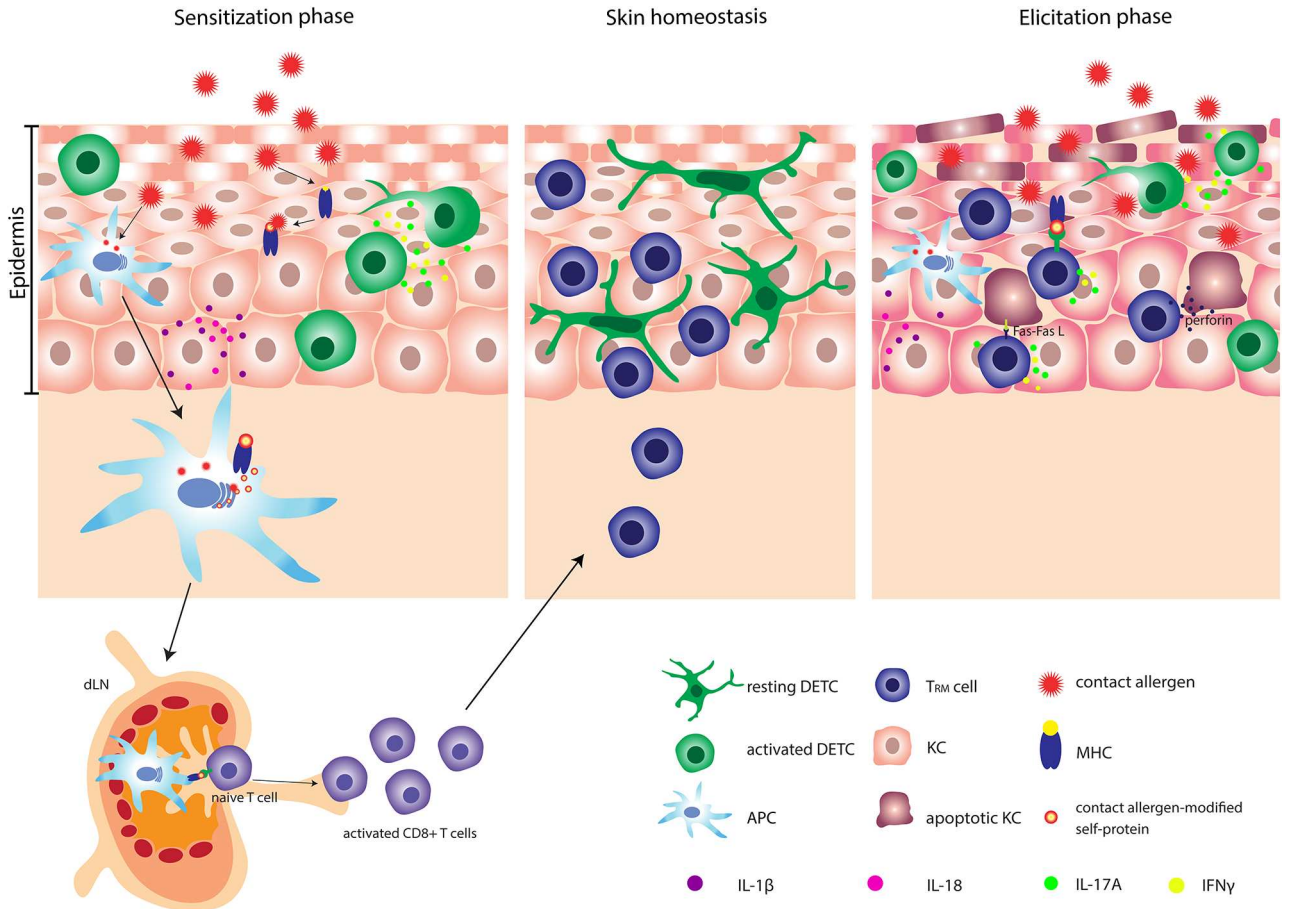

FIGURE 1 | Pathophysiology of allergic contact dermatitis (ACD). After penetrating the skin, contact allergens react with or modify self-proteins into immunogenic proteins. Contact allergen-stressed KC produce profound amount of IL-1β and IL-18 and activated DETC produce IL-17A and IFN-γ. Simultaneously, APC are activated and can subsequently carry contact allergen-modified self-protein-MHC complexes to the dLN, where the priming of naïve T cells occur. Activated $CD8^+$ T cells undergo clonal expansion and several memory subsets are developed. One specific subset of memory $CD8^+$ T cells e.g., T_{RM} cells migrates back to the site of primary allergen exposure. At the second exposure to the contact allergen, a faster and stronger inflammatory response is induced. Activated DETC and T_{RM} cells produce significant amounts of IL-17A and IFN-γ and stressed KC produce large amounts of IL-1β. Additionally, T_{RM} cells can induce apoptosis in allergen-modified KC via either the Fas-FasL-dependent or the perforin-dependent pathway. DETC, dendritic epidermal γδ T cell; APC, antigen presenting cell; T_{RM} cells, tissue-resident memory T cells; KC, keratinocyte.

ear-thickness (20, 58). Interestingly, the γδ T cells that assist the αβ T cells were shown to be DETC (55, 56). In accordance, we have shown that exposure of the skin to DNFB results in activation of DETC (20). The contact allergen-induced DETC activation was mediated by an indirect pathway, probably via the KC, involving IL-1β (20). In addition to DETC, the role of dermal γδ T cells in inflammatory skin disorders has been extensively investigated (58, 68–70). It has been shown that chimeric mice deficient in dermal γδ T cell but with normal amount of DETC have reduced ear swelling in response to DNFB treatment compared to WT mice. This indicated that dermal γδ T cells play an important pro-inflammatory role in the acute CHS response (58). In accordance, it was shown that dermal γδ T cells play a significant role in recruitment of neutrophils to the skin upon exposure to DNFB via an IL-17-dependent pathway (58).

Although these studies provide a strong evidence for a pro-inflammatory role of γδ T cells in the response to contact allergens, other studies have suggested an anti-inflammatory role of γδ T cells in ACD (63–66, 71). *Guan et al.* found that a lack of γδ T cells correlated with an increased ear swelling following OXA challenge (65). They used two different models to investigate this; TCRδ deficient C57BL/6 mice and depletion of the γδ T cells using the antibody UC7-13D5 (65). Interestingly, it has subsequently been shown that UC7-13D5 does not deplete γδ T cells, but instead induces TCR internalization and thereby generates "invisible" γδ T cells (72). Furthermore, as they only analyzed the response by changes in ear thickness, it is hard to conclude whether γδ T cells are in fact anti-inflammatory. In contrast, *Girardi et al.* observed no significant difference in ear thickness between TCRδ-deficient C57BL/6 mice and C57BL/6 mice following DNFB challenge (66). However, in the

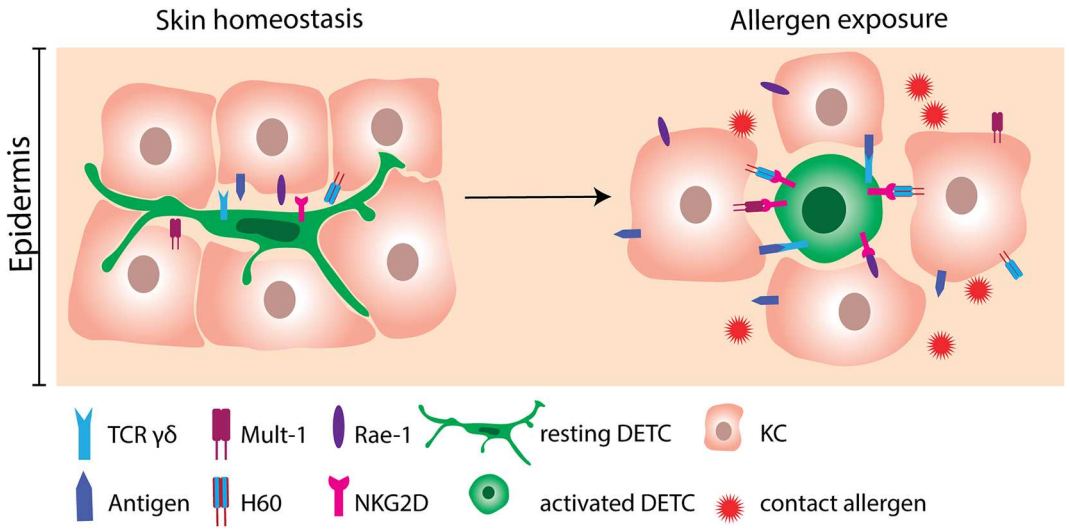

FIGURE 2 | Activation of DETC by contact allergen-induced stress molecules. DETC constantly monitor the epidermis with their dendrites in order to respond rapidly to the incoming environmental triggers. At homeostatic conditions, expression of stress molecules on KC is scarce. Following allergen exposure, KC greatly upregulate NKG2D ligands as Mult-1, H60 and Rae-1. NKG2D ligands consequently bind to NKG2D receptors expressed on DETC. Engagement of NKG2D is not sufficient to activate DETC. Therefore, interaction of the γδ TCR with yet unknown antigens probably needs to occur simultaneously. DETC, dendritic epidermal γδ T cell; KC, keratinocyte.

same study, an increase in ear thickness was found in TCRδ-deficient FVB-Tac mice compared to FVB-Tac mice using the same experimental setting. At first sight, this study suggested that DETC in C57BL/6 mice play a neutral role in ACD, whereas they play an anti-inflammatory role in FVB-Tac mice (66). However, it was subsequently shown that FVB-Tac mice have a defect in the development of DETC due to the lack of Skint-1 expression in the thymus (73–75). Interestingly, FVB-Tac mice develop spontaneous skin inflammation that correlates with infiltration of αβ T cells in the epidermis (73). Furthermore, an increased spontaneous skin inflammation was seen in TCRδ-deficient FVB-Tac mice compared to FVB-Tac mice (66). We have recently shown that a low-grade steady-state inflammation in mice with normal DETC increases the responsiveness to various contact allergens (76). Therefore, we find it difficult to conclude whether the increased response to contact allergens found in TCRδ-deficient FVB-Tac mice compared to FVB-Tac mice is directly mediated by an anti-inflammatory role of DETC or caused by the increased spontaneous skin inflammation observed in TCRδ-deficient FVB-Tac mice. *Sullivan et al.* found that intravenously (i.v.) injection of Thy-1$^+$ epidermal cells primed with 2,4,6-trinitrobenzenesulfonic acid (TNBS) *in vitro* resulted in decreased ear swelling in C57BL/6 mice sensitized with TNCB, and that the size of the ear swelling was inversely correlated with the numbers of Thy-1$^+$ epidermal cells injected (63). However, it was later shown that only 27% of Thy-1$^+$ epidermal cells co-expressed Vγ3 (64). This makes it difficult to conclude whether the tolerance induction is mediated by DETC or by another cell type. In addition, it was shown that *in vitro* FITC-conjugated Thy-1$^+$ epidermal cells clones (AU4 and AU16) could induce tolerance in FITC-challenged C3H mice when injected subcutaneously (s.c.) but not when injected i.v. (64). In contrast, unconjugated AU4 and AU16 were not able to suppress ear swelling when administered s.c. but only when injected i.v. However, whereas both AU4 and AU16 expressed Thy-1, neither of them expressed Vγ3 (64). Therefore, it is difficult to conclude that the induced tolerance is mediated by DETC. However, both studies showed that the tolerance was mediated in an allergen-specific manner (63, 64). Thus, it was found that downregulation of the CHS response occurred in C57BL/6 mice that received i.v. injections of TNBS-primed Thy-1$^+$ epidermal cells prior to sensitization and challenge with TNBS but not in mice sensitized and challenged with OXA (63). Furthermore, FITC-conjugated AU16 cells ameliorated the ear swelling in C3H mice that were challenged with FITC, but not in the C3H mice challenged with DNFB (64).

The conflicting results on the role of γδ T cells in the response to contact allergens seen in studies using TCRδ-deficient mice, might as discussed above be due to the level of spontaneous inflammation seen depending on different genetic background (20, 58, 65, 66). However, the diverging results on the role of γδ T cells in the response to contact allergens were also seen in studies with TCRδ-deficient mice on a C57BL/6 background: a pro-inflammatory role (20, 58), an anti-inflammatory role (65) and no effect (66). The explanation for this might be the use of different mouse models to induce CHS. *Girardi et al.* and *Guan et al.* sensitized and challenged on different skin areas whereas *Nielsen et al.* and *Jiang et al.* sensitized and challenged on the same skin area. Finally, lack of γδ T cells have been shown to lead to a repopulation of the skin by other cells, including innate lymphoid cells (ILC) and different types of αβ T cells e.g., DETC-like αβ T cells within the epidermis (48, 77). Interestingly, dermal ILC3 and CD4$^+$ T helper 17 cells (Th17)

TABLE 1 | Pro-inflammatory and anti-inflammatory roles of γδ T cells. DETC; dendritic epidermal γδ T cell.

Species	Model	Cell type	Cell markers	Localization	Effect	Reference
Pro-inflammatory role in ACD						
Mouse	*In vivo* depletion with anti-TCRδ (UC7-13D5) in CBA/J mice	γδ T cells	TCR γδ (UC7-13D5)	Skin	Assist αβ T cells in the transfer of CHS in an allergen-unspecific manner	(55)
	Adoptive transfer of allergen-derivatized lymphocytes in CHS model in CBA/J mice		TCR γδ (GL3)			(56)
	In vivo allergen-derivatized γδ T cells for adoptive transfer to CBA/J mice		TCR γδ (UC7-13D5/GL4)			(57)
	C57BL/6 TCRδKO mice		TCR γδ (GL3), Vγ3 (536), Vγ4 (UC3-10A6)	Dermis	Produce IL-17, recruit neutrophils to the skin in the acute CHS reponse	(58)
	CHS model in CBA/J mice	DETC	TCR γδ (UC7-13D5), Vγ3 (536)	Lymph nodes and peritoneal cavity	Assist αβ T cells in the transfer of CHS in an allergen-unspecific manner	(55)
	Adoptive transfer of allergen-derivatized lymphocytes in CHS model in CBA/J mice		TCR γδ (GL3), Vγ3 (536)	Skin		(56)
	C57BL/6 TCRδKO mice and epidermis-derived DETC short-term cell line		TCR γδ (GL3), Vγ3 (536)	Epidermis	Produce IFN-γ and IL-17A following allergen treatment	(20)
	DETC cell line 7-17				Produce IFN-γ following allergen treatment	(59)
Human	Skin biopsies from allergen-induced skin lesions of healthy donors	Vδ2$^+$ Vγ9$^+$ T cells	TCR δ1, Vδ1 (δTCS1), Vδ2 (BB3), Vγ9 (TiyA)	Epidermis and dermis	Amplification or resolution of ACD	(60)
	Skin biopsies from patients with allergy to heavy metal salts				Mediate skin defense against highly reactive heavy metals	(61)
	Skin biopsies from patients with nickel allergy	γδ T cells	TCR γδ (γ3.20)		Produce IFN-γ, IL-17A and IL-22	(62)
Anti-inflammatory role in ACD						
Mouse	Allergen-derivatized Thy$^+$ cells used for adoptive transfer to C57BL/6 mice	Thy1$^+$ cells	Thy-1.2 (30-H12)	Epidermis	Ameliorate CHS response in an allergen-specific manner	(63)
	Allergen-derivatized/non-derivatized epidermis-derived cell lines AU4, AU16 used for adoptive transfer to C3H mice					(64)
	C57BL/6 TCRδKO mice and *in vivo* depletion with anti-TCRδ (UC7-13D5)	γδ T cells		Skin		(65)
	FVB TCRδKO mice	DETC	TCR γδ (GL3), Vγ3 (536)	Epidermis		(66)

were found able to take over the functions of acutely depleted γδ T cells (77). Additionally, the repopulation is likely to depend on the microbiotic environment within the specific animal facility. Thus, further investigations using combination of various γδ T cells ablation strategies like conditional depletion of γδ T cells and TCRδ-deficient model, together with CHS model might bring new insights to the role of DETC during the response to contact allergens.

ALLERGEN-INDUCED ACTIVATION OF DETC

DETC were first described as Thy-1$^+$ dendritic epidermal T cells (37, 38) and since then their role has been intensively investigated in healthy skin (48, 52, 78) and in different skin pathologies (20, 59, 66, 67, 79–84). Interestingly, contact allergens cannot directly activate DETC but must act via an indirect pathway to activate the DETC (20). Contact allergen-induced stress molecules expressed or secreted by KC likely mediate the contact allergen-induced DETC activation.

With their dendrites, DETC monitor the microenvironment of the epidermis (**Figure 2**). Since each DETC is in direct contact to multiple KC, they respond rapidly to stressed and damaged KC (42, 59, 78, 85). In line with this, KC and epidermal cells (EC) primed with DNFB *in vitro* or *in vivo* can activate cultured DETC. Additionally, EC primed with different contact allergens like TNCB, OXA can activate DETC, whereas EC primed with irritants like sodium lauryl sulfate (SLS) and croton oil cannot activate DETC (67). If the DETC are physically separated from the EC by a cytokine permeable membrane, their proliferation rate is reduced (67), showing the importance of cell to cell contact for proper DETC activation. Several molecules, such as the NKG2D ligands mouse UL-16-binding protein-like transcript 1 (Mult-1), histocompatibility 60 (H60) and retinoic acid early inducible-1 (Rae-1) α-ε family in mice and MHC class I-chain-related A (MICA) in humans have been investigated for their ability to provide activation signals to DETC (59, 86–90). In the absence of cellular stress, the NKG2D ligands are scarcely expressed (91). However, following treatment with 2,4-dinitrobenzenesulfonic acid (DNBS) the murine KC cell line PAM2.12 upregulates Mult-1, H60 and Rae-1 (59). Likewise, treatment of mice with DNFB resulted in upregulation of Mult-1 in the epidermis (59). Importantly, DETC seem to be the only cells expressing NKG2D in the skin of mice (59). In humans, MICA is upregulated on primary KC following exposure to nickel (59). Interestingly, in addition to cutaneous lymphocyte-associated antigen (CLA) positive γδ T cells, a majority of CLA$^+$ CD8$^+$ T cells and of CLA$^+$ natural killer (NK) cells express NKG2D in humans (59). Blocking NKG2D on DETC resulted in a reduction in allergen-induced IFN-γ production (59), which provides strong evidence for an important role of NKG2D and its ligands in allergen-induced DETC activation. In addition to NKG2D, two other co-stimulatory receptors, JAML and CD100, play important roles in DETC activation during wound repair (79, 87). Although, the role of JAML and CD100 have not been investigated in ACD, it may be speculated that they play a similar role in DETC activation and the inflammatory response in ACD as seen in wound repair.

γδ T CELLS IN HUMAN ACD

The role of γδ T cells in ACD in humans is poorly characterized. In normal adult skin, γδ T cells represent around 1–15% of the CD3$^+$ lymphocytes (41, 60, 92, 93) with γδ T cells located within the basal KC layer of the epidermis and in the perivascular areas of the dermis. The localization of γδ T cells in perivascular areas suggests their origin from the circulation (39, 41, 92). In accordance, γδ T cells infiltrate the epidermis and dermis at later time point after challenge with DNCB, namely at 48 h post challenge (60). This suggested a role for γδ T cells in the amplification or the resolution of ACD reaction and not its initiation (60). In addition, γδ T cells are found in the epidermis and the dermis in allergic skin reactions to gold chloride and some of them exert dendritic morphology (61). Finally, challenge of the skin with nickel in nickel-allergic patients, resulted in a rise of γδ T cells that produced IFN-γ, IL-17A, and IL-22 in the dermis (62). This suggested a pro-inflammatory role of γδ T cells in the response to nickel. Although αβ T cells certainly are central mediators of ACD, more studies on human γδ T cells should be conducted to fully characterize their role in ACD.

TARGETING NKG2D AS A POTENTIAL NOVEL TREATMENT FOR ACD

Avoidance of exposure to the specific contact allergen is the optimal preventive treatment and will ease the symptoms in many patients with ACD. However, some patients cannot avoid the contact allergen and others need active treatment in addition to allergen avoidance to resolve the symptoms. Thus, treatment with broad anti-inflammatory/immunosuppressive drugs such as corticosteroids and cyclosporine is crucial to restrain ACD symptoms. To date, no biologics have been proven successful in the treatment of ACD in man (94). A central role of NKG2D in responses to contact allergens suggests that targeting NKG2D might be a more specific way to treat ACD than the broad anti-inflammatory/immunosuppressive drugs used today (59). Thus, locally applied NKG2D blockers or antagonist should be tested for their use in treatment of ACD. Furthermore, it has been shown that ligation of NKG2D leads to DETC degranulation-mediated cytotoxicity (88) and consequently degranulation inhibitors might be alternative promising compounds in ACD. In brief, further basic and clinical studies investigating precision therapeutics are crucial for the development of more specific treatments of ACD.

CONCLUSION

γδ T cells, including DETC, are crucial regulators of immune responses to contact allergens. Although some studies have suggested that γδ T cells might play an anti-inflammatory role during ACD, the majority of studies point to a pro-inflammatory role of γδ T cells in ACD. Contact allergens induce activation of DETC in mice via an indirect pathway that involves both cytokines and stress molecules expressed by KC which are subsequently recognized by NKG2D on the DETC. As human KC also produce and express cytokines and stress molecules in response to allergens, it is likely that these molecules could be potential targets in more specific treatments of ACD.

AUTHOR CONTRIBUTIONS

VM, CG and CB wrote the paper. VM made the table and figures.

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REFERENCES

1. Lachapelle JM. Historical Aspects. In: Frosch PJ, Menné T, Lepoittevin JP, editors. *Contact Dermatitis*. Berlin; Heidelberg: Springer (2006). doi: 10.1007/3-540-31301-X_1
2. Castagne D. *Dermatoses Professionnelles Provoquées par Les Bois Tropicaux*. Thèse de Médecine, University of Bordeaux, Bordeaux (1976).
3. Rustemeyer T, Hoogstraten IMW, Van Blomberg BME, Von Scheper RJ. Dermatitis In *Contact Dermatitis* (Heidelberg: Springer, Berlin), 11–43. doi: 10.1007/3-540-31301-X_2
4. Martin SF, Esser PR, Weber FC, Jakob T, Freudenberg MA, Schmidt M, et al. Mechanisms of chemical-induced innate immunity in allergic contact dermatitis. *Allergy Eur J Allergy Clin Immunol.* (2011) 66:1152–63. doi: 10.1111/j.1398-9995.2011.02652.x
5. Han JH, Lee HJ, Bang CH, Lee JHJY, Park YM, Lee JHJY. P-phenylenediamine hair dye allergy and its clinical characteristics. *Ann Dermatol.* (2018) 30:316–21. doi: 10.5021/ad.2018.30.3.316
6. Ahlström MG, Thyssen JP, Wennervaldt M, Menné T, Johansen JD. Nickel allergy and allergic contact dermatitis: a clinical review of immunology, epidemiology, exposure, and treatment. *Contact Dermatitis*. (2019) 81:227–41. doi: 10.1111/cod.13327
7. Jacob SE, Barland C, ElSaie ML. Patch-test-induced "flare-up" reactions to neomycin at prior biopsy sites. *Dermatitis.* (2008) 19:46–8. doi: 10.2310/6620.2008.08023
8. Tan CH, Rasool S, Johnston GA. Contact dermatitis: allergic and irritant. *Clin Dermatol.* (2014) 32:116–24. doi: 10.1016/j.clindermatol.2013.05.033
9. Blauvelt A, Hwang ST, Udey MC. Allergic and immunologic diseases of the skin. *J Allergy Clin Immunol.* (2003) 111:560–70. doi: 10.1067/mai.2003.78
10. Alinaghi F, Bennike NH, Egeberg A, Thyssen JP, Johansen JD. Prevalence of contact allergy in the general population: a systematic review and meta-analysis. *Contact Derm.* (2019) 80:77–85. doi: 10.1111/cod.13119
11. Karlberg AT, Bergström MA, Börje A, Luthman K, Nilsson JLG. Allergic contact dermatitis - Formation, structural requirements, and reactivity of skin sensitizers. *Chem Res Toxicol.* (2008) 21:53–69. doi: 10.1021/tx7002239
12. Vocanson M, Hennino A, Rozières A, Poyet G, Nicolas JF. Effector and regulatory mechanisms in allergic contact dermatitis. *Allergy Eur J Allergy Clin Immunol.* (2009) 64:1699–714. doi: 10.1111/j.1398-9995.2009.02082.x
13. Enk AH, Katz SI. Early molecular events in the induction phase of contact sensitivity. *Proc Natl Acad Sci USA.* (1992) 89:1398–402. doi: 10.1111/1523-1747.ep12668608
14. Sutterwala FS, Ogura Y, Szczepanik M, Lara-Tejero M, Lichtenberger GS, Grant EP, et al. Critical role for NALP3/CIAS1/cryopyrin in innate and adaptive immunity through its regulation of caspase-1. *Immunity.* (2006) 24:317–27. doi: 10.1016/j.immuni.2006.02.004
15. Watanabe H, Gaide O, Pétrilli V, Martinon F, Contassot E, Roques S, et al. Activation of the IL-1β-processing inflammasome is involved in contact hypersensitivity. *J Invest Dermatol.* (2007) 127:1956–63. doi: 10.1038/sj.jid.5700819
16. Vennegaard MT, Dyring-Andersen B, Skov L, Nielsen MM, Schmidt JD, Bzorek M, et al. Epicutaneous exposure to nickel induces nickel allergy in mice via a MyD88-dependent and interleukin-1-dependent pathway. *Contact Derm.* (2014) 71:224–32. doi: 10.1111/cod.12270
17. Watanabe H, Gehrke S, Contassot E, Roques S, Tschopp J, Friedmann PS, et al. Danger signaling through the inflammasome acts as a master switch between tolerance and sensitization. *J Immunol.* (2008) 180:5826–32. doi: 10.4049/jimmunol.180.9.5826
18. Enk AH, Angeloni VL, Udey MC, Katz SI. An essential role for Langerhans cell-derived IL-1 beta in the initiation of primary immune responses in skin. *J Immunol.* (1993) 150:3698–704.
19. Shornick LP, De Togni P, Mariathasan S, Goellner J, Strauss-Schoenberger J, Karr RW, et al. Mice deficient in IL-1β manifest impaired contact hypersensitivity to trinitrochlorobenzene. *J Exp Med.* (1996) 183:1427–36. doi: 10.1084/jem.183.4.1427
20. Nielsen MM, Lovato P, MacLeod AS, Witherden DA, Skov L, Dyring-Andersen B, et al. IL-1beta -Dependent activation of dendritic epidermal T cells in contact hypersensitivity. *J Immunol.* (2014) 192:2975–83. doi: 10.4049/jimmunol.1301689
21. Kripke ML, Munn CG, Jeevan A, Tang J, Bucana C. Evidence that cutaneous antigen-presenting cells migrate to regional lymph nodes during contact sensitization. *J Immunol.* (1990) 145:2833–8.
22. Weinlich G, Heine M, Stossel H, Zanella M, Stoitzner P, Ortner U, et al. Entry into afferent lymphatics and maturation *in situ* of migrating murine cutaneous dendritic cells. *J Invest Dermatol.* (1998) 110:441–8. doi: 10.1046/j.1523-1747.1998.00161.x
23. Honda T, Nakajima S, Egawa G, Ogasawara K, Malissen B, Miyachi Y, et al. Compensatory role of Langerhans cells and langerin-positive dermal dendritic cells in the sensitization phase of murine contact hypersensitivity. *J Allergy Clin Immunol.* (2010) 125: 1154–6.e2. doi: 10.1016/j.jaci.2009.12.005
24. Gocinski BL, Tigelaar RE. Roles of CD4 + and CD8 + T cells in murine contact sensitivity revealed by in vivo monoclonal antibody depletion. *J Immunol.* (1990) 144:4121–8.
25. Wang B, Fujisawa H, Zhuang L, Freed I, Howell BG, Shahid S, et al. CD4 + Th1 and CD8 + Type 1 Cytotoxic T Cells Both Play a Crucial Role in the Full Development of Contact Hypersensitivity. *J Immunol.* (2000) 165:6783–90. doi: 10.4049/jimmunol.165.12.6783
26. Abe M, Kondo T, Xu H, Fairchild RL. Interferon-γ inducible protein (IP-10) expression is mediated by CD8+ T cells and is regulated by CD4+ T cells during the elicitation of contact hypersensitivity. *J Invest Dermatol.* (1996) 107:360–6. doi: 10.1111/1523-1747.ep12363337
27. Xu H, Dilulio NA, Fairchild RL. T cell populations primed by hapten sensitization in contact sensitivity are distinguished by polarized patterns of cytokine production: interferon gamma-producing (Tc1) effector CD8+ T cells and interleukin (Il) 4/Il-10-producing (Th2) negative regulator. *J Exp Med.* (1996) 183:1001–12. doi: 10.1084/jem.183.3.1001
28. Nakae S, Komiyama Y, Nambu A, Sudo K, Iwase M, Homma I, et al. Antigen-specific T cell sensitization is impaired in Il-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity.* (2002) 17:375–87. doi: 10.1016/S1074-7613(02)00391-6
29. He D, Wu L, Kim HK, Li H, Elmets CA, Xu H. CD8 + IL-17-Producing T Cells Are Important in Effector Functions for the Elicitation of Contact Hypersensitivity Responses. *J Immunol.* (2006) 177:6852–8. doi: 10.4049/jimmunol.177.10.6852
30. Kish DD, Li X, Fairchild RL. CD8 T cells producing IL-17 and IFN-γ initiate the innate immune response required for responses to antigen skin challenge. *J Immunol.* (2009) 182:5949–59. doi: 10.4049/jimmunol.0802830
31. He D, Wu L, Kim HK, Li H, Elmets CA, Xu H. IL-17 and IFN-γ mediate the elicitation of contact hypersensitivity responses by different mechanisms and both are required for optimal responses. *J Immunol.* (2009) 2:1463–70. doi: 10.4049/jimmunol.0804108
32. Gaide O, Emerson RO, Jiang X, Gulati N, Nizza S, Desmarais C, et al. Common clonal origin of central and resident memory T cells following skin immunization. *Nat Med.* (2015) 21:647–53. doi: 10.1038/nm.3860
33. Schmidt JD, Ahlstrom MG, Johansen JD, Dyring-Andersen B, Agerbeck C, Nielsen MM, et al. Rapid allergen-induced interleukin-17 and gamma interferon secretion by skin-resident memory CD8+ T cells. *Contact Derm.* (2017) 76:218–27. doi: 10.1111/cod.12715
34. Gamradt P, Laoubi L, Nosbaum A, Mutez V, Lenief V, Grande S, et al. Inhibitory checkpoint receptors control CD8+ resident memory T cells to prevent skin allergy. *J Allergy Clin Immunol.* (2019) 143:2147–57.e9. doi: 10.1016/j.jaci.2018.11.048

35. Asarnow DM, Kuziel WA, Bonyhad M, Tigelaar RE, Tucker PW, Allison JP. Limited diversity of γδ antigen receptor genes of thy-1+ dendritic epidermal cells. *Cell*. (1988) 55:837–47. doi: 10.1016/0092-8674(88)90139-0
36. Havran WL, Allison JP. Developmentally ordered appearance of thymocytes expressing different T-cell antigen receptors. *Nature*. (1988) 335:443–5. doi: 10.1038/335443a0
37. Bergstresser PR, Tigelaar RE, Dees JH, Streilein JW. Thy-1 antigen-bearing dendritic cells populate murine epidermis. *J Invest Dermatol*. (1983) 81:286–8. doi: 10.1111/1523-1747.ep12518332
38. Tschachler E, Schuler G, Hutterer J, Leibl H, Wolff K. Expression of Thy-1 Antigen by Murine Epidermal Cells. *J Invest Dermatol*. (1983) 81:282–5. doi: 10.1111/1523-1747.ep12518326
39. Dupuy P, Heslan M, Fraitag S, Hercend T, Dubertret L, Bagot M. T-cell receptor-γ/d bearing lymphocytes in normal and inflammatory human skin. *J Invest Dermatol*. (1990) 94:764–8. doi: 10.1111/1523-1747.ep12874626
40. Toulon A, Breton L, Taylor KR, Tenenhaus M, Bhavsar D, Lanigan C, et al. A role for human skin-resident T cells in wound healing. *J Exp Med*. (2009) 206:743–50. doi: 10.1084/jem.20081787
41. Dyring-Andersen B, Nielsen MM, Geisler C, Bonefeld CM, Skov L. IL-17A- and IFNγ-producing T cells in healthy skin. *Scand J Immunol*. (2016) 83:297–9. doi: 10.1111/sji.12412
42. Nielsen MM, Witherden DA, Havran WL. γδ T cells in homeostasis and host defence of epithelial barrier tissues. *Nat Rev Immunol*. (2017) 17:733–45. doi: 10.1038/nri.2017.101
43. Jameson J, Havran WL. Skin γδ T-cell functions in homeostasis and wound healing. *Immunol Rev*. (2007) 215:114–22. doi: 10.1111/j.1600-065X.2006.00483.x
44. MacLeod AS, Havran WL. Functions of skin-resident γδ T cells. *Cell Mol Life Sci*. (2011) 68:2399–408. doi: 10.1007/s00018-011-0702-x
45. Garman RD, Doherty PJ, Raulet DH. Diversity, rearrangement, and expression of murine T cell gamma genes. *Cell*. (1986) 45:733–42. doi: 10.1016/0092-8674(86)90787-7
46. Heilig JS, Tonegawa S. Diversity of murine gamma genes and expression in fetal and adult T lymphocytes. *Nature*. (1986) 322:836–40. doi: 10.1038/322836a0
47. Holtmeier W, Pfänder M, Hennemann A, Zollner TM, Kaufmann R, Caspary WF. The TCR δ repertoire in normal human skin is restricted and distinct from the TCR δ repertoire in the peripheral blood. *J Invest Dermatol*. (2001) 116:275–80. doi: 10.1046/j.1523-1747.2001.01250.x
48. Jameson JM, Cauvi G, Witherden DA, Havran WL. A Keratinocyte-Responsive TCR Is Necessary for Dendritic Epidermal T Cell Activation by Damaged Keratinocytes and Maintenance in the Epidermis. *J Immunol*. (2004) 172:3573–9. doi: 10.4049/jimmunol.172.6.3573
49. Komori HK, Witherden DA, Kelly R, Sendaydiego K, Jameson JM, Teyton L, et al. Cutting Edge: Dendritic Epidermal γδ T Cell Ligands Are Rapidly and Locally Expressed by Keratinocytes following Cutaneous Wounding. *J Immunol*. (2012) 188:2972–6. doi: 10.4049/jimmunol.1100887
50. Witherden DA, Ramirez K, Havran WL. Multiple receptor-ligand interactions direct tissue-resident yd T cell activation. *Front Immunol*. (2014) 5:602. doi: 10.3389/fimmu.2014.00602
51. Ramirez K, Witherden DA, Havran WL. All hands on DE(T)C: epithelial-resident γδ T cells respond to tissue injury. *Cell Immunol*. (2015) 296:57–61. doi: 10.1016/j.cellimm.2015.04.003
52. Zhang B, Wu J, Jiao Y, Bock C, Dai M, Chen B, et al. Differential requirements of TCR signaling in homeostatic maintenance and function of dendritic epidermal T cells. *J Immunol*. (2019) 195:4282–91. doi: 10.4049/jimmunol.1501220
53. Born WK, Kemal Aydintug M, O'brien RL. Diversity of γδ T-cell antigens. *Cell Mol Immunol*. (2013) 10:13–20. doi: 10.1038/cmi.2012.45
54. Wiest DL. Development of γδ T Cells, the Special-Force Soldiers of the Immune System. In: Bosselut R, Vacchio M, editors. *T-Cell development-Methods in Molecular Biology*. New Yorl, NY: Humana Press (2016). p. 23–32. doi: 10.1007/978-1-4939-2809-5_2
55. Dieli F, Asherson GL, Sireci G, Dominici R, Gervasi F, Vendetti S, et al. γδ cells involved in contact sensitivity preferentially rearrange the Vγ3 region and require interleukin-7. *Eur J Immunol*. (1997) 27:206–14. doi: 10.1002/eji.1830270131
56. Dieli F, Ptak W, Sireci G, Romano GC, Potestio M, Salerno A, et al. Cross-talk between Vβ8+ and γδ+ T lymphocytes in contact sensitivity. *Immunology*. (1998) 93:469–77. doi: 10.1046/j.1365-2567.1998.00435.x
57. Ptak W, Askenase PW. Gamma delta T cells assist alpha beta T cells in adoptive transfer of contact sensitivity. *J Immunol*. (1992) 149:3503–8.
58. Jiang X, Park CO, Sweeney JG, Yoo MJ, Gaide O, Kupper TS. Dermal γδ T cells do not freely re-circulate out of skin and produce IL-17 to promote neutrophil infiltration during primary contact hypersensitivity. *PLoS ONE*. (2017) 12:1–20. doi: 10.1371/journal.pone.0169397
59. Nielsen MM, Dyring-Andersen B, Schmidt JD, Witherden D, Lovato P, Woetmann A, et al. NKG2D-dependent activation of dendritic epidermal T cells in contact hypersensitivity. *J Invest Dermatol*. (2015) 135:1311–9. doi: 10.1038/jid.2015.23
60. Fujita M, Miyachi Y, Nakata K, Imamura S. γδ T-cell receptor-positive cells of human skin. II Appearance in delayed-type hypersensitivity reaction. *Arch Dermatol Res*. (1993) 285:436–40. doi: 10.1007/BF00372140
61. Nordlind K, Lidén S. Gamma/delta T cells and human skin reactivity to heavy metals. *Arch Dermatol Res*. (1995) 287:137–41. doi: 10.1007/BF01262321
62. Dyring-Andersen B, Skov L, Løvendorf MB, Bzorek M, Søndergaard K, Lauritsen JPH, et al. CD4+ T cells producing interleukin (IL)-17, IL-22 and interferon-γ are major effector T cells in nickel allergy. *Contact Derm*. (2013) 68:339–47. doi: 10.1111/cod.12043
63. Sullivan S, Bergstresser PR, Tigelaar RE, Streilein JW. Induction and regulation of contact hypersensitivity by resident, bone marrow-derived, dendritic epidermal cells: Langerhans cells and Thy-1+ epidermal cells. *J Immunol*. (1986) 137:2460–7.
64. Welsh EA, Kripke ML. Murine Thy-1 + dendritic epidermal cells induce immunologic tolerance *in vivo*. *J Immunol*. (1990) 144:883–91.
65. Guan H, Zu G, Slater M, Elmets C, Xu H. γδT cells regulate the development of hapten-specific CD8+ effector T cells in contact hypersensitivity responses. *J Invest Dermatol*. (2002) 119:137–42. doi: 10.1046/j.1523-1747.2002.01830.x
66. Girardi M, Lewis J, Glusac E, Filler RB, Geng L, Hayday AC, et al. Resident skin-specific gammadelta T cells provide local, nonredundant regulation of cutaneous inflammation. *J Exp Med*. (2002) 195:855–67. doi: 10.1084/jem.20012000
67. Huber H, Descossy P, van Brandwijk R, Knop J. Activation of murine epidermal TCR-gamma delta+ T cells by keratinocytes treated with contact sensitizers. *J Immunol*. (1995) 155:2888–94.
68. Gray EE, Suzuki K, Cyster JG. Cutting Edge: Identification of a Motile IL-17–Producing γδ T Cell Population in the Dermis. *J Immunol*. (2011) 186:6091–5. doi: 10.4049/jimmunol.1100427
69. Sumaria N, Roediger B, Ng LG, Qin J, Pinto R, Cavanagh LL, et al. Cutaneous immunosurveillance by self-renewing dermal γδ T cells. *J Exp Med*. (2011) 208:505–18. doi: 10.1084/jem.20101824
70. Yihua C, Xiaoyan S, Chuanlin D, Chunjian Q, Kejia L, Xia L, et al. Pivotal Role of Dermal IL-17-producing γδ T Cells in Skin Inflammation. *Immunity*. (2011) 35:596–610. doi: 10.1016/j.immuni.2011.08.001.Pivotal
71. Tamaki K, Fujiwara H, Katz SI. The Role of Epidermal Cells in the Induction and Suppression of Contact Sensitivity. *J Invest Dermatol*. (1981) 76:275–8. doi: 10.1111/1523-1747.ep12526115
72. Koenecke C, Czeloth N, Bubke A, Schmitz S, Kissenpfennig A, Malissen B, et al. Alloantigen-specific de novo-induced Foxp3+ Treg revert in vivo and do not protect from experimental GVHD. *Eur J Immunol*. (2009) 39:3091–6. doi: 10.1002/eji.200939432
73. Lewis JM, Girardi M, Roberts SJ, Barbee SD, Hayday AC, Tigelaar RE. Selection of the cutaneous intraepithelial γδ+ T cell repertoire by a thymic stromal determinant. *Nat Immunol*. (2006) 7:843–50. doi: 10.1038/ni1363
74. Boyden LM, Lewis JM, Barbee SD, Bas A, Girardi M, Hayday AC, et al. Skint1, the prototype of a newly identified immunoglobulin superfamily gene cluster, positively selects epidermal γδ T cells. *Nat Genet*. (2008) 40:656–62. doi: 10.1038/ng.108
75. Barbee SD, Woodward MJ, Turchinovich G, Mention JJ, Lewis JM, Boyden LM, et al. Skint-1 is a highly specific, unique selecting component for epidermal T cells. *Proc Natl Acad Sci USA*. (2011) 108:3330–5. doi: 10.1073/pnas.1010890108
76. Petersen TH, Jee MH, Gadsbøll ASØ, Schmidt JD, Sloth JJ, Sonnenberg GF, et al. Mice with epidermal filaggrin deficiency show increased immune

reactivity to nickel. *Contact Derm*. (2019) 80:139–48. doi: 10.1111/cod.13153
77. Sandrock I, Reinhardt A, Ravens S, Binz C, Wilharm A, Martins J, et al. Genetic models reveal origin, persistence and nonredundant functions of IL-17-producing γδ T cells. *J Exp Med*. (2018) 215:3006–18. doi: 10.1084/jem.20181439
78. Sharp LL, Jameson JM, Cauvi G, Havran WL. Dendritic epidermal T cells regulate skin homeostasis through local production of insulin-like growth factor 1. *Nat Immunol*. (2005) 6:73–9. doi: 10.1038/ni1152
79. Witherden DA, Verdino P, Rieder SE, Garijo O, Robyn E, Teyton L, et al. The adhesion molecule JAML is a costimulatory receptor for epithelial gammadelta T cell activation. *Science*. (2010) 329:1205–1210. doi: 10.1126/science.1192698.The
80. Yoshida S, Mohamed RH, Kajikawa M, Koizumi J, Tanaka M, Fugo K, et al. Involvement of an NKG2D ligand H60c in epidermal dendritic T cell-mediated wound repair. *J Immunol*. (2012) 188:3972–9. doi: 10.4049/jimmunol.1102886
81. Macleod AS, Witherden DA, Wendy L, Macleod AS, Hemmers S, Garijo O, et al. Dendritic epidermal T cells regulate skin antimicrobial barrier function. *J Clin Invest*. (2013) 123:4364–74. doi: 10.1172/JCI70064.4364
82. Keyes BE, Liu S, Asare A, Naik S, Levorse J, Polak L, et al. Impaired epidermal to dendritic T cell signaling slows wound repair in aged skin. *Cell*. (2016) 167:1323–38.e14. doi: 10.1016/j.cell.2016.10.052
83. Liu Z, Liang G, Gui L, Li Y, Liu M, Bai Y, et al. Weakened IL-15 production and impaired mTOR activation alter dendritic epidermal T cell homeostasis in diabetic mice. *Sci Rep*. (2017) 7:6028. doi: 10.1038/s41598-017-05950-5
84. Ø Gadsbøll AS, Jee MH, Funch AB, Alhede M, Mraz V, Weber JF, et al. Pathogenic CD8+ epidermis-resident memory T cells displace dendritic epidermal T cells in allergic dermatitis. *J Invest Dermatol*. (2019) 140:806–15.e5. doi: 10.1016/j.jid.2019.07.722
85. Chodaczek G, Papanna V, Zal MA, Zal T. Body-barrier surveillance by epidermal γδ TCRs. *Nat Immunol*. (2012) 13:272–82. doi: 10.1038/ni.2240
86. Verdino P, Witherden DA, Ferguson MS, Corper AL, Schiefner A, Havran WL, et al. Molecular insights into γδ T cell costimulation by an anti-JAML antibody. *Structure*. (2011) 19:80–9. doi: 10.1016/j.str.2010.10.007
87. Witherden DA, Watanabe M, Garijo O, Rieder SE, Sarkisyan G, Cronin SJF, et al. The CD100 receptor interacts with its plexin B2 ligand to regulate epidermal γδ T cell function. *Immunity*. (2012) 37:314–25. doi: 10.1016/j.immuni.2012.05.026
88. Ibusuki A, Kawai K, Yoshida S, Uchida Y, Nitahara-Takeuchi A, Kuroki K, et al. NKG2D triggers cytotoxicity in murine epidermal γδ T cells via PI3K-dependent, Syk/ZAP70-independent signaling pathway. *J Invest Dermatol*. (2014) 134:396–404. doi: 10.1038/jid.2013.353
89. Zhang C, Xiao C, Dang E, Cao J, Zhu Z, Fu M, et al. CD100-Plexin-B2 promotes the inflammation in psoriasis by activating NF-κB and the inflammasome in keratinocytes. *J Invest Dermatol*. (2018) 138:375–83. doi: 10.1016/j.jid.2017.09.005
90. Verdino P, Witherden DA, Havran WL, Wilson IA. The molecular interaction of CAR and JAML recruits the central cell signal transducer PI3K. *Science*. (2010) 329:1210–4. doi: 10.1126/science.1187996
91. Raulet DH, Gasser S, Gowen BG, Deng W, Jung H. Regulation of ligands for the NKG2D activating receptor. *Annu Rev Immunol*. (2013) 31:413–41. doi: 10.1146/annurev-immunol-032712-095951
92. Groh V, Porcelli S, Fabbi IM, Lanier LL, Picker LJ, Anderson T, et al. Human lymphocytes bearing T cell receptor y/d are phenotypically diverse and evenly distributed throughout the lymphoid system. *J Exp Med*. (1989) 169:1277–94. doi: 10.1084/jem.169.4.1277
93. Alaibac M, Morris J, Yu R, Chu AC. T lymphocytes bearing the γδ T-cell receptor: a study in normal human skin and pathological skin conditions. *Br J Dermatol*. (1992) 127:458–62. doi: 10.1111/j.1365-2133.1992.tb14840.x
94. Martin SF, Rustemeyer T, Thyssen JP. Recent advances in understanding and managing contact dermatitis. *F1000Res*. (2018) 7:F1000 Faculty Rev-810. doi: 10.12688/f1000research.17242.1

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REVIEW
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T Cells in Fibrosis and Fibrotic Diseases

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Fibrosis is the extensive deposition of fibrous connective tissue, and it is characterized by the accumulation of collagen and other extracellular matrix (ECM) components. Fibrosis is essential for wound healing and tissue repair in response to a variety of triggers, which include infection, inflammation, autoimmune disorder, degenerative disease, tumor, and injury. Fibrotic remodeling in various diseases, such as liver cirrhosis, pulmonary fibrosis, renal interstitial fibrosis, myocardial infarction, systemic sclerosis (SSc), and graft-versus-host disease (GVHD), can impair organ function, causing high morbidity and mortality. Both innate and adaptive immunity are involved in fibrogenesis. Although the roles of macrophages in fibrogenesis have been studied for many years, the underlying mechanisms concerning the manner in which T cells regulate fibrosis are not completely understood. The T cell receptor (TCR) engages the antigen and shapes the repertoire of antigen-specific T cells. Based on the divergent expression of surface molecules and cell functions, T cells are subdivided into natural killer T (NKT) cells, γδ T cells, $CD8^+$ cytotoxic T lymphocytes (CTL), regulatory T (Treg) cells, T follicular regulatory (Tfr) cells, and T helper cells, including Th1, Th2, Th9, Th17, Th22, and T follicular helper (Tfh) cells. In this review, we summarize the pro-fibrotic or anti-fibrotic roles and distinct mechanisms of different T cell subsets. On reviewing the literature, we conclude that the T cell regulations are commonly disease-specific and tissue-specific. Finally, we provide perspectives on microbiota, viral infection, and metabolism, and discuss the current advancements of technologies for identifying novel targets and developing immunotherapies for intervention in fibrosis and fibrotic diseases.

Keywords: T cells, fibrosis, fibrotic diseases, T helper, CD8, Treg, NKT, γδ T

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INTRODUCTION

As a leading cause of mortality, fibrotic diseases can occur in virtually every organ and tissue. Extracellular matrix (ECM) component deposition is observed in many fibroproliferative diseases, including liver cirrhosis (LC), pulmonary fibrosis, renal interstitial fibrosis, myocardial infarction, systemic sclerosis (SSc), and graft-versus-host disease (GVHD) (1, 2). Numerous studies have demonstrated that the immune response plays an essential role in fibrosis and fibrotic diseases. Systematic investigation of the immune cells and signaling pathways remains fundamental for developing novel therapies (3). Therefore, it is essential to understand the key factors influencing fibrosis.

After hepatic injuries, immune cells participate in wound healing and tissue repair by initiating inflammation. The infiltrated T cells, macrophages, neutrophils, dendritic cells (DCs), and the

liver-resident macrophages, Kupffer cells, cooperatively contribute to the liver fibrotic cascade and lead to the activation of hepatic stellate cells (HSCs) and the generation of myofibroblasts (4, 5). Non-alcoholic fatty liver disease (NAFLD) is characterized by hepatic steatosis with the presence of T cells, including natural killer T (NKT) cells, γδ T cells, $CD8^+$ cytotoxic T lymphocytes (CTL), regulatory T (Treg) cells, and T helper cells. These T cells exert their function by attenuating or aggravating the liver injury and fibrosis progression (6).

Further, pulmonary fibrosis is a highly lethal pathological process, in which T cell responses contribute to the pathogenesis of idiopathic pulmonary fibrosis (IPF), cystic fibrosis (CF), and various other lung diseases (7, 8). However, the functions of each T cell subset appear to be perplexing and are influenced by interactions with epithelial cells or fibroblasts, the pulmonary localization of fibrosis in the bronchial or alveolar region, and the disease progression stage (9, 10). T cells can regulate the pulmonary fibrosis outcome through cAMP-regulated chloride channels (11), Fas-Fas ligand (FasL) interactions (12), or T cell exhaustion (13). Given the strong relationship between fibrosis and inflammation, the determination of precise functions of T cells may also be beneficial for inflammatory and autoimmune diseases.

Cardiovascular disease (CVD) is a class of heart or blood vessel-related disease. T cells modulate cardiac fibroblasts and MMP activity during cardiac fibrosis and hypertension (14–16). Using T cell-deficient mice disease model, previous studies have demonstrated the pivotal role of T cells in heart failure (HF) (17, 18), myocardial fibrosis (19), ischemia (20), and myocardial infarction (21).

Furthermore, intestinal fibrosis occurs in the gastrointestinal tract during chronic diseases such as Crohn's disease (CD), ulcerative colitis (UC), ulcerative jejunoileitis, and radiation enteritis. T cells participate in persistent dysregulated inflammation and lead to excessive myofibroblast proliferation, ECM deposition, and scar tissue formation (15, 22).

Systemic sclerosis (SSc) affects the skin and multiple internal organs, causing excessive ECM deposition and vasculopathy. Many studies have suggested that some T cell subsets, such as Th17, Treg, Th2, Th9, and Th22, can serve as a hallmark of SSc. Therefore, discussing the mechanisms of each T cell subset may help identify leash the pro-fibrotic T cells and cytokines in SSc (2, 23).

Renal fibrosis is considered to be a consequence of immune response involving myofibroblast accumulation and matrix deposition. Researchers have observed that T cell activation and infiltration can cause interstitial fibrosis and glomerular injury in the kidney (24).

Furthermore, GVHD patients may suffer vascular injury caused by the immune response between recipient endothelial cells and circulating alloreactive donor T cells. T helper cells, including Th17 and Tfh cells, secrete IL-17 and IL-21 cytokines and augment this immune response and fibrosis outcome (25).

T cell-dependent fibrosis plays a crucial role in muscle regeneration, and sclerotic scar tissue in muscles is attributed to Duchenne muscular dystrophy (DMD). Although T cells can undermine the muscle regeneration (26), they are also important for biomaterial scaffold muscle tissue repair (27).

In summary, T cells are essential for fibrosis and fibrotic diseases. The orchestration of fibrotic tissue remodeling is programed by multiple cytokines, chemokines, and growth factors (28, 29). In this review, we briefly discuss the role of all T cell subsets and their underlying mechanisms in fibrosis and fibrotic diseases (**Table 1**, **Figure 1**).

THE ROLE AND MECHANISMS OF T CELLS IN FIBROSIS AND FIBROTIC DISEASES

Th1 Cells and Fibrosis

Inflammation is considered to be one of the major steps leading to fibrosis (29). However, the production of pro-inflammatory cytokines is not always pro-fibrotic. IL-12 induces the differentiation of naïve CD4 cells to Th1 cells to produce the pro-inflammatory cytokine IFNγ. IFNγ suppresses fibroblast-induced collagen synthesis and attenuates fibrosis. Therefore, Th1 cells are largely considered to play an anti-fibrotic role (85). Wynn et al. previously used IL-12 for treating *Schistosoma mansoni* infection in mice. This not only inhibited Th2-dominated immune response by elevating Th1 cytokine expression but also drastically ameliorated fibrosis (86). IFNγ production up-regulates the expression of matrix metalloproteinases (MMPs), including MMP-2, MMP-7, MMP-9, and MMP-13, to degrade ECM components. This proteolytic activity helps alter ECM remodeling and ameliorates fibrosis (87). Th1 cells and cytokine IFNγ are not always anti-fibrotic. On the contrary, they can also play a harmful role in bone regeneration (88), liver injury (89), and fibrotic diseases (30, 31). In cardiac fibrosis, Th1 cell infiltration leads to the activation of cardiac fibroblasts (CFBs) which then transform into myofibroblasts via integrin alpha4. Further, Th1 cells induce TGFβ expression in myofibroblasts, which forms a fibrillary ECM in the myocardium (31).

Th2 Cells and Fibrosis

Th2 cells are characterized by the production of signature cytokines IL-4, IL-5, and IL-13. Th2 cells, along with eosinophils, basophils, macrophages, and type 2 innate lymphoid cells (ILC2), contribute to the type 2 immunity-induced pathological process of fibrosis (90). As a commonly recognized opponent of Th1 cells, Th2 cells can alter Th1-associated IFNγ expression levels. In *Pseudomonas aeruginosa*-infected cystic fibrosis patients, an elevated ratio of pulmonary $CCR4^+$ Th2 cells to $CXCR3^+$ Th1 lymphocytes was found in the bronchoalveolar lavage fluid, with significantly higher levels of Th2 cytokines IL-4 and IL-13 (32). In addition to infection, allergic inflammation also triggers a Th2 response. Asthma is a chronic allergic inflammatory disease with fibrotic airway remodeling. However, the mechanism underlying airway fibrosis remains poorly understood. Morimoto et al. found that IL-33 induces the production of $ST2^{hi}$ memory-type pathogenic Th2 cells which enhance amphiregulin (Areg) levels. Furthermore, amphiregulin-epidermal growth factor receptor

TABLE 1 | The role of T cells in fibrosis and fibrotic diseases.

T cell subset	Tissue/ organ	Species	Disease	Effect in fibrosis	References
Th1	Peritoneal membrane	Mouse	SES-induced inflammation	Pro-fibrotic	(30)
Th1	Heart	Human Mouse	Cardiac fibrosis	Pro-fibrotic	(31)
Th2	Lung	Human	Cystic fibrosis	Pro-fibrotic	(32)
Th2	Lung	Human	Cystic fibrosis	Pro-fibrotic	(33)
Th2	Lung	Human Mouse	ECRS HDM	Pro-fibrotic	(34)
Th17	Liver	Mouse	BDL,CCl_4	Pro-fibrotic	(35)
Th17	Lung	Mouse	*S. rectivirgula*-induced lung fibrosis	Pro-fibrotic	(36)
Treg	Lung	Mouse	Bleomycin-induced pulmonary fibrosis	Anti-fibrotic	(37)
Treg	Heart	Mouse	Angiotensin II-infused hypertension	Anti-fibrotic	(38)
Treg	Lung	Mouse	Silica-induced lung fibrosis	Pro-fibrotic	(39)
Treg	Heart	Mouse	MI	Pro-fibrotic	(40)
Treg	Lymphatic tissues	Macaque	SIV infection	Pro-fibrotic	(41)
Treg	Liver	Human	Chronic HCV	Anti-fibrotic	(42)
Treg	Liver	Humanized mouse	HIV-1 infection	Anti-fibrotic	(43)
Treg	Liver	Mouse	Biliary fibrosis in murine sclerosing cholangitis	Anti-fibrotic	(44)
Treg	Lung	Mouse	*A. fumigatus*-induced lung fibrosiss	Anti-fibrotic	(45)
Treg	Kidney	Mouse	IRI	Anti-fibrotic	(46)
Tfh	Liver	Mouse	*S. japonicu*m infection	Pro-fibrotic	(47)
Tfh	Skin	Human Mouse	SSc GvHD	Pro-fibrotic	(48)
Tfh	Lung	Human	PBC	Pro-fibrotic	(49)
Tfh	Lung	Human	IPF	Pro-fibrotic	(50)
Th9	Lung	Mouse Human	Silica-induced lung fibrosis IPF	Pro-fibrotic	(51)
Th9	Liver	Mouse	CCl_4	Pro-fibrotic	(52)
Th9	Lung	Human	Cystic fibrosis	Pro-fibrotic	(53)
Th22	Heart	Mouse	CVB3 infection	Anti-fibrotic	(54)
Th22	Lung	Human	Cystic fibrosis	Anti-fibrotic	(55)
Th22	Liver	Mouse	CCl_4	Anti-fibrotic	(56)
Th22	Liver	Mouse	MCD diet	Anti-fibrotic	(57)
CD8	Microvessels in the skin	Human	GVHD	Pro-fibrotic	(58)
CD8	Thyroids	Mouse	Thyroid epithelial cell fibrosis	Pro-fibrotic	(59)
CD8	Lung	Mouse	Bleomycin-induced pulmonary fibrosis	Pro-fibrotic	(60)
CD8	Kidney	Mouse	UUO	Anti-fibrotic	(61)
NKT	Lung	Mouse	Bleomycin-induced pulmonary fibrosis	Anti-fibrotic	(62)
NKT	Liver	Mouse	TAA, CCl_4	Pro-fibrotic	(63)
NKT	Liver	Mouse	α-GalCer, CCl_4	Pro-fibrotic	(64)
NKT	Liver	Mouse Human	NASH	Pro-fibrotic	(65)
NKT	Liver	Mouse	CCl_4	Pro-fibrotic	(66)
NKT	Liver	Mouse	PBC	Pro-fibrotic	(67)
NKT	Liver	Mouse	NASH	Pro-fibrotic	(68)
NKT	Liver	Mouse	CCl_4	Anti-fibrotic	(69)
NKT	Liver	Mouse	CCl_4, MCD diet	Pro-fibrotic	(70)
NKT	Lung	Human	ILDs	Anti-fibrotic	(71)
γδ T	Lung	Human	IPF	Anti-fibrotic	(72)
γδ T	Lung	Human	Cystic fibrosis	Anti-fibrotic	(73)
γδ T	Liver	Mouse	CCl_4, MCD diet	Anti-fibrotic	(74)
γδ T	Lung	Mouse	Bleomycin-induced pulmonary fibrosis	Anti-fibrotic	(75)

(Continued)

TABLE 1 | Continued

T cell subset	Tissue/ organ	Species	Disease	Effect in fibrosis	References
γδ T	Lung	Human	Cystic fibrosis	Anti-fibrotic	(76)
γδ T	Lung	Mouse	Bleomycin-induced pulmonary fibrosis	Anti-fibrotic	(77)
γδ T	Lung	Mouse	*B. subtilis*-induced pulmonary fibrosis	Anti-fibrotic	(78)
γδ T	Lung	Mouse	Bleomycin-induced pulmonary fibrosis	Anti-fibrotic	(79)
γδ T	Kidney	Mouse	UUO	Pro-fibrotic	(80)
γδ T	Liver	Mouse	CCl_4	Pro-fibrotic	(81)
γδ T	Liver	Mouse	CCl_4	Anti-fibrotic	(82)
γδ T	Liver	Mouse	*S. japonicum* infection	Pro-fibrotic	(83)
γδ T	Kidney	Human	Tubulointerstitial fibrosis	Pro-fibrotic	(84)

SES, Staphylococcus epidermidis; ECRS, eosinophilic chronic rhinosinusitis; HDM, house dust mite; BDL, bile duct ligation; CCl_4, carbon tetrachloride; IPF, idiopathic pulmonary fibrosis; MI, myocardial infarction; SIV, simian immunodeficiency virus; IRI, ischemia/reperfusion injury; SSc, Systemic sclerosis; GVHD, graft-versus-host disease; PBC, primary biliary cirrhosis; CVB3, Coxsackie virus B3; MCD, methionine-choline–deficient; UUO, unilateral ureteric obstruction; TAA, thioacetamide; NASH, nonalcoholic steatohepatitis; ILDs, interstitial lung diseases.

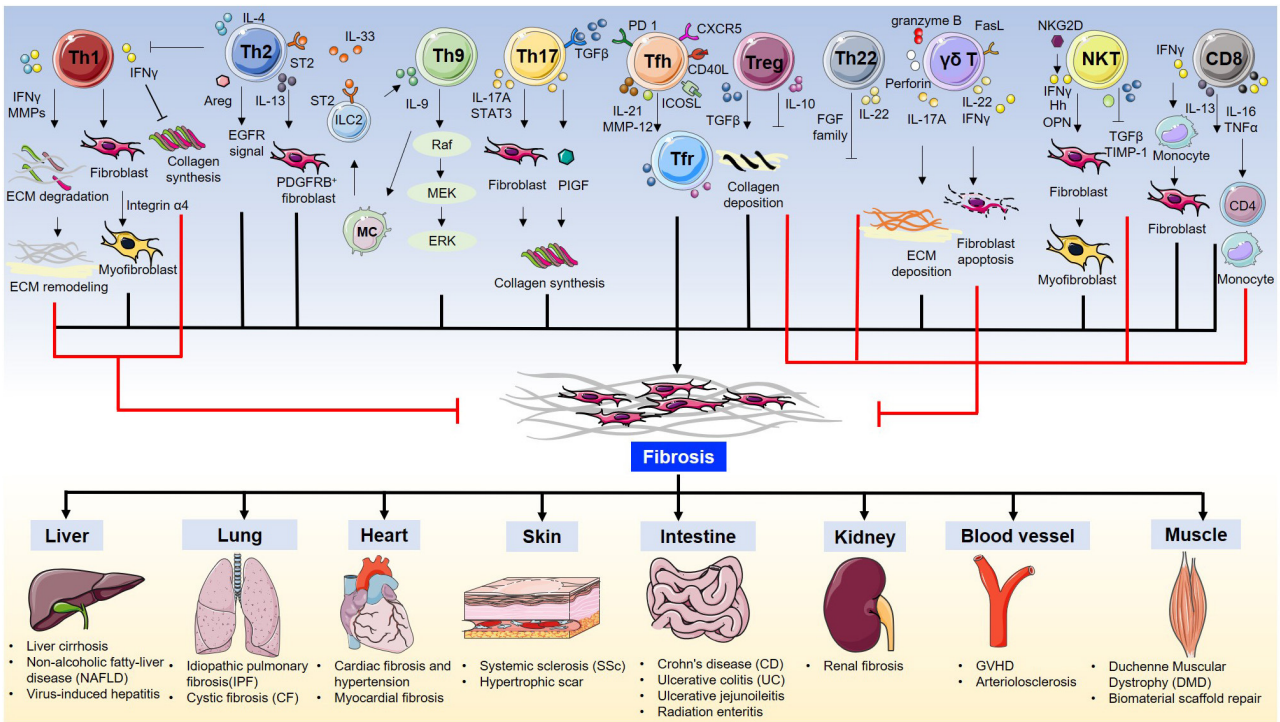

FIGURE 1 | T cells in fibrosis and fibrotic diseases.

(EGFR) signal results in osteopontin-producing eosinophils and fibrotic responses. Their study highlighted how Th2 memory cells are critical for allergy-induced airway fibrosis (34). It has been reported that aberrant and spontaneous development of Th2 cells in the lamina propria of TRAF6-knockout mice with eosinophilic enteritis causes fibrosis in the small intestine (91). Further, IL-13 is considered to be an essential Th2 cytokine for fibrosis (90). By specifically disrupting IL-13 signaling in liver-resident tissue fibroblasts, also known as hepatic stellate cells (HSCs), Gieseck et al. demonstrated that PDGFRB$^+$ fibroblasts are necessary for mediating IL-13-induced pathological fibrosis in mice (92). Furthermore, IL-13-regulated lipogenesis, bile acid synthesis, and biliary-dependent steatosis seem to be distinct cellular pathways from fibrosis, suggesting the possible intervention of IL-13 for the promotion of hepatobiliary expansion without aggravating fibrosis (92). Th2-targeted treatment has been tested in fibrosis-related diseases. For example, vitamin D3 was found to attenuate Th2 response in cystic fibrosis patients with allergic bronchopulmonary aspergillosis by substantially reducing DC-expressed costimulatory

molecule OX40 ligand (OX40L) and increasing TGFβ expression (33).

Th9 Cells and Fibrosis

Th9 cells were originally described in parasitic infections and allergic diseases. As a newly defined subset of T helper cells, it responds to environmental cues and cytokine milieu to produce IL-9 (93). Pleiotropic cytokine IL-9 activates various target cells including dendritic cells, mast cells, and $CD8^+$ T cells, and is involved in the pathological processes of multiple diseases including inflammatory diseases, infectious diseases, autoimmune diseases, and cancer (93–95). Elevated serum IL-9 levels have been reported in patients with periportal fibrosis caused by *Schistosoma mansoni* infection (96). In both the silica-induced lung fibrosis mouse model and human patients with idiopathic pulmonary fibrosis (IPF) and cystic fibrosis, IL-9 levels were found to be correspondingly elevated (51, 53). While the administration of IL-9 neutralizing antibody protects mice from IPF and cystic fibrosis (CF) (51, 53). During an infection event, IL-33 activates ILC2 to produce IL-9, and the Th9 cells and IL-9 cytokine production is further amplified by IL-9-activated mast cell-driven ILC2 expansion (53). In liver cirrhosis patients, IL-9 is also significantly increased and has been proven to play an important role in hepatic fibrosis progression. IL-9 has further been reported to activate Raf/MEK/ERK signaling pathway in a commonly used carbon tetrachloride (CCl_4)-induced liver fibrosis mouse model. Consistent with studies in lung fibrotic diseases, IL-9 antibody inactivates hepatic stellate cells (HSCs) and ameliorates liver fibrosis (52). These results offer a possible treatment strategy for reducing fibrosis by blocking IL-9 signaling.

Th17 Cells and Fibrosis

Th17 cells have been discovered and characterized as the third subset of T helper cells. Characterized by their IL-17-producing ability, Th17 cells are critically involved in inflammatory responses (97). The relation between Th17 cells and fibrosis has been investigated in recent years. Th17 cells serve as a key component in mucosal immunity including the respiratory tract (98). In a hypersensitivity pneumonitis mouse model established by repeated exposure to *Saccharopolyspora rectivirgula*, increased Th17 were responsible for the inflammatory and fibrotic responses (36). Similarly, Th17 cells infiltrated into the bronchial submucosa of human patients with cystic fibrosis (CF) (99, 100). In the liver, IL-17 targets multiple types of cells, including Kupffer cells and hepatic stellate cells (HSCs). Gao et al. observed increased levels of IL-17A and IL-17 receptor in a liver fibrosis model induced by intragastric gavage with CCl_4 or bile duct ligation (35). Mechanistically, IL-17 activates the STAT3 signaling pathway in HSCs to produce type 1 collagen. Interestingly, the deletion of IL-23, or administration of IL-17E (also known as IL-25), attenuates liver fibrosis (35). Given the fact that IL-17 is not only produced by Th17 but also by γδ T cells (101), NKT cells (102), and type 3 innate lymphoid cells (ILC3) (103), and the differentiation and function of Th17 cells highly rely on the cytokine milieu, such as IL-1β, IL-6, IL-23, and TGFβ (104), it is important to identify novel factors that may regulate Th17 cell differentiation in fibrosis-related diseases. Angiogenesis contributes to fibroproliferative diseases. Recently, Jameson et al. reported that placental growth factor (PlGF), an angiogenic factor of the VEGF family, is specifically secreted from Th17 cells. PIGF is also required for the progression of collagen-induced arthritis in mice (105). Th17 cell differentiation program is highly heterogeneous (106) and is controlled by numerous factors, including febrile temperature (107). The fever shapes Th17 cell differentiation by SUMOylation of the transcription factor SMAD4 (107), which is tightly associated with T cell function during inflammation (108, 109). SMAD4 has been reported to control Th17 function with an oncoprotein SKI (110, 111), which has been linked to wound healing and fibrosis (112). This evidence suggests that TGFβ signaling in T cells also plays an important role in regulating inflammatory responses (104). TGFβ controls fibrosis in different cell types. Elevated mechanical tension activates the TGFβ signaling loop in mouse alveolar stem cells (AT2) and causes progressive pulmonary fibrosis (113). The complexity of the cytokine regimen responsible for Th17 differentiation may be harnessed to treat fibrotic diseases.

Th22 Cells and Fibrosis

Th22 cells are characterized by the production of cytokine IL-22. Although there exists a Th17-expressed IL-22 population, the Th22 cells exhibit transcriptomes distinct from those of Th17 cells (114). Importantly, Th22 cells express several members of the fibroblast growth factor (FGF) family, such as FGF1, FGF-5, FGF-12, and FGF-13, which are master regulators for wound healing, tissue repair, tissue regeneration, and fibrosis (114). Th22 represents a key T cell subset for epidermal immunity, and its levels are significantly increased in the epidermis of patients with morphea and SSc (115). Th22 cells are also increased in patients with cystic fibrosis in response to *P. aeruginosa* infection; this suggests the involvement of Th22 cells in pulmonary immunity (55). Th22 cells are also highly associated with liver fibrosis and are considered to play a hepatoprotective role (116). Th22 cell levels are elevated in mice with CCl_4-induced liver fibrosis (56), mice with methionine choline-deficient (MCD) diet-induced non-alcoholic steatohepatitis (NASH)(57), and human patients with liver cirrhosis (LC) (117). Liver-infiltrated Th22 cells, or recombinant IL-22 treatment, ameliorates fibrogenesis by attenuating hepatic stellate cell activation (56). Similarly, increased levels of Th22 and IL-22 are observed in mice with Coxsackie virus B3 (CVB3)-induced chronic myocarditis and dilated cardiomyopathy. Consistently, the expression of matrix metalloproteinase-9 (MMP9) is increased and that of metalloproteinase-1 (TIMP-1) inhibitor is decreased (54). Furthermore, the administration of an IL-22-neutralizing antibody exacerbated myocardial fibrosis as well as mortality. It is important to note that although IL-22 is mainly produced by immune cells including Th22 cells, Th17 cells, γδ T cells, NKT cells, and type 3 innate lymphoid cells (ILC3), it primarily targets non-immune cells (118). These facts suggest that targeting crosstalk between immune cells and tissues via the IL-22 signaling pathway is possible in fibrogenesis.

Regulatory T Cells and Fibrosis

Regulatory T cells (Tregs) play a pivotal role in modulating self-tolerance and immune homeostasis. It has been reported that cystic fibrosis patients with *P. aeruginosa* infection have impaired Tregs (119). Further, depletion of Treg cells by anti-CD25 antibody in silica-induced lung fibrosis attenuates fibrosis, and this process is probably dependent on the indirect function of Treg-secreted IL-10 and TGFβ (39). However, intranasal administration of TGFβ1-expressing plasmid results in increased TGFβ1- and IL-10-producing Treg cells and ameliorates bleomycin-induced lung fibrosis in mice (37). Recently, Ichikawa et al. found that depletion of $CD69^{hi}CD103^{hi}Foxp3^{+}$ Treg cells resulted in substantially higher levels of lung fibrosis in mice exposed to *Aspergillus fumigatus* because of the pathology promoted by tissue-resident $CD103^{lo}CD44^{hi}CD69^{hi}CD4^{+}$ T cells, which express high level of fibrosis-related genes. Their work thus defined a new tissue-resident Treg subpopulation in the lungs (45). The role of Tregs in fibrosis can be controversial and may be associated with a specific type of disease model. In angiotensin II-infused hypertensive mice, the adoptive transfer of $CD4^{+}CD25^{+}$ Treg cells improves cardiac hypertrophy and ameliorates cardiac fibrosis (38). However, in mice with ischemic cardiomyopathy, Treg ablation alleviates hypertrophy and cardiac fibrosis (40). Under the context of viral infections, $TGFβ1^{+}$ Treg cells induce deleterious collagen deposition and lymphatic tissue fibrosis in simian immunodeficiency virus (SIV)-infected rhesus macaques, showing a pro-fibrotic effect (41). However, in hepatitis C virus (HCV)-infected human patients, or in human immunodeficiency virus type 1 (HIV-1)-infected humanized mice, Treg cells prevent liver immunopathogenesis and limit liver fibrosis (42, 43). Treg cells exhibit different transcriptional changes in response to regenerative or fibrogenic environmental cues. During kidney fibrosis, remarkably increased levels of tissue-resident Treg cells express elevated fibrosis-related transcription factors, such as *Id2*, *Nfκb*, *Rgs2*, and *Junb* (46). Depletion or restoration of Treg cells may become a viable approach in controlling fibrosis in this case. In contrast, in Mdr2 (Abcb4) deficient mice with sclerosing cholangitis, a low dose of IL-2 treatment diminishes biliary injury and fibrosis by the expansion of intrahepatic Tregs (44).

Tfh Cells and Fibrosis

T-follicular helper (Tfh) cells, characterized by the expression of the lineage-specific transcription factor Bcl6 and production of IL-21, are essential for B cell function. They express high level of surface markers, such as CXCR5, CD40L, ICOS, and PD-1 (120). Upon *Schistosoma japonicum* infection, macrophages drive the differentiation of Tfh cells through CD40-CD40L and ICOS-ICOSL interactions. Following this, the infiltrated Tfh cells increase the hepatic granuloma formation and lead to severe liver fibrosis (47). The levels of these cell are also increased in patients with primary biliary cirrhosis (PBC) (49). In patients with idiopathic pulmonary fibrosis, the levels of $CXCR5^{+}ICOS^{+}PD\text{-}1^{+}$ Tfh cells are increased in the peripheral blood (50). Further, the levels of $CXCR5^{+}ICOS^{+}PD\text{-}1^{+}$ Tfh cells are strongly associated with dermal fibrosis in patients with systemic sclerosis (SSc). A mouse sclerodermatous GVHD (GVHD-SSc) model suggested that the level of these profibrotic Tfh cells are IL-21- and MMP-12-dependent. Furthermore, it has been shown that both IL-21 and ICOS antibody administration can effectively reduce skin fibrosis (48). As a new subset of T cells, Tfh cells may bring novel insights into fibrotic disease therapies.

Tfr Cells and Fibrosis

T follicular regulatory (Tfr) cells, sharing Tfh makers including CXCR5, Bcl6, ICOS, and PD-1, are characterized by Foxp3 expression and have a regulatory function (121). In patients with primary biliary cholangitis (PBC), the levels of $CD4^{+}CXCR5^{+}CD127^{lo}CD25^{hi}$ Tfr cells, as well as $CCR7^{hi}PD\text{-}1^{lo}$ central memory Tfr cells, are dramatically decreased, whereas those of $CCR7^{lo}PD\text{-}1^{hi}$ effector memory Tfr cells are increased. This evidence suggests the involvement of Tfr cells in primary biliary cholangitis regulation (122). In patients with chronic hepatitis B (CHB) infection, levels of circulating Tfr cells are significantly increased and are positively associated with FIB-4, which is the fibrosis index based on four factors (123). Another study including patients with CHB and chronic hepatitis C (CHC) also indicated that Tfr cells possibly modulated liver fibrosis by secreting the regulatory cytokine IL-10 and TGFβ (124). Thus, evidence suggests an emerging role of Tfr cells in virus-induced liver fibrosis (123).

Cytotoxic T Cells (CTLs, $CD8^{+}$ T Cells) and Fibrosis

Cytotoxic T cells (CTLs, $CD8^{+}$ T cells), expressing CD8 glycoprotein as an identity marker, are vital for killing infected cells and tumor cells. They also play important roles in many fibrosis-related diseases. Perivascular infiltrated $CD8^{+}$ T cells are found in patients with GVHD of the skin (58). In a mouse model of acute cerebral ischemia, $CD8^{+}$ T cells infiltrated into the perivascular space and expressed IL-16 to recruit monocytes and $CD4^{+}$ T cells, resulting in reduced hindlimb muscle fibrosis (125). Furthermore, in a renal fibrosis model, $CD8^{+}$ T cells and IFNγ reduced the $CD4^{+}$ T cell-induced monocyte-to-fibroblast transition (61). Activated $CD8^{+}$ T cells can produce TNFα and induce thyroid fibrosis (59). They can also secrete IL-13 to mediate bleomycin-induced pulmonary fibrogenesis in an IL-21-dependent manner (60). In contrast, fibrosis also has an impact on the immunosurveillance functions of $CD8^{+}$ T cells. For example, mouse liver fibrosis during HBV infection can jeopardize hepatocellular antigen recognition by intravascular $CD8^{+}$ T cells when crawling along liver sinusoids (126). Further, liver fibrosis and non-alcoholic steatohepatitis (NASH) lead to the accumulation of liver resident $IgA^{+}PD\text{-}L1^{+}IL\text{-}10^{+}$ cells that directly impair CTL/$CD8^{+}$ T cell functions against tumor-associated antigens, resulting in the development of hepatocellular carcinoma (HCC) (127). The fibrosis of secondary lymph nodes may result in $CD8^{+}$ T cell depletion after vaccine responses (128). Therefore, while developing vaccine strategies and triggering pro-fibrotic inflammatory responses for infectious diseases, the impact on $CD8^{+}$ T cells must be considered.

NKT Cells and Fibrosis

Natural killer T (NKT) cells express the αβ T cell receptor and recognize the glycolipid antigens presented by MHC I-liked protein CD1d. Being largely presented in the liver, NKT cells are central components of the immune response during liver injury, repair, inflammation, and fibrosis (129–131). A xenobiotics-induced liver fibrosis model is commonly used for determining the effect of NKT cells on liver fibrogenesis. NKT cells are critically involved in liver fibrosis induced by thioacetamide (TAA) (63), CCl_4 (63, 64, 66, 69), α-galactosylceramide (α-GalCer) (64), or 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) (69). It seems that the function of NKT cells in fibrosis is subject to different contexts. In TAA-induced liver fibrosis, the CD1d deficient mice show ameliorated liver fibrogenesis with blunted TIMP-1 expression (63), whereas in CCl_4-induced liver fibrosis, NKT-deficient mice are more susceptible (64). Furthermore, infiltrated NKT cells increase NKG2D ligand expression to activate HSCs and ameliorate liver fibrosis (66, 69). The participation of NKT cells at different fibrosis stages may also cause divergence (64). In a methionine choline-deficient (MCD) diet-induced non-alcoholic steatohepatitis (NASH) model, NKT cells accumulated, Hedgehog (Hh) and osteopontin (OPN) levels increased, and HSCs activated and differentiate to myofibroblasts (65, 68). Additionally, in a xenobiosis-induced model of primary biliary cirrhosis (PBC), the invariant natural killer T cell activator α-GalCer induced exacerbated fibrosis (67). Moreover, it has been reported that IL-30 treatment (69) or targeting CXCR6/CLCL16 (70) may serve as a potential approach for the amelioration of liver fibrosis. Further, NKT cells play a role in lung diseases (62, 71). In a bleomycin-induced pulmonary mouse fibrosis model, the genetic deletion of CD1d was reported to result in severe fibrosis, although adoptive transfer of NKT cells protected the mice from fibrosis. NKT cells attenuate pulmonary fibrosis by producing IFNγ and reducing TGFβ levels (62). Given the unique characteristics of NKT cells, the precise mechanism for NKT cells in fibrosis warrants further investigation.

γδ T Cells and Fibrosis

γδ T cells are unconventional T cells characterized by a T cell receptor γ chain and δ chain, which are not restricted by MHC. These cells may either instigate fibrosis or resolve fibrosis in a cytokine-dependent and disease-dependent manner (132). Although the overall number of γδ T cells is small, these cells represent a major T cell population in the skin, gut, and lung. The dendritic γδ T cells in the dermis are also named DETC, dendritic epidermal T cells. Further, it has been reported that skin fibroblasts exhibit higher proliferative activity and stronger collagen synthesis ability when cultured with γδ T cell supernatant (133). Studies on patients with systemic sclerosis have demonstrated the involvement of γδ T cells (134–136). The level of pathogenic $CD27^+$ γδ T cells were reported to increase, with upregulation of granzyme B or perforin expression (134). Further, Vγ9Vδ2, a subset of human γδ T cells, shows anti-fibrotic potential with the production of IFNγ (135, 136).

An abundance of approximately 8–20% γδ T cells in resident pulmonary lymphocytes shows the possible engagement of γδ T cells in lung infection, chronic inflammation, and subsequent pulmonary fibrosis (137). Sarcoidosis and idiopathic pulmonary fibrosis have shown to cause an increase in γδ T cell levels in the bronchoalveolar lavage fluid of patients (72). γδ T cells are also expanded in both cystic fibrosis patients with *P. aeruginosa* infection and mice with *Bacillus subtilis* infection (76, 138). In a bleomycin-induced fibrosis model, ablation of γδ T cells results in severe pulmonary fibrosis. Moreover, γδ T cells may prevent fibrosis by expressing CXCL10 (79). An important response of γδ T cells to infection and injury is the production of cytokine IL-17. Thus, γδ T cells were found to be the predominant source of IL-17 in mice with fibrosis induced by bleomycin (77), *B. subtilis* (139), and silica (140). IL-22 also contributes to the inhibition of pulmonary fibrosis. Deficiency in aryl hydrocarbon receptor (AhR) signaling or IL-22 enhances collagen deposition and accelerates fibrosis. Vγ6Vδ1 γδ T cells are the predominant source of IL-22 in protecting the lung from pulmonary fibrosis (78). Another major cytokine produced by γδ T cells is IFNγ. In a bleomycin-induced pulmonary fibrosis model, IFNγ produced by γδ T cells was found to attenuate fibrosis by indirectly inhibiting IL-17-secreting Th17 cells (75).

γδ T cells are also enriched in the liver and play an important role in liver fibrosis and cirrhosis (141). IL-17-producing γδ T cells are crucial in a variety of liver diseases (101). The chemokine receptor CCR6 is required for generating IL-17-producing γδ T cells. Hepatic γδ T cells contact and promote the FasL-induced HSC apoptosis to protect the liver from excessive fibrosis in an IL-17-dependent manner (74). IFNγ-producing γδ T cells are also protective in liver fibrosis by showing direct cytotoxicity against activated HSCs (82). Furthermore, hepatocyte-derived exosomes may mediate the activation of TLR3, which in turn enhances the production of IL-17 in γδ T cells (81). IL-17-producing γδ T cells are also enriched in patients with biliary atresia (BA), which is characterized by the destruction of the biliary system and liver fibrosis (142).

In addition, γδ T cells also display critical roles during fibrosis of other tissues. As γδ T cell is a key source of IL-17, the number of γδ T cells is elevated in patients with tubulointerstitial fibrosis, as determined by their biopsies (84). Further, in mice with kidney obstructive injury, γδ T cells are a major source of IL-17 and contribute to the pathogenesis of renal fibrosis with myofibroblast activation and ECM deposition (80). Moreover, in another study on a myocardial infarction mouse model, the IL-17-producing γδ T cells were found to promote fibroblast proliferation and aggravate fibrosis (143).

The aforementioned studies suggest that γδ T cells may be protective or deleterious in fibrosis in a cytokine-specific and tissue-specific manner. Thus, understanding the roles and underlying mechanisms of γδ T cells in the pathogenesis of fibrotic diseases would be useful for developing γδ T cell-based immunotherapies.

CONCLUDING REMARKS AND PERSPECTIVES

As a key process of wound healing, a variety of immune cells engage in the manifestation of inflammation and fibrosis. To date, the involvement of T cells has been well-established in orchestrating the fibrous tissue microenvironment. Although

extensive studies have been conducted on T cells and fibrosis, many questions remain elusive.

What is the initial trigger of fibrosis? How do we identify risk factors leading to a severe fibrotic disease? How do we utilize T cells in other fibrosis-related narratives? The pandemic of 2019 coronavirus disease COVID-19, caused by the SARS-CoV-2 virus infection, has caused worldwide mortality (144). COVID-19 is accompanied by fibrosis (145, 146), and is particularly dangerous for patients with pulmonary fibrotic diseases including cystic fibrosis (147). Pulmonary fibrosis is also commonly developed in patients with other virus-induced respiratory diseases, including severe acute respiratory syndrome coronavirus (SARS-CoV) (148–150) and Middle East respiratory syndrome (MERS) (151). A better understanding of cellular and molecular mechanisms will help treat fibrosis in patients with severe virus-induced diseases. Some T cell subsets participate in tissue repair and wound healing (152, 153) and are useful for tissue engineering. For instance, the recruitment of antigen-specific T cells followed by Th2 adjuvant vaccination can help biomaterials for tissue repair (154). Studies on the influence of T cells on fibrosis are especially useful for the treatment of hypertrophic scarring, which is a severe form of fibrosis frequently developed in case of severe burn injuries. In early studies, T cells were found to be heavily infiltrated into the dermis and epidermis of human patients (155). In burn patients with hypertrophic scars, Th1 and Th2 cell subsets and cytokines were identified to be strongly associated with the development of fibrosis (156, 157). In a previous study, TGFβ-producing T cells were found in burn patients (158); Th22 cells were also found to promote fibroblast-mediated wound repair in an acute skin wounding mouse model (159). These T cells interact with and remodel ECM and shape the local immune and fibrogenic responses in both the epidermis and dermis of hypertrophic tissues (160). The initial trigger event for T cell activation and the crosstalk between T cells and keratinocytes, fibroblast, and epithelial cells thus become critical questions for resolving the niche and local environment. Although these types of studies are largely limited in human patients, several animal models have been developed to serve as powerful tools to study human hypertrophic scarring. Momtazi et al. grafted human skin onto several immune-deficient mice, including TCR$\alpha\beta^{-/-}\gamma\delta^{-/-}$, RAG-$1^{-/-}$, and RAG-$2^{-/-}\gamma c^{-/-}$ mice. The proliferative scars showed histological and immunohistochemical similarities to human hypertrophic scars. The study therefore not only proves the importance of T cells in scar formation but also provides a useful tool for human study (161). Similar studies were also performed with nude mice and SCID pig as animal models for studying the roles and functions of T cells in hypertrophic scar tissue (162, 163). Moreover, given the fact that T cells and T cell associated cytokines and chemokines differ over the period of scar formation (164), the findings of these animal model studies are particularly important for studying the temporal and spatial characteristic of fibrosis in terms of T cell-shaped local environment.

Are there novel pathways and mechanisms that are essential for T cell-mediated fibrotic diseases? How do we render T cells capable of halting the irreversible fibrotic response? There have been all kinds of approaches and clinical trials for fibrosis-related diseases (25, 165). T cell function can be modulated by lowering the level of cytokines, such as IFNγ (166), or by inhibiting kinases, such as hyperactivated focal adhesion kinase (FAK) (167). In some cases, altering the intestinal microbiota can reduce fibrosis (168). The checkpoint inhibitor for cancer therapy has drawn extensive attention in recent years and is also a promising way for fibrosis remodeling. The blockade of costimulatory signals such as OX40L or CTLA4 prevents fibrosis and induces the regression of established fibrosis (169, 170). Targeting a metabolic regulator is a prominent strategy for fibrosis reduction (171). Furthermore, mitochondrial biogenesis and endoplasmic reticulum (ER) stress have been associate with fibrosis (172, 173). Obesity-associated oxidative stress also contributes to fibrosis and fibrotic diseases (174). Accordingly, discoveries on fibrosis mechanisms warrant new opportunities to develop metabolic reprogramming drugs.

Technological advances shed light on the research as well as approaches for controlling fibrotic diseases. Single-cell sequencing provides the necessary tools to delineate the transcriptomic profiles of all types of individual cells. Thus, it has become possible to identify subpopulations that are critical for fibrogenesis and reveal new fibrogenic pathways (175). Chimeric antigen receptor (CAR) T cell therapy has been used in cancer treatment. Further, T cells engineered with fibroblast activation protein CAR have shown great potential to ablate cardiac fibroblasts, and significantly reduce cardiac fibrosis (176). In summary, this review discusses recent notable studies, and provides a framework for T cell-mediated fibrosis paving the way for rational targets and effective immunotherapies.

AUTHOR CONTRIBUTIONS

MZ prepared and wrote the manuscript. SZ edited the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *J Clin Investig.* (2007) 117:524–9. doi: 10.1172/JCI31487
2. Chizzolini C. T cells, B cells, and polarized immune response in the pathogenesis of fibrosis and systemic sclerosis. *Curr Opin Rheumatol.* (2008) 20:707–12. doi: 10.1097/BOR.0b013e32830c45ae
3. Sugimoto MA, Vago JP, Perretti M, Teixeira MM. Mediators of the Resolution of the Inflammatory Response. *Trends Immunol.* (2019) 40:212–7. doi: 10.1016/j.it.2019.01.007
4. Pellicoro A, Ramachandran P, Iredale JP, Fallowfield JA. Liver fibrosis and repair: immune regulation of wound healing in a solid organ. *Nat Rev Immunol.* (2014) 14:181–94. doi: 10.1038/nri3623
5. Koyama Y, Brenner DA. Liver inflammation and fibrosis. *J Clin Investig.* (2017) 127:55–64. doi: 10.1172/JCI88881

6. Van Herck MA, Weyler J, Kwanten WJ, Dirinck EL, De Winter BY, Francque SM, et al. The differential roles of t cells in non-alcoholic fatty liver disease and obesity. *Front Immunol.* (2019) 10:82. doi: 10.3389/fimmu.2019.00082
7. Desai O, Winkler J, Minasyan M, Herzog EL. The role of immune and inflammatory cells in idiopathic pulmonary fibrosis. *Front Med.* (2018) 5:43. doi: 10.3389/fmed.2018.00043
8. Wynn TA. Integrating mechanisms of pulmonary fibrosis. *J Exp Med.* (2011) 208:1339–50. doi: 10.1084/jem.20110551
9. Lo Re S, Lison D, Huaux F. CD4+ T lymphocytes in lung fibrosis: diverse subsets, diverse functions. *J Leukocyte Biol.* (2013) 93:499–510. doi: 10.1189/jlb.0512261
10. Luzina IG, Todd NW, Iacono AT, Atamas SP. Roles of T lymphocytes in pulmonary fibrosis. *J Leukocyte Biol.* (2008) 83:237–44. doi: 10.1189/jlb.0707504
11. Chen JH, Schulman H, Gardner P. A cAMP-regulated chloride channel in lymphocytes that is affected in cystic fibrosis. *Science.* (1989) 243:657–60. doi: 10.1126/science.2464852
12. Hao Z, Hampel B, Yagita H, Rajewsky K. T cell-specific ablation of Fas leads to Fas ligand-mediated lymphocyte depletion and inflammatory pulmonary fibrosis. *J Exp Med.* (2004) 199:1355–65. doi: 10.1084/jem.20032196
13. McKinney EF, Lee JC, Jayne DR, Lyons PA, Smith KG. T-cell exhaustion, co-stimulation and clinical outcome in autoimmunity and infection. *Nature.* (2015) 523:612–6. doi: 10.1038/nature14468
14. Wei L. Immunological aspect of cardiac remodeling: T lymphocyte subsets in inflammation-mediated cardiac fibrosis. *Exp Mol Pathol.* (2011) 90:74–8. doi: 10.1016/j.yexmp.2010.10.004
15. Norlander AE, Madhur MS, Harrison DG. The immunology of hypertension. *J Exp Med.* (2018) 215:21–33. doi: 10.1084/jem.20171773
16. Abdullah CS, Jin ZQ. Targeted deletion of T-cell S1P receptor 1 ameliorates cardiac fibrosis in streptozotocin-induced diabetic mice. *FASEB J.* (2018) 32:5426–35. doi: 10.1096/fj.201800231R
17. Laroumanie F, Douin-Echinard V, Pozzo J, Lairez O, Tortosa F, Vinel C, et al. CD4+ T cells promote the transition from hypertrophy to heart failure during chronic pressure overload. *Circulation.* (2014) 129:2111–24. doi: 10.1161/CIRCULATIONAHA.113.007101
18. Kaya Z, Goser S, Buss SJ, Leuschner F, Ottl R, Li J, et al. Identification of cardiac troponin I sequence motifs leading to heart failure by induction of myocardial inflammation and fibrosis. *Circulation.* (2008) 118:2063–72. doi: 10.1161/CIRCULATIONAHA.108.788711
19. Ramos G, Hofmann U, Frantz S. Myocardial fibrosis seen through the lenses of T-cell biology. *J Mol Cell Cardiol.* (2016) 92:41–5. doi: 10.1016/j.yjmcc.2016.01.018
20. Stabile E, Burnett MS, Watkins C, Kinnaird T, Bachis A, la Sala A, et al. Impaired arteriogenic response to acute hindlimb ischemia in CD4-knockout mice. *Circulation.* (2003) 108:205–10. doi: 10.1161/01.CIR.0000079225.50817.71
21. Borg N, Alter C, Gorldt N, Jacoby C, Ding Z, Steckel B, et al. CD73 on T cells orchestrates cardiac wound healing after myocardial infarction by purinergic metabolic reprogramming. *Circulation.* (2017) 136:297–313. doi: 10.1161/CIRCULATIONAHA.116.023365
22. Lenti MV, Di Sabatino A. Intestinal fibrosis. *Mol Aspects Med.* (2019) 65:100–9. doi: 10.1016/j.mam.2018.10.003
23. Liu M, Wu W, Sun X, Yang J, Xu J, Fu W, et al. New insights into CD4(+) T cell abnormalities in systemic sclerosis. *Cytokine Growth Factor Rev.* (2016) 28:31–6. doi: 10.1016/j.cytogfr.2015.12.002
24. Nikolic-Paterson DJ. CD4+ T cells: a potential player in renal fibrosis. *Kidney Int.* (2010) 78:333–5. doi: 10.1038/ki.2010.182
25. MacDonald KP, Blazar BR, Hill GR. Cytokine mediators of chronic graft-versus-host disease. *J Clin Investig.* (2017) 127:2452–63. doi: 10.1172/JCI90593
26. Morrison J, Lu QL, Pastoret C, Partridge T, Bou-Gharios G. T-cell-dependent fibrosis in the mdx dystrophic mouse. *Lab Investig.* (2000) 80:881–91. doi: 10.1038/labinvest.3780092
27. Sadtler K, Estrellas K, Allen BW, Wolf MT, Fan H, Tam AJ, et al. Developing a pro-regenerative biomaterial scaffold microenvironment requires T helper 2 cells. *Science.* (2016) 352:366–70. doi: 10.1126/science.aad9272
28. Eming SA, Wynn TA, Martin P. Inflammation and metabolism in tissue repair and regeneration. *Science.* (2017) 356:1026–30. doi: 10.1126/science.aam7928
29. Distler JHW, Gyorfi AH, Ramanujam M, Whitfield ML, Konigshoff M, Lafyatis R. Shared and distinct mechanisms of fibrosis. *Nat Rev Rheumatol.* (2019) 15:705–30. doi: 10.1038/s41584-019-0322-7
30. Fielding CA, Jones GW, McLoughlin RM, McLeod L, Hammond VJ, Uceda J, et al. Interleukin-6 signaling drives fibrosis in unresolved inflammation. *Immunity.* (2014) 40:40–50. doi: 10.1016/j.immuni.2013.10.022
31. Nevers T, Salvador AM, Velazquez F, Ngwenyama N, Carrillo-Salinas FJ, Aronovitz M, et al. Th1 effector T cells selectively orchestrate cardiac fibrosis in nonischemic heart failure. *J Exp Med.* (2017) 214:3311–29. doi: 10.1084/jem.20161791
32. Hartl D, Griese M, Kappler M, Zissel G, Reinhardt D, Rebhan C, et al. Pulmonary T(H)2 response in *Pseudomonas aeruginosa*-infected patients with cystic fibrosis. *J Allergy Clin Immunol.* (2006) 117:204–11. doi: 10.1016/j.jaci.2005.09.023
33. Kreindler JL, Steele C, Nguyen N, Chan YR, Pilewski JM, Alcorn JF, et al. Vitamin D3 attenuates Th2 responses to Aspergillus fumigatus mounted by CD4+ T cells from cystic fibrosis patients with allergic bronchopulmonary aspergillosis. *J Clin Investig.* (2010) 120:3242–54. doi: 10.1172/JCI42388
34. Morimoto Y, Hirahara K, Kiuchi M, Wada T, Ichikawa T, Kanno T, et al. Amphiregulin-producing pathogenic memory t helper 2 cells instruct eosinophils to secrete osteopontin and facilitate airway fibrosis. *Immunity.* (2018) 49:134–50 e6. doi: 10.1016/j.immuni.2018.04.023
35. Meng F, Wang K, Aoyama T, Grivennikov SI, Paik Y, Scholten D, et al. Interleukin-17 signaling in inflammatory, Kupffer cells, and hepatic stellate cells exacerbates liver fibrosis in mice. *Gastroenterology.* (2012) 143:765–76 e3. doi: 10.1053/j.gastro.2012.05.049
36. Simonian PL, Roark CL, Wehrmann F, Lanham AK, Diaz del Valle F, Born WK, et al. Th17-polarized immune response in a murine model of hypersensitivity pneumonitis and lung fibrosis. *J Immunol.* (2009) 182:657–65. doi: 10.4049/jimmunol.182.1.657
37. Kitani A, Fuss I, Nakamura K, Kumaki F, Usui T, Strober W. Transforming growth factor (TGF)-beta1-producing regulatory T cells induce Smad-mediated interleukin 10 secretion that facilitates coordinated immunoregulatory activity and amelioration of TGF-beta1-mediated fibrosis. *J Exp Med.* (2003) 198:1179–88. doi: 10.1084/jem.20030917
38. Kvakan H, Kleinewietfeld M, Qadri F, Park JK, Fischer R, Schwarz I, et al. Regulatory T cells ameliorate angiotensin II-induced cardiac damage. *Circulation.* (2009) 119:2904–12. doi: 10.1161/CIRCULATIONAHA.108.832782
39. Liu F, Liu J, Weng D, Chen Y, Song L, He Q, et al. CD4+CD25+Foxp3+ regulatory T cells depletion may attenuate the development of silica-induced lung fibrosis in mice. *PLoS ONE.* (2010) 5:e15404. doi: 10.1371/journal.pone.0015404
40. Bansal SS, Ismahil MA, Goel M, Zhou G, Rokosh G, Hamid T, et al. Dysfunctional and proinflammatory regulatory T-lymphocytes are essential for adverse cardiac remodeling in ischemic cardiomyopathy. *Circulation.* (2019) 139:206–21. doi: 10.1161/CIRCULATIONAHA.118.036065
41. Estes JD, Wietgrefe S, Schacker T, Southern P, Beilman G, Reilly C, et al. Simian immunodeficiency virus-induced lymphatic tissue fibrosis is mediated by transforming growth factor beta 1-positive regulatory T cells and begins in early infection. *J Infect Dis.* (2007) 195:551–61. doi: 10.1086/510852
42. Claassen MA, de Knegt RJ, Tilanus HW, Janssen HL, Boonstra A. Abundant numbers of regulatory T cells localize to the liver of chronic hepatitis C infected patients and limit the extent of fibrosis. *J Hepatol.* (2010) 52:315–21. doi: 10.1016/j.jhep.2009.12.013
43. Nunoya J, Washburn ML, Kovalev GI, Su L. Regulatory T cells prevent liver fibrosis during HIV type 1 infection in a humanized mouse model. *J Infect Dis.* (2014) 209:1039–44. doi: 10.1093/infdis/jit548
44. Taylor AE, Carey AN, Kudira R, Lages CS, Shi T, Lam S, et al. Interleukin 2 promotes hepatic regulatory T cell responses and protects from biliary fibrosis in murine sclerosing cholangitis. *Hepatology.* (2018) 68:1905–21. doi: 10.1002/hep.30061
45. Ichikawa T, Hirahara K, Kokubo K, Kiuchi M, Aoki A, Morimoto Y, et al. CD103(hi) Treg cells constrain lung fibrosis induced by CD103(lo)

tissue-resident pathogenic CD4 T cells. *Nat Immunol.* (2019) 20:1469–80. doi: 10.1038/s41590-019-0494-y
46. do Valle Duraes F, Lafont A, Beibel M, Martin K, Darribat K, Cuttat R, et al. Immune cell landscaping reveals a protective role for regulatory T cells during kidney injury and fibrosis. *JCI insight.* (2020) 5:130651. doi: 10.1172/jci.insight.130651
47. Chen X, Yang X, Li Y, Zhu J, Zhou S, Xu Z, et al. Follicular helper T cells promote liver pathology in mice during Schistosoma japonicum infection. *PLoS Pathog.* (2014) 10:e1004097. doi: 10.1371/journal.ppat.1004097
48. Taylor DK, Mittereder N, Kuta E, Delaney T, Burwell T, Dacosta K, et al. T follicular helper-like cells contribute to skin fibrosis. *Sci Transl Med.* (2018) 10:aaf5307. doi: 10.1126/scitranslmed.aaf5307
49. Wang L, Sun Y, Zhang Z, Jia Y, Zou Z, Ding J, et al. CXCR5+ CD4+ T follicular helper cells participate in the pathogenesis of primary biliary cirrhosis. *Hepatology.* (2015) 61:627–38. doi: 10.1002/hep.27306
50. Asai Y, Chiba H, Nishikiori H, Kamekura R, Yabe H, Kondo S, et al. Aberrant populations of circulating T follicular helper cells and regulatory B cells underlying idiopathic pulmonary fibrosis. *Respir Res.* (2019) 20:244. doi: 10.1186/s12931-019-1216-6
51. Sugimoto N, Suzukawa M, Nagase H, Koizumi Y, Ro S, Kobayashi K, et al. IL-9 Blockade suppresses silica-induced lung inflammation and fibrosis in mice. *Am J Respir Cell Mol Biol.* (2019) 60:232–43. doi: 10.1165/rcmb.2017-0287OC
52. Guo X, Cen Y, Wang J, Jiang H. CXCL10-induced IL-9 promotes liver fibrosis via Raf/MEK/ERK signaling pathway. *Biomed Pharmacother.* (2018) 105:282–9. doi: 10.1016/j.biopha.2018.05.128
53. Moretti S, Renga G, Oikonomou V, Galosi C, Pariano M, Iannitti RG, et al. A mast cell-ILC2-Th9 pathway promotes lung inflammation in cystic fibrosis. *Nat Commun.* (2017) 8:14017. doi: 10.1038/ncomms14017
54. Guo Y, Wu W, Cen Z, Li X, Kong Q, Zhou Q. IL-22-producing Th22 cells play a protective role in CVB3-induced chronic myocarditis and dilated cardiomyopathy by inhibiting myocardial fibrosis. *Virol J.* (2014) 11:230. doi: 10.1186/s12985-014-0230-z
55. Bayes HK, Bicknell S, MacGregor G, Evans TJ. T helper cell subsets specific for *Pseudomonas aeruginosa* in healthy individuals and patients with cystic fibrosis. *PLoS ONE.* (2014) 9:e90263. doi: 10.1371/journal.pone.0090263
56. Lu DH, Guo XY, Qin SY, Luo W, Huang XL, Chen M, et al. Interleukin-22 ameliorates liver fibrogenesis by attenuating hepatic stellate cell activation and downregulating the levels of inflammatory cytokines. *World J Gastroenterol.* (2015) 21:1531–45. doi: 10.3748/wjg.v21.i5.1531
57. Rolla S, Alchera E, Imarisio C, Bardina V, Valente G, Cappello P, et al. The balance between IL-17 and IL-22 produced by liver-infiltrating T-helper cells critically controls NASH development in mice. *Clin Sci.* (2016) 130:193–203. doi: 10.1042/CS20150405
58. Biedermann BC, Sahner S, Gregor M, Tsakiris DA, Jeanneret C, Pober JS, et al. Endothelial injury mediated by cytotoxic T lymphocytes and loss of microvessels in chronic graft versus host disease. *Lancet.* (2002) 359:2078–83. doi: 10.1016/S0140-6736(02)08907-9
59. Yu S, Fang Y, Sharav T, Sharp GC, Braley-Mullen H. CD8+ T cells induce thyroid epithelial cell hyperplasia and fibrosis. *J Immunol.* (2011) 186:2655–62. doi: 10.4049/jimmunol.1002884
60. Brodeur TY, Robidoux TE, Weinstein JS, Craft J, Swain SL, Marshak-Rothstein A. IL-21 Promotes Pulmonary Fibrosis through the Induction of Profibrotic CD8+ T Cells. *J Immunol.* (2015) 195:5251–60. doi: 10.4049/jimmunol.1500777
61. Dong Y, Yang M, Zhang J, Peng X, Cheng J, Cui T, et al. Depletion of CD8+ T Cells Exacerbates CD4+ T Cell-Induced Monocyte-to-Fibroblast Transition in Renal Fibrosis. *J Immunol.* (2016) 196:1874–81. doi: 10.4049/jimmunol.1501232
62. Kim JH, Kim HY, Kim S, Chung JH, Park WS, Chung DH. Natural killer T (NKT) cells attenuate bleomycin-induced pulmonary fibrosis by producing interferon-gamma. *Am J Pathol.* (2005) 167:1231–41. doi: 10.1016/S0002-9440(10)61211-4
63. Ishikawa S, Ikejima K, Yamagata H, Aoyama T, Kon K, Arai K, et al. CD1d-restricted natural killer T cells contribute to hepatic inflammation and fibrogenesis in mice. *J Hepatol.* (2011) 54:1195–204. doi: 10.1016/j.jhep.2010.08.022
64. Park O, Jeong WI, Wang L, Wang H, Lian ZX, Gershwin ME, et al. Diverse roles of invariant natural killer T cells in liver injury and fibrosis induced by carbon tetrachloride. *Hepatology.* (2009) 49:1683–94. doi: 10.1002/hep.22813
65. Syn WK, Oo YH, Pereira TA, Karaca GF, Jung Y, Omenetti A, et al. Accumulation of natural killer T cells in progressive nonalcoholic fatty liver disease. *Hepatology.* (2010) 51:1998–2007. doi: 10.1002/hep.23599
66. Jin Z, Sun R, Wei H, Gao X, Chen Y, Tian Z. Accelerated liver fibrosis in hepatitis B virus transgenic mice: involvement of natural killer T cells. *Hepatology.* (2011) 53:219–29. doi: 10.1002/hep.23983
67. Wu SJ, Yang YH, Tsuneyama K, Leung PS, Illarionov P, Gershwin ME, et al. Innate immunity and primary biliary cirrhosis: activated invariant natural killer T cells exacerbate murine autoimmune cholangitis and fibrosis. *Hepatology.* (2011) 53:915–25. doi: 10.1002/hep.24113
68. Syn WK, Agboola KM, Swiderska M, Michelotti GA, Liaskou E, Pang H, et al. NKT-associated hedgehog and osteopontin drive fibrogenesis in non-alcoholic fatty liver disease. *Gut.* (2012) 61:1323–9. doi: 10.1136/gutjnl-2011-301857
69. Mitra A, Satelli A, Yan J, Xueqing X, Gagea M, Hunter CA, et al. IL-30 (IL27p28) attenuates liver fibrosis through inducing NKG2D-rae1 interaction between NKT and activated hepatic stellate cells in mice. *Hepatology.* (2014) 60:2027–39. doi: 10.1002/hep.27392
70. Wehr A, Baeck C, Heymann F, Niemietz PM, Hammerich L, Martin C, et al. Chemokine receptor CXCR6-dependent hepatic NK T Cell accumulation promotes inflammation and liver fibrosis. *J Immunol.* (2013) 190:5226–36. doi: 10.4049/jimmunol.1202909
71. Bergantini L, Cameli P, d'Alessandro M, Vagaggini C, Refini RM, Landi C, et al. NK and NKT-like cells in granulomatous and fibrotic lung diseases. *Clin Exp Med.* (2019) 19:487–94. doi: 10.1007/s10238-019-00578-3
72. Utsumi K, Kawanishi K, Kuriyama Y, Nakano M, Ichinose Y, Toyama K. [Gamma delta T cells in peripheral blood and in bronchoalveolar lavage fluid from patients with sarcoidosis and idiopathic pulmonary fibrosis]. *Nihon Kyobu Shikkan Gakkai zasshi.* (1995) 33:1186–90.
73. Perez-Payarols J, Julia Benique MR, Matamoros Flori N, Roman Pinana JM. [An increase in gamma-delta T-lymphocytes in the peripheral blood of cystic fibrosis patients]. *Anales espanoles de pediatria.* (1996) 44:239–41.
74. Hammerich L, Bangen JM, Govaere O, Zimmermann HW, Gassler N, Huss S, et al. Chemokine receptor CCR6-dependent accumulation of gammadelta T cells in injured liver restricts hepatic inflammation and fibrosis. *Hepatology.* (2014) 59:630–42. doi: 10.1002/hep.26697
75. Segawa S, Goto D, Iizuka A, Kaneko S, Yokosawa M, Kondo Y, et al. The regulatory role of interferon-gamma producing gamma delta T cells via the suppression of T helper 17 cell activity in bleomycin-induced pulmonary fibrosis. *Clin Exp Immunol.* (2016) 185:348–60. doi: 10.1111/cei.12802
76. Raga S, Julia MR, Crespi C, Figuerola J, Martinez N, Mila J, et al. Gammadelta T lymphocytes from cystic fibrosis patients and healthy donors are high TNF-alpha and IFN-gamma-producers in response to *Pseudomonas aeruginosa*. *Respir Res.* (2003) 4:9. doi: 10.1186/1465-9921-4-9
77. Braun RK, Ferrick C, Neubauer P, Sjoding M, Sterner-Kock A, Kock M, et al. IL-17 producing gammadelta T cells are required for a controlled inflammatory response after bleomycin-induced lung injury. *Inflammation.* (2008) 31:167–79. doi: 10.1007/s10753-008-9062-6
78. Simonian PL, Wehrmann F, Roark CL, Born WK, O'Brien RL, Fontenot AP. gammadelta T cells protect against lung fibrosis via IL-22. *J Exp Med.* (2010) 207:2239–53. doi: 10.1084/jem.20100061
79. Pociask DA, Chen K, Choi SM, Oury TD, Steele C, Kolls JK. gammadelta T cells attenuate bleomycin-induced fibrosis through the production of CXCL10. *Am J Pathol.* (2011) 178:1167–76. doi: 10.1016/j.ajpath.2010.11.055
80. Peng X, Xiao Z, Zhang J, Li Y, Dong Y, Du J. IL-17A produced by both gammadelta T and Th17 cells promotes renal fibrosis via RANTES-mediated leukocyte infiltration after renal obstruction. *J Pathol.* (2015) 235:79–89. doi: 10.1002/path.4430
81. Seo W, Eun HS, Kim SY, Yi HS, Lee YS, Park SH, et al. Exosome-mediated activation of toll-like receptor 3 in stellate cells stimulates interleukin-17 production by gammadelta T cells in liver fibrosis. *Hepatology.* (2016) 64:616–31. doi: 10.1002/hep.28644
82. Liu M, Hu Y, Yuan Y, Tian Z, Zhang C. gammadeltaT cells suppress liver fibrosis via strong cytolysis and enhanced NK cell-mediated

cytotoxicity against hepatic stellate cells. *Front Immunol.* (2019) 10:477. doi: 10.3389/fimmu.2019.00477
83. Zheng L, Hu Y, Wang Y, Huang X, Xu Y, Shen Y, et al. Recruitment of Neutrophils Mediated by Vgamma2 gammadelta T Cells Deteriorates Liver Fibrosis Induced by Schistosoma japonicum Infection in C57BL/6 Mice. *Infect Immunity.* (2017) 85:16. doi: 10.1128/IAI.01020-16
84. Law BM, Wilkinson R, Wang X, Kildey K, Lindner M, Beagley K, et al. Effector gammadelta T cells in human renal fibrosis and chronic kidney disease. *Nephrol Dialysis, Transpl.* (2019) 34:40–8. doi: 10.1093/ndt/gfy098
85. Wynn TA. Fibrotic disease and the T(H)1/T(H)2 paradigm. *Nat Rev Immunol.* (2004) 4:583–94. doi: 10.1038/nri1412
86. Wynn TA, Cheever AW, Jankovic D, Poindexter RW, Caspar P, Lewis FA, et al. An IL-12-based vaccination method for preventing fibrosis induced by schistosome infection. *Nature.* (1995) 376:594–6. doi: 10.1038/376594a0
87. Roderfeld M, Rath T, Pasupuleti S, Zimmermann M, Neumann C, Churin Y, et al. Bone marrow transplantation improves hepatic fibrosis in Abcb4-/- mice via Th1 response and matrix metalloproteinase activity. *Gut.* (2012) 61:907–16. doi: 10.1136/gutjnl-2011-300608
88. Liu Y, Wang L, Kikuiri T, Akiyama K, Chen C, Xu X, et al. Mesenchymal stem cell-based tissue regeneration is governed by recipient T lymphocytes via IFN-gamma and TNF-alpha. *Nat Med.* (2011) 17:1594–601. doi: 10.1038/nm.2542
89. Zhang S, Liang R, Luo W, Liu C, Wu X, Gao Y, et al. High susceptibility to liver injury in IL-27 p28 conditional knockout mice involves intrinsic interferon-gamma dysregulation of CD4+ T cells. *Hepatology.* (2013) 57:1620–31. doi: 10.1002/hep.26166
90. Gieseck RL, III, Wilson MS, Wynn TA. Type 2 immunity in tissue repair and fibrosis. *Nat Rev Immunol.* (2018) 18:62–76. doi: 10.1038/nri.2017.90
91. Han D, Walsh MC, Cejas PJ, Dang NN, Kim YF, Kim J, et al. Dendritic cell expression of the signaling molecule TRAF6 is critical for gut microbiota-dependent immune tolerance. *Immunity.* (2013) 38:1211–22. doi: 10.1016/j.immuni.2013.05.012
92. Gieseck RL, 3rd, Ramalingam TR, Hart KM, Vannella KM, Cantu DA, Lu WY, et al. Interleukin-13 activates distinct cellular pathways leading to ductular reaction, steatosis, and fibrosis. *Immunity.* (2016) 45:145–58. doi: 10.1016/j.immuni.2016.06.009
93. Kaplan MH. Th9 cells: differentiation and disease. *Immunol Rev.* (2013) 252:104–15. doi: 10.1111/imr.12028
94. Noelle RJ, Nowak EC. Cellular sources and immune functions of interleukin-9. *Nat Rev Immunol.* (2010) 10:683–7. doi: 10.1038/nri2848
95. Vegran F, Apetoh L, Ghiringhelli F. Th9 cells: a novel CD4 T-cell subset in the immune war against cancer. *Cancer Res.* (2015) 75:475–9. doi: 10.1158/0008-5472.CAN-14-2748
96. Barreto AV, Lacerda GA, Figueiredo AL, Diniz GT, Gomes EC, Domingues AL, et al. Evaluation of serum levels of IL-9 and IL-17 in human Schistosoma mansoni infection and their relationship with periportal fibrosis. *Immunobiology.* (2016) 221:1351–4. doi: 10.1016/j.imbio.2016.07.014
97. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Ann Rev Immunol.* (2009) 27:485–517. doi: 10.1146/annurev.immunol.021908.132710
98. Iwanaga N, Kolls JK. Updates on T helper type 17 immunity in respiratory disease. *Immunology.* (2019) 156:3–8. doi: 10.1111/imm.13006
99. Tan HL, Regamey N, Brown S, Bush A, Lloyd CM, Davies JC. The Th17 pathway in cystic fibrosis lung disease. *Am J Respir Critic Care Med.* (2011) 184:252–8. doi: 10.1164/rccm.201102-0236OC
100. Bacher P, Hohnstein T, Beerbaum E, Rocker M, Blango MG, Kaufmann S, et al. Human anti-fungal Th17 immunity and pathology rely on cross-reactivity against *Candida albicans*. *Cell.* (2019) 176:1340–55 e15. doi: 10.1016/j.cell.2019.01.041
101. Xi C, Jia Z, Xiaoli W, Na Z, He W, Hao J. New aspect of Liver IL-17(+)gammadelta T Cells. *Mol Immunol.* (2019) 107:41–3. doi: 10.1016/j.molimm.2018.12.030
102. Tsagaratou A. Unveiling the regulation of NKT17 cell differentiation and function. *Mol Immunol.* (2019) 105:55–61. doi: 10.1016/j.molimm.2018.11.013
103. Domingues RG, Hepworth MR. Immunoregulatory Sensory Circuits in Group 3 Innate Lymphoid Cell (ILC3) Function and Tissue Homeostasis. *Front Immunol.* (2020) 11:116. doi: 10.3389/fimmu.2020.00116
104. Zhang S. The role of transforming growth factor beta in T helper 17 differentiation. *Immunology.* (2018) 155:24–35. Epub 2018/04/24. doi: 10.1111/imm.12938
105. Jameson J, Ugarte K, Chen N, Yachi P, Fuchs E, Boismenu R, et al. A role for skin gammadelta T cells in wound repair. *Science.* (2002) 296:747–9. Epub 2002/04/27. doi: 10.1126/science.1069639
106. Zhang S. Hero or villain? The heterogeneity of Th17 cells. *Mol Immunol.* (2019) 112:358–9. doi: 10.1016/j.molimm.2019.06.014
107. Wang X, Ni L, Wan S, Zhao X, Ding X, Dejean A, et al. Febrile temperature critically controls the differentiation and pathogenicity of T Helper 17 Cells. *Immunity.* (2020) 52, 1–14. doi: 10.1016/j.immuni.2020.01.006
108. Gu AD, Wang Y, Lin L, Zhang SS, Wan YY. Requirements of transcription factor Smad-dependent and -independent TGF-beta signaling to control discrete T-cell functions. *Proc Natl Acad Sci USA.* (2012) 109:905–10. doi: 10.1073/pnas.1108352109
109. Gu AD, Zhang S, Wang Y, Xiong H, Curtis TA, Wan YY. A critical role for transcription factor Smad4 in T cell function that is independent of transforming growth factor beta receptor signaling. *Immunity.* (2015) 42:68–79. doi: 10.1016/j.immuni.2014.12.019
110. Zhang S, Takaku M, Zou L, Gu AD, Chou WC, Zhang G, et al. Reversing SKI-SMAD4-mediated suppression is essential for TH17 cell differentiation. *Nature.* (2017) 551:105–9. doi: 10.1038/nature24283
111. Zhang S, Zhang G, Wan YY. SKI and SMAD4 are essential for IL-21-induced Th17 differentiation. *Mol Immunol.* (2019) 114:260–8. doi: 10.1016/j.molimm.2019.07.029
112. Tecalco-Cruz AC, Rios-Lopez DG, Vazquez-Victorio G, Rosales-Alvarez RE, Macias-Silva M. Transcriptional cofactors Ski and SnoN are major regulators of the TGF-beta/Smad signaling pathway in health and disease. *Signal Transduc Targeted Therap.* (2018) 3:15. doi: 10.1038/s41392-018-0015-8
113. Wu H, Yu Y, Huang H, Hu Y, Fu S, Wang Z, et al. Progressive pulmonary fibrosis is caused by elevated mechanical tension on alveolar stem cells. *Cell.* (2020) 180:107–21 e17. doi: 10.1016/j.cell.2019.11.027
114. Eyerich S, Eyerich K, Pennino D, Carbone T, Nasorri F, Pallotta S, et al. Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *J Clin Investig.* (2009) 119:3573–85. doi: 10.1172/JCI40202
115. Brembilla NC, Dufour AM, Alvarez M, Hugues S, Montanari E, Truchetet ME, et al. IL-22 capacitates dermal fibroblast responses to TNF in scleroderma. *Ann Rheum Dis.* (2016) 75:1697–705. doi: 10.1136/annrheumdis-2015-207477
116. Khawar MB, Azam F, Sheikh N, Abdul Mujeeb K. How does interleukin-22 mediate liver regeneration and prevent injury and fibrosis? *J Immunol Res.* (2016) 2016:2148129. doi: 10.1155/2016/2148129
117. Qin S, Chen M, Guo X, Luo W, Wang J, Jiang H. The clinical significance of intrahepatic Th22 cells in liver cirrhosis. *Adv Clin Exp Med.* (2019) 28:765–70. doi: 10.17219/acem/94062
118. Dudakov JA, Hanash AM, van den Brink MR. Interleukin-22: immunobiology and pathology. *Ann Rev Immunol.* (2015) 33:747–85. doi: 10.1146/annurev-immunol-032414-112123
119. Hector A, Schafer H, Poschel S, Fischer A, Fritzsching B, Ralhan A, et al. Regulatory T-cell impairment in cystic fibrosis patients with chronic pseudomonas infection. *Am J Respir Crit Care Med.* (2015) 191:914–23. doi: 10.1164/rccm.201407-1381OC
120. Liu X, Nurieva RI, Dong C. Transcriptional regulation of follicular T-helper (Tfh) cells. *Immunol Rev.* (2013) 252:139–45. doi: 10.1111/imr.12040
121. Fonseca VR, Ribeiro F, Graca L. T follicular regulatory (Tfr) cells: Dissecting the complexity of Tfr-cell compartments. *Immunol Rev.* (2019) 288:112–27. doi: 10.1111/imr.12739
122. Zheng J, Wang T, Zhang L, Cui L. Dysregulation of circulating Tfr/Tfh ratio in primary biliary cholangitis. *Scand J Immunol.* (2017) 86:452–61. doi: 10.1111/sji.12616
123. Wu X, Su Z, Cai B, Yan L, Li Y, Feng W, et al. Increased circulating follicular regulatory t-like cells may play a critical role in chronic hepatitis B virus infection and disease progression. *Viral immunol.* (2018) 31:379–88. doi: 10.1089/vim.2017.0171
124. Wang L, Qiu J, Yu L, Hu X, Zhao P, Jiang Y. Increased numbers of CD5+CD19+CD1dhighIL-10+ Bregs, CD4+Foxp3+ Tregs,

CD4+CXCR5+Foxp3+ follicular regulatory T (TFR) cells in CHB or CHC patients. *J Transl Med*. (2014) 12:251. doi: 10.1186/s12967-014-0251-9
125. Stabile E, Kinnaird T, la Sala A, Hanson SK, Watkins C, Campia U, et al. CD8+ T lymphocytes regulate the arteriogenic response to ischemia by infiltrating the site of collateral vessel development and recruiting CD4+ mononuclear cells through the expression of interleukin-16. *Circulation*. (2006) 113:118–24. doi: 10.1161/CIRCULATIONAHA.105.576702
126. Guidotti LG, Inverso D, Sironi L, Di Lucia P, Fioravanti J, Ganzer L, et al. Immunosurveillance of the liver by intravascular effector CD8(+) T cells. *Cell*. (2015) 161:486–500. doi: 10.1016/j.cell.2015.03.005
127. Shalapour S, Lin XJ, Bastian IN, Brain J, Burt AD, Aksenov AA, et al. Inflammation-induced IgA+ cells dismantle anti-liver cancer immunity. *Nature*. (2017) 551:340–5. doi: 10.1038/nature24302
128. Kityo C, Makamdop KN, Rothenberger M, Chipman JG, Hoskuldsson T, Beilman GJ, et al. Lymphoid tissue fibrosis is associated with impaired vaccine responses. *J Clin Investig*. (2018) 128:2763–73. doi: 10.1172/JCI97377
129. Notas G, Kisseleva T, Brenner D. NK and NKT cells in liver injury and fibrosis. *Clin Immunol*. (2009) 130:16–26. doi: 10.1016/j.clim.2008.08.008
130. Mattner J. Natural killer T (NKT) cells in autoimmune hepatitis. *Curr Opin Immunol*. (2013) 25:697–703. doi: 10.1016/j.coi.2013.09.008
131. Gao B, Radaeva S, Park O. Liver natural killer and natural killer T cells: immunobiology and emerging roles in liver diseases. *J Leukocyte Biol*. (2009) 86:513–28. doi: 10.1189/jlb.0309135
132. Bank I. The Role of gammadelta T Cells in Fibrotic Diseases. *Rambam Maimonides Med J*. (2016) 7:10256. doi: 10.5041/RMMJ.10256
133. Ohtsuka T. Effect of gammadelta T cell supernatant on human skin fibroblast proliferation and collagen production–possible role of transforming growth factor-beta and basic fibroblast growth factor. *Int J Dermatol*. (2008) 47:1135–40. doi: 10.1111/j.1365-4632.2008.03805.x
134. Henriques A, Silva C, Santiago M, Henriques MJ, Martinho A, Trindade H, et al. Subset-specific alterations in frequencies and functional signatures of gammadelta T cells in systemic sclerosis patients. *Inflamm Res*. (2016) 65:985–94. doi: 10.1007/s00011-016-0982-6
135. Markovits N, Bendersky A, Loebstein R, Brusel M, Kessler E, Bank I. Anti-fibrotic characteristics of Vgamma9+ gammadelta T cells in systemic sclerosis. *Clin Exp Rheumatol*. (2016) 34 Suppl 100:23–9.
136. Migalovich Sheikhet H, Villacorta Hidalgo J, Fisch P, Balbir-Gurman A, Braun-Moscovici Y, Bank I. Dysregulated CD25 and cytokine expression by gammadelta T cells of systemic sclerosis patients stimulated with cardiolipin and zoledronate. *Front Immunol*. (2018) 9:753. doi: 10.3389/fimmu.2018.00753
137. Cheng M, Hu S. Lung-resident gammadelta T cells and their roles in lung diseases. *Immunology*. (2017) 151:375–84. doi: 10.1111/imm.12764
138. Simonian PL, Roark CL, Diaz del Valle F, Palmer BE, Douglas IS, Ikuta K, et al. Regulatory role of gammadelta T cells in the recruitment of CD4+ and CD8+ T cells to lung and subsequent pulmonary fibrosis. *J Immunol*. (2006) 177:4436–43. doi: 10.4049/jimmunol.177.7.4436
139. Simonian PL, Roark CL, Born WK, O'Brien RL, Fontenot AP. Gammadelta T cells and Th17 cytokines in hypersensitivity pneumonitis and lung fibrosis. *Transl Res*. (2009) 154:222–7. doi: 10.1016/j.trsl.2009.08.006
140. Lo Re S, Dumoutier L, Couillin I, Van Vyve C, Yakoub Y, Uwambayinema F, et al. IL-17A-producing gammadelta T and Th17 lymphocytes mediate lung inflammation but not fibrosis in experimental silicosis. *J Immunol*. (2010) 184:6367–77. doi: 10.4049/jimmunol.0900459
141. Wang X, Tian Z. gammadelta T cells in liver diseases. *Front Med*. (2018) 12:262–8. doi: 10.1007/s11684-017-0584-x
142. Klemann C, Schroder A, Dreier A, Mohn N, Dippel S, Winterberg T, et al. Interleukin 17, produced by gammadelta T cells, contributes to hepatic inflammation in a mouse model of biliary atresia and is increased in livers of patients. *Gastroenterology*. (2016) 150:229–41 e5. doi: 10.1053/j.gastro.2015.09.008
143. Yan X, Shichita T, Katsumata Y, Matsuhashi T, Ito H, Ito K, et al. Deleterious effect of the IL-23/IL-17A axis and gammadeltaT cells on left ventricular remodeling after myocardial infarction. *J Am Heart Assoc*. (2012) 1:e004408. doi: 10.1161/JAHA.112.004408
144. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med*. (2020) 382:1708–20. doi: 10.1101/2020.02.06.20020974
145. Wu J, Feng CL, Xian XY, Qiang J, Zhang J, Mao QX, et al. [Novel coronavirus pneumonia (COVID-19) CT distribution and sign features]. *Zhonghua jie he he hu xi za zhi*. (2020) 43:E030.
146. Xu YH, Dong JH, An WM, Lv XY, Yin XP, Zhang JZ, et al. Clinical and computed tomographic imaging features of novel coronavirus pneumonia caused by SARS-CoV-2. *J Infect*. (2020). doi: 10.1016/j.jinf.2020.02.017
147. Deschamp AR, Hatch JE, Slaven JE, Gebregziabher N, Storch G, Hall GL, et al. Early respiratory viral infections in infants with cystic fibrosis. *J Cystic Fibrosis*. (2019) 18:844–50. doi: 10.1016/j.jcf.2019.02.004
148. Xie L, Liu Y, Fan B, Xiao Y, Tian Q, Chen L, et al. Dynamic changes of serum SARS-coronavirus IgG, pulmonary function and radiography in patients recovering from SARS after hospital discharge. *Respir Res*. (2005) 6:5. doi: 10.1186/1465-9921-6-5
149. Venkataraman T, Coleman CM, Frieman MB. Overactive epidermal growth factor receptor signaling leads to increased fibrosis after severe acute respiratory syndrome coronavirus infection. *J Virol*. (2017) 91(12). doi: 10.1128/JVI.00182-17
150. Venkataraman T, Frieman MB. The role of epidermal growth factor receptor (EGFR) signaling in SARS coronavirus-induced pulmonary fibrosis. *Antiviral Res*. (2017) 143:142–50. doi: 10.1016/j.antiviral.2017.03.022
151. Kim J, Yang YL, Jeong Y, Jang YS. Middle East respiratory syndrome-coronavirus infection into established hDDP4-transgenic mice accelerates lung damage via activation of the pro-inflammatory response and pulmonary fibrosis. *J Microbiol Biotechnol*. (2019) 30, 427–38. doi: 10.4014/jmb.1910.10055
152. Burzyn D, Kuswanto W, Kolodin D, Shadrach JL, Cerletti M, Jang Y, et al. A special population of regulatory T cells potentiates muscle repair. *Cell*. (2013) 155:1282–95. doi: 10.1016/j.cell.2013.10.054
153. Ali N, Zirak B, Rodriguez RS, Pauli ML, Truong HA, Lai K, et al. Regulatory T cells in skin facilitate epithelial stem cell differentiation. *Cell*. (2017) 169:1119–29 e11. doi: 10.1016/j.cell.2017.05.002
154. Kwee BJ, Seo BR, Najibi AJ, Li AW, Shih TY, White D, et al. Treating ischemia via recruitment of antigen-specific T cells. *Sci Adv*. (2019) 5:eaav6313. doi: 10.1126/sciadv.aav6313
155. Castagnoli C, Trombotto C, Ondei S, Stella M, Calcagni M, Magliacani G, et al. Characterization of T-cell subsets infiltrating post-burn hypertrophic scar tissues. *Burns*. (1997) 23:565–72. doi: 10.1016/S0305-4179(97)00070-3
156. Bernabei P, Rigamonti L, Ariotti S, Stella M, Castagnoli C, Novelli F. Functional analysis of T lymphocytes infiltrating the dermis and epidermis of post-burn hypertrophic scar tissues. *Burns*. (1999) 25:43–8. doi: 10.1016/S0305-4179(98)00128-4
157. Tredget EE, Yang L, Delehanty M, Shankowsky H, Scott PG. Polarized Th2 cytokine production in patients with hypertrophic scar following thermal injury. *J Interferon Cytokine Res*. (2006) 26:179–89. doi: 10.1089/jir.2006.26.179
158. Wang J, Jiao H, Stewart TL, Shankowsky HA, Scott PG, Tredget EE. Increased TGF-beta-producing CD4+ T lymphocytes in postburn patients and their potential interaction with dermal fibroblasts in hypertrophic scarring. *Wound Repair Regen*. (2007) 15:530–9. doi: 10.1111/j.1524-475X.2007.00261.x
159. McGee HM, Schmidt BA, Booth CJ, Yancopoulos GD, Valenzuela DM, Murphy AJ, et al. IL-22 promotes fibroblast-mediated wound repair in the skin. *J Invest Dermatol*. (2013) 133:1321–9. doi: 10.1038/jid.2012.463
160. Kwan PO, Tredget EE. Biological principles of scar and contracture. *Hand Clin*. (2017) 33:277–92. doi: 10.1016/j.hcl.2016.12.004
161. Momtazi M, Ding J, Kwan P, Anderson CC, Honardoust D, Goekjian S, et al. Morphologic and histologic comparison of hypertrophic scar in nude mice, T-cell receptor, and recombination activating gene knockout mice. *Plast Reconstr Surg*. (2015) 136:1192–204. doi: 10.1097/PRS.0000000000001782
162. Ding J, Tredget EE. Transplanting human skin grafts onto nude mice to model skin scars. *Methods Mol Biol*. (2017) 1627:65–80. doi: 10.1007/978-1-4939-7113-8_5
163. Singer AJ, Tuggle C, Ahrens A, Sauer M, McClain SA, Tredget E, et al. Survival of human cadaver skin on severe combined immune deficiency pigs: Proof of concept. *Wound Repair Regen*. (2019) 27:426–30. doi: 10.1111/wrr.12715

164. Chen B, Li H, Xia W. The role of Th1/Th2 cell chemokine expression in hypertrophic scar. *Int Wound J.* (2020) 17:197–205. doi: 10.1111/iwj.13257
165. Schuppan D, Kim YO. Evolving therapies for liver fibrosis. *J Clin Investig.* (2013) 123:1887–901. doi: 10.1172/JCI66028
166. Kosaka H, Yoshimoto T, Yoshimoto T, Fujimoto J, Nakanishi K. Interferon-gamma is a therapeutic target molecule for prevention of postoperative adhesion formation. *Nat Med.* (2008) 14:437–41. doi: 10.1038/nm1733
167. Jiang H, Hegde S, Knolhoff BL, Zhu Y, Herndon JM, Meyer MA, et al. Targeting focal adhesion kinase renders pancreatic cancers responsive to checkpoint immunotherapy. *Nat Med.* (2016) 22:851–60. doi: 10.1038/nm.4123
168. Tedesco D, Thapa M, Chin CY, Ge Y, Gong M, Li J, et al. Alterations in intestinal microbiota lead to production of interleukin 17 by intrahepatic gammadelta T-cell receptor-positive cells and pathogenesis of cholestatic liver disease. *Gastroenterology.* (2018) 154:2178–93. doi: 10.1053/j.gastro.2018.02.019
169. Elhai M, Avouac J, Hoffmann-Vold AM, Ruzehaji N, Amiar O, Ruiz B, et al. OX40L blockade protects against inflammation-driven fibrosis. *Proc Natl Acad Sci USA.* (2016) 113:E3901–10. doi: 10.1073/pnas.1523512113
170. Boleto G, Guignabert C, Pezet S, Cauvet A, Sadoine J, Tu L, et al. T-cell costimulation blockade is effective in experimental digestive and lung tissue fibrosis. *Arthritis Res Therap.* (2018) 20:197. doi: 10.1186/s13075-018-1694-9
171. Zhao X, Kwan JYY, Yip K, Liu PP, Liu FF. Targeting metabolic dysregulation for fibrosis therapy. *Nat Rev Drug Discov.* (2020) 19:57–75. doi: 10.1038/s41573-019-0040-5
172. Dai DF, Santana LF, Vermulst M, Tomazela DM, Emond MJ, MacCoss MJ, et al. Overexpression of catalase targeted to mitochondria attenuates murine cardiac aging. *Circulation.* (2009) 119:2789–97. doi: 10.1161/CIRCULATIONAHA.108.822403
173. Kropski JA, Blackwell TS. Endoplasmic reticulum stress in the pathogenesis of fibrotic disease. *J Clin Investig.* (2018) 128:64–73. doi: 10.1172/JCI93560
174. Grohmann M, Wiede F, Dodd GT, Gurzov EN, Ooi GJ, Butt T, et al. Obesity Drives STAT-1-Dependent NASH and STAT-3-Dependent HCC. *Cell.* (2018) 175:1289–306 e20. doi: 10.1016/j.cell.2018.09.053
175. Ramachandran P, Dobie R, Wilson-Kanamori JR, Dora EF, Henderson BEP, Luu NT, et al. Resolving the fibrotic niche of human liver cirrhosis at single-cell level. *Nature.* (2019) 575:512–8. doi: 10.1038/s41586-019-1631-3
176. Aghajanian H, Kimura T, Rurik JG, Hancock AS, Leibowitz MS, Li L, et al. Targeting cardiac fibrosis with engineered T cells. *Nature.* (2019) 573:430–3. doi: 10.1038/s41586-019-1546-z

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Role of Dendritic Epidermal T Cells in Cutaneous Carcinoma

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Dendritic epidermal T cells (DETCs) are γδ T cells expressing invariant Vγ5Vδ1 T cell receptor (TCR) in murine epidermis. Initially, the development and the maturation of DETC progenitors are mediated by skint-1, TCR, and cytokines in the fetal thymus. Then, the DETC progenitors migrate to the epidermis with the guidance of selectins, CCR10, CCR4, *etc.* Eventually, mature DETCs proliferate and maintain a homeostatic population in the epidermis through IL-15 and aryl hydro-carbon receptor signaling. In "stressed" skin, DETCs are activated, exhibiting features such as a round morphology, cytotoxicity, and production of cytokines. In cutaneous carcinoma, DETCs generally inhibit tumor development directly in non-major histocompatibility complex-restricted manner, with the assistance of cytokines. DETCs also recognize and inhibit tumor via TCR, non-TCR receptors (such as 2B4 and NKG2D), or both. This study summarizes the biogenesis and the function of DETCs in cutaneous carcinoma and clarifies the essential surveillance role in the epidermis that DETCs play. As there are no DETCs in human epidermis but only human epidermis γδ T cells, we need to understand the anti-tumor pathways used by DETCs to find analogous immune pathways in human skin, which could be exploited for novel therapeutics.

Keywords: dendritic epidermal T cells, γδ T cells, epidermis, squamous cell carcinoma, melanoma

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INTRODUCTION

The γδ T cells are abundant in epithelial surfaces of the skin, intestine, lung, *etc.* (1). The skin is comprised of the epidermis, the basement membrane, and the dermis. The epidermis consists of 95% keratinocytes and 5% immune cells, including Langerhans cells and T cells that are predominantly epidermal γδ T cells (2, 3). The dermis contains dermal γδ T cells and variant immune cells, including αβ T cells, macrophages, dendritic cells, *etc.* The epidermal γδ T cells are different from the dermal γδ T cells in the T cell receptor (TCR) chains and shapes. In mouse, compared with the round dermal γδ T cells expressing Vγ4, Vγ2 but not Vγ5 TCR (4), the epidermal γδ T cells are dendritic and exclusively express Vγ5 TCR, therefore termed as dendritic epidermal γδ T cells (DETCs). This dendritic morphology of DETCs may be localization specific as the skin-resident memory $CD8^+$ T cells are also dendritic in the epidermis (3, 5). The dendritic morphology of DETCs may be shaped by CD103 and E-cadherin (6, 7).

DETCs are unique in rodents, and similar γδ T cells reside in the epidermis of some species (8), and DETCs are reported to play an important role in wound healing and surveillance on tumors (8). In rat epidermis, the majority of T cells are dendritic γδ T cells, with Vγ and Vδ chains highly similar

to DETCs (9). In cattle epidermis, the skin-resident γδ T cells are still dendritic but with different Vγ chain and Vδ chain compared with DETCs (10). In humans, the γδ T cells equivalent to DETCs are to be discovered; only a small subset of γδ T cells expressing the Vδ1 TCR reside in the epidermis, termed as human epidermal γδ T cells. The human epidermal γδ T cells also promote wound healing by secreting insulin-like growth factor 1 and are also cytotoxic to cutaneous carcinoma as DETCs (11–13). However, the human epidermal γδ T cells are still different from DETCs in terms of the molecular mechanisms of homing to the epidermis, activation, and antigen recognition (14, 15), and human epidermal γδ T cells are round in morphology instead of dendritic. In this study, we summarize the biogenesis of DETCs and their function roles in cutaneous carcinoma and hope that these mechanisms can provide cues to the study of human epidermal γδ T cells in parallel.

BIOGENESIS OF DETC

DETCs are derived from DETC progenitors that are the first T cells generated in the thymus at embryonic day 13 (8). A few mechanisms are reported about the development and the maturation of the DETC progenitors. Skint-1, a member of the butyrophilin-like (Btnl) family proteins derived from mature thymic epithelial cells with activated rank signaling (16), is identified as the key molecule in promoting the selective development of $V\gamma5^+$ DETC progenitors (17). Skint-1 determines the differentiation direction of fetal thymocytes through a CDR3-like loop-dependent manner (17). After receiving the Skint-1 signal, the DETC progenitors provoke differentiation and produce IFN-γ by activating the Egr3-mediated pathway while suppressing Sox13 and RORγt that are essential for other γδ T cells that produce IL-17 (18). Although Skint-1 is not a γδTCR ligand, the Skint-1-mediated selection might be through a TCR-related manner because Egr3, Sox13, and Rorc are downstream molecules of TCR signaling (18). TCR–ligands interaction is also essential for the maturation of DETC progenitors. TCRs induce the expression of sphingosine-1-phosphate receptor 1 in DETC progenitors (19). The downstream of TCR signaling in mice only have a delayed DETC accumulation but not any effect on the DETC compartment in the epidermis (20). Therefore, the TCR–ligands signaling might only regulate the development of DETC progenitors in the thymus. Besides the cell–cell communication, the cytokines derived from fetal thymocytes promote the development of DETC precursors. IL-7 and IL-7R signaling is essential for TCR gene transcription in a JAK/STAT pathway-dependent manner (21). IL-2 and IL-15 promote the survival of DETC precursors (21).

DETCs are located in the basal layer of the epidermis. Therefore, DETC precursors need to migrate from the thymus to the epidermis via the following steps: (1) adhering to the endothelial capillary in the dermis and (2) extravasation and locating to the epidermis (22). For the first step, DETC precursors express ligands to bind to the selectins expressed on the vascular endothelium. Although the exact ligands have not been identified, evidences show that DETCs are dramatically reduced in mice lacking E-selectins and P-selectins (23). For the second step, the DETC precursors express high levels of CC-chemokine receptor 10 (CCR10), which is the receptor of CC-chemokine ligand 27 expressed by keratinocytes (24). DETCs are markedly reduced in mice lacking CCR10 because the DETC precursors are halted in the dermis (25). A small subset of DETC precursors is homing to the epidermis in a CCR4-dependent manner (23). The Vγ5 TCRs might be important for the DETC precursor migration and epidermal localization (20); however, TCR is also reported to be not specific for DETC migration and homing to the epidermis. Further investigations are needed (26–28).

Once the DETCs home in the epidermis, they proliferate exponentially along with the growth of the skin after birth in an IL-15-dependent manner as DETCs are decreased in IL-15- or IL-15R-deficient mice, while IL-15 is secreted by keratinocytes (29). In adults, DETCs are not supplied by circulating γδ T cells from hematopoietic stem cell but keep a homeostatic number by self-renewal in an aryl hydro-carbon receptor (AHR)-dependent manner. The AHRs are activated by ligands from the DETC cytoplasm. When lacking the AHR signaling, the DETCs cannot proliferate after homing in the epidermis (30). DETCs also produce insulin-like growth factors (IGFs) to prevent themselves from apoptosis (13). The Vγ5 TCR is important for the homeostatic maintenance of mature DETCs in adults (31). Therefore, after homing to the epidermis, the mature DETCs proliferate and maintain a homeostatic population.

DETCs need to be activated to play a functional role in damaged skin and cancer. In steady-state skin, DETCs extend their dendrites to the suprabasal layers and closely contact with keratinocytes (32). In pathological-state skin, activated DETCs become motile by losing the dendrites (32). DETCs may be activated by co-culturing with transformed keratinocytes and protect keratinocytes from apoptosis in an IGF1-dependent manner (13, 33). The TCRs are essential for DETC activation by recognizing antigens from keratinocyte or Langerhans cells (34, 35). Damaged or stressed keratinocytes express TCR ligands that can activate DETCs in a non-major histocompatibility complex (MHC)-restricted manner (35–37). Beyond TCR, the complete activation of DETCs requires co-stimulatory signals such as junctional adhesion molecule-like (JAML) (38), CD100 (39), 2B4 (40), and natural killer group 2D (NKG2D) (41). JAML expressed in DETC is similar to CD28/B7 in αβ T cell (38). CD100 expressed in DETC is a receptor for plexin B2-mediated signaling in keratinocyte to initiate DETC activation, with a morphology change (39). 2B4 expressed in DETC is associated with tumor target recognition (40). NKG2D expressed in DETC is a receptor for stress-induced proteins to activate DETC in responding to tumor or cutaneous wound (42, 43). The cytokines are also important for DETC activation. DETCs freshly isolated from skin can be activated by IL-2 (40), and activated DETCs produce IL-2 (44). IL-7 and IL-15 from keratinocytes and fibroblast activate DETCs (45–47). In contrast, the activation of DETCs is inhibited by the E-cadherin of keratinocytes (7). The DETC expression of JAML (38), CD100 (39), and NKG2D (43) are critical for

wound healing. The DETC expression of 2B4 (40), NKG2D (41, 42), and IL-2 (44) may facilitate the cytotoxic potential to tumor cells.

ROLE OF DETC IN CUTANEOUS CARCINOMA

A total of 90% of cutaneous carcinomas are comprised of basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma. In general, the inhibition of cutaneous carcinoma by activated DETCs relies on three consecutive signals: TCR in MHC—restriction independent (15), non-TCR receptors such as NKG2D (48), 2B4 (40), or cytokines such as IL-2 (44), and IFNγ (38, 48, 49).

DETC in Non-melanoma Skin Cancer

BCC and SCC are usually categorized as non-melanoma skin cancer (50). BCC is the most common skin cancer, which starts from the base cell layer of the epidermis. SCC is the second common cutaneous carcinoma from damaged keratinocytes (50, 51). The non-melanoma skin cancer may be caused by solar UV radiation or chemicals such as arsenic (52, 53).

Majority of BCCs and 50% of SCCs are caused by solar UV radiation. In chronic UV radiation, the DNA repairing mechanism caused gene mutation and genome instability, which are responses for tumor formation. PTCH1 and P53 mutations drive BCC and SCC initiation, respectively (54, 55). DETC is the major antitumor player in murine epidermis. DETC can directly lyse the SCC cell line Pam 212 monolayer effectively (56) or inhibit the tumor cells by inducing CD8+T cells (57). DETC can lyse the PDV tumorigenic keratinocyte cell line (42) but not the normal keratinocyte cells *in vitro* (56). Therefore, the DETC's cytolytic activity may be tumor cell specific. The DETCs protect the keratinocyte from UV-caused DNA damage by reducing γH2AX, a cyclobutane pyrimidine dimer. UV-damaged keratinocytes secrete IL-1β, which triggers DETCs to produce IL-17A, and in turn, IL-17A upregulates molecules linked to DNA repair response and limits γH2AX expression in keratinocyte cells (58). The DETC population is decreased in UV-irradiated epidermis (57). Therefore, DETCs might have a potential role in preventing UV-induced skin cancer, and further studies are needed. However, IL-17A plays a dual role in promoting both tumor growth and antitumor immunity in skin cancer. On one hand, IL-17A accelerates the proliferation of skin epithelial cells to promote tumorigenesis (59, 60). IL-17A also promotes the tumor microenvironment formation by attracting an infiltration of immune cells (61). In murine models of ovarian cancer and pancreatic ductal adenocarcinoma, the IL-17-producing γδ T cells (not DETCs) are proliferative, active, and may directly inhibit adaptive antitumor immunity by producing PD-L1 and Galectin-9 (62, 63). Whether the tumor-infiltrating immune cells together with DETCs can promote tumorigenesis and tumor progression needs to be investigated. On the other hand, the IL-17-producing $CD8^+$ T induces tumor regression in mice with vascularized B16 melanoma (64). The IL-17-producing γδ T cells enhance chemotherapy to mice with fibrosarcoma (65). Th17 cells activate endogenous cytotoxic $CD8^+$ T cells, leading to tumor regression in melanoma (66). The generation of IL-17-producing T cells with different phenotypes in response to variant tumor contexts would explain the conflicting observations. Whether IL-17 plays a role in DETC-mediated antitumor immunity needs to be studied. In an UV-induced SCC model, DETCs can inhibit the activation of $CD4^+$T cells, but not $CD8^+$T cells, within 3 days after UV radiation, resulting in an accelerated tumor growth (67).

Aside from UV, SCC may also be induced by chemicals. In a 7,12-dimethylbenz(a)anthracene (DMBA)/12-O-tetradecanoylphorbol-13-acetate (TPA)-induced SCC model, the DETCs show an anti-tumor role as γδ T-depleted mice are more acceptable for tumors than the wild-type mice. DETCs eliminate DMBA/TPA-induced SCC by expressing IFNγ and NKG2D, therefore promoting the therapeutic effect of rapamycin on SCC (68). IFNγ promotes the migration, activation, and cytotoxicity of DETCs in SCC. NKG2D, a receptor of natural killer cells, is only expressed in DETC in murine epidermis. The expression of ligands for NAG2D, such as Rae-1 and H60, is inducible in SCC by DMBA/TPA treatment (42). Blocking NKG2D can inhibit DETC activation, but whether these ligands activate DETCs directly or indirectly needs more investigation. Rae-1 can activate DETCs directly without TCR signaling (69, 70). H60c can directly activate DETCs to produce IL-13 (71), but H60c is also reported to only provide co-stimulatory signals for DETC activation, failing to activate DETCs directly (69, 72). Thus, DETCs eliminate tumor mediated by NKG2D, but the NKG2D signaling of DETCs may response differently in a different stimulation content.

Cutaneous lymphoma, a rare subtype of non-Hodgkin lymphoma, starts from the lymphocytes in the skin but is not classified as cutaneous carcinoma. As for the well-established non-Hodgkin lymphoma cell line YAC, DETCs directly kill YAC cells by producing perforin and granzymes (56), and this cytotoxicity is not MHC-restricted (73, 74). The anti-tumor potential of DETCs is strengthened in the presence of cytokines. 2B4, initially found in NK cells and T cells, associate with non-MHC-restricted recognition to tumor targets (75, 76), is expressed in DETC, and mediates the killing of tumor cells by DETC (40). IL-2 enhances the cytotoxicity of DETC to lymphoma cells by stimulating 2B4 expression (40). The DETCs activated by CoA produce IL-2, which stimulates DETCs to kill YAC cells (40, 44). IL-7, produced by keratinocytes, is also critical for DETC activation to acquire a cytotoxic capability to lymphomas (56). The cytotoxicity of DETC directly to lymphoma cells can be strengthened by cytokines.

DETC in Melanoma

Melanoma starts from melanocytes and is very aggressive and metastatic. Melanoma occupies 1% of cutaneous carcinoma cases but is the most lethal event in a cutaneous carcinoma patient. DETC cell line AU16 inhibits melanoma progression *in vivo* and kills melanoma cells in cytotoxicity *in vitro* (77). The DETC cell line AU16, derived from C3H mice, is an IL-2-dependent cell line and cytotoxic to melanoma cell lines and chemo-induced fibrosarcoma *in vitro* (77). The injection of mixed AU16 cells

and melanoma cells can delay the melanoma growth *in vivo* (77). In another study, the inhibition of DETCs to melanoma may be tumor specific as normal keratinocytes are not affected (56). The inhibition of DETCs on melanoma is IL-2 dependent and needs a close contact between DETCs and melanoma (56). Microscopically, DETCs destruct melanoma monolayers by adhering to tumor cells first and then gradually forming enlarged discrete foci to disrupt the melanoma cells. DETCs may inhibit melanoma in a NKG2D-dependent manner as NKG2D ligands are largely expressed in melanoma (78). The studies of DETCs on inhibiting melanoma are limited, and further investigations are needed.

CONCLUSION AND PERSPECTIVE

DETCs are the resident γδ T cells, with a dendritic morphology, in murine epidermis. Once the skin is damaged or has tumor, DETCs are activated by cytokines (such as IL-17, IL-15, and IL-2) or signaling directly from keratinocytes and Langerhans cells. The activated DETCs generally inhibit tumor progress but also promote tumor development in a certain tumor microenvironment. However, our understanding of the biology of DETCs is still largely limited, particularly in the area of DETCs responding to a skin tumor microenvironment. How do the DETCs maintain hemostasis in a skin tumor microenvironment? How do the DETCs communicate with the tumor-infiltrated immune cells and with the neighbor cells in the epidermis? We need to understand the anti-tumor pathways used by DETCs to find analogous immune pathways in human skin which could be exploited for novel therapeutics.

AUTHOR CONTRIBUTIONS

JX participated in the design of this study and organized the manuscript. MQ wrote the draft of the manuscript. HZ designed and revised the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Chien YH, Meyer C, Bonneville M. γδ T cells: first line of defense and beyond. *Annu Rev Immunol.* (2014) 32:121–55. doi: 10.1146/annurev-immunol-032713-120216
2. Nguyen AV, Soulika AM. The dynamics of the skin's immune system. *Int J Mol Sci.* (2019) 20:1811. doi: 10.3390/ijms20081811
3. Zaid A, Mackay LK, Rahimpour A, Braun A, Veldhoen M, Carbone FR, et al. Persistence of skin-resident memory T cells within an epidermal niche. *Proc Natl Acad Sci USA.* (2014) 111:5307–12. doi: 10.1073/pnas.1322292111
4. Sumaria N, Roediger B, Ng LG, Qin J, Pinto R, Cavanagh LL, et al. Cutaneous immunosurveillance by self-renewing dermal γδ T cells. *J Exp Med.* (2011) 208:505–18. doi: 10.1084/jem.20101824
5. Hayday A, Tigelaar R. Immunoregulation in the tissues by γδ T cells. *Nat Rev Immunol.* (2003) 3:233–42. doi: 10.1038/nri1030
6. Schon MP, Schon M, Parker CM, Williams IR. Dendritic epidermal T cells (DETC) are diminished in integrin alphaE(CD103)-deficient mice. *J Invest Dermatol.* (2002) 119:190–3. doi: 10.1046/j.1523-1747.2002.17973.x
7. Uchida Y, Kawai K, Ibusuki A, Kanekura T. Role for E-cadherin as an inhibitory receptor on epidermal γδ T cells. *J Immunol.* (2011) 186:6945–54. doi: 10.4049/jimmunol.1003853
8. Sutoh Y, Mohamed RH, Kasahara M. Origin and evolution of dendritic epidermal T cells. *Front Immunol.* (2018) 9:1059. doi: 10.3389/fimmu.2018.01059
9. Elbe A, Kilgus O, Hunig T, Stingl G. T-cell receptor diversity in dendritic epidermal T cells in the rat. *J Invest Dermatol.* (1994) 102:74–9. doi: 10.1111/1523-1747.ep12371735
10. Hein WR, Dudler L. TCR gamma delta+ cells are prominent in normal bovine skin and express a diverse repertoire of antigen receptors. *Immunology.* (1997) 91:58–64. doi: 10.1046/j.1365-2567.1997.00224.x
11. Toulon A, Breton L, Taylor KR, Tenenhaus M, Bhavsar D, Lanigan C, et al. A role for human skin-resident T cells in wound healing. *J Exp Med.* (2009) 206:743–50. doi: 10.1084/jem.20081787
12. Pang DJ, Neves JF, Sumaria N, Pennington DJ. Understanding the complexity of γδ T-cell subsets in mouse and human. *Immunology.* (2012) 136:283–90. doi: 10.1111/j.1365-2567.2012.03582.x
13. Sharp LL, Jameson JM, Cauvi G, Havran WL. Dendritic epidermal T cells regulate skin homeostasis through local production of insulin-like growth factor 1. *Nat Immunol.* (2005) 6:73–9. doi: 10.1038/ni1152
14. Davey MS, Willcox CR, Baker AT, Hunter S, Willcox BE. Recasting human Vδ1 lymphocytes in an adaptive role. *Trends Immunol.* (2018) 39:446–59. doi: 10.1016/j.it.2018.03.003
15. Adams EJ, Gu S, Luoma AM. Human gamma delta T cells: evolution and ligand recognition. *Cell Immunol.* (2015) 296:31–40. doi: 10.1016/j.cellimm.2015.04.008
16. Roberts NA, White AJ, Jenkinson WE, Turchinovich G, Nakamura K, Withers DR, et al. Rank signaling links the development of invariant gammadelta T cell progenitors and Aire(+) medullary epithelium. *Immunity.* (2012) 36:427–37. doi: 10.1016/j.immuni.2012.01.016
17. Salim M, Knowles TJ, Hart R, Mohammed F, Woodward MJ, Willcox CR, et al. Characterization of a putative receptor binding surface on skint-1, a critical determinant of dendritic epidermal t cell selection. *J Biol Chem.* (2016) 291:9310–21. doi: 10.1074/jbc.M116.722066
18. Turchinovich G, Hayday AC. Skint-1 identifies a common molecular mechanism for the development of interferon-γ-secreting versus interleukin-17-secreting γδ T cells. *Immunity.* (2011) 35:59–68. doi: 10.1016/j.immuni.2011.04.018
19. Xiong N, Kang C, Raulet DH. Positive selection of dendritic epidermal gammadelta T cell precursors in the fetal thymus determines expression of skin-homing receptors. *Immunity.* (2004) 21:121–31. doi: 10.1016/j.immuni.2004.06.008
20. Xia M, Qi Q, Jin Y, Wiest DL, August A, Xiong N. Differential roles of IL-2-inducible T cell kinase-mediated TCR signals in tissue-specific localization and maintenance of skin intraepithelial T cells. *J Immunol.* (2010) 184:6807–14. doi: 10.4049/jimmunol.1000453
21. Ye SK, Maki K, Lee HC, Ito A, Kawai K, Suzuki H, et al. Differential roles of cytokine receptors in the development of epidermal gamma delta T cells. *J Immunol.* (2001) 167:1929–34. doi: 10.4049/jimmunol.167.4.1929
22. Nielsen MM, Witherden DA, Havran WL. γδ T cells in homeostasis and host defence of epithelial barrier tissues. *Nat Rev Immunol.* (2017) 17:733–45. doi: 10.1038/nri.2017.101
23. Jiang X, Campbell JJ, Kupper TS. Embryonic trafficking of gammadelta T cells to skin is dependent on E/P selectin ligands and CCR4. *Proc Natl Acad Sci USA.* (2010) 107:7443–8. doi: 10.1073/pnas.0912943107
24. Jin Y, Xia M, Saylor CM, Narayan K, Kang J, Wiest DL, et al. Cutting edge: Intrinsic programming of thymic gammadeltaT cells for specific peripheral tissue localization. *J Immunol.* (2010) 185:7156–60. doi: 10.4049/jimmunol.1002781

25. Jin Y, Xia M, Sun A, Saylor CM, Xiong N. CCR10 is important for the development of skin-specific γδT cells by regulating their migration and location. *J Immunol.* (2010) 185:5723–31. doi: 10.4049/jimmunol.1001612
26. Hara H, Kishihara K, Matsuzaki G, Takimoto H, Tsukiyama T, Tigelaar RE, et al. Development of dendritic epidermal T cells with a skewed diversity of gamma delta TCRs in V delta 1-deficient mice. *J Immunol.* (2000) 165:3695–705. doi: 10.4049/jimmunol.165.7.3695
27. Bonneville M, Itohara S, Krecko EG, Mombaerts P, Ishida I, Katsuki M, et al. Transgenic mice demonstrate that epithelial homing of gamma/delta T cells is determined by cell lineages independent of T cell receptor specificity. *J Exp Med.* (1990) 171:1015–26. doi: 10.1084/jem.171.4.1015
28. Mallick-Wood CA, Lewis JM, Richie LI, Owen MJ, Tigelaar RE, Hayday AC. Conservation of T cell receptor conformation in epidermal gammadelta cells with disrupted primary Vgamma gene usage. *Science.* (1998) 279:1729–33. doi: 10.1126/science.279.5357.1729
29. Edelbaum D, Mohamadzadeh M, Bergstresser PR, Sugamura K, Takashima A. Interleukin (IL)-15 promotes the growth of murine epidermal gamma delta T cells by a mechanism involving the beta- and gamma c-chains of the IL-2 receptor. *J Invest Dermatol.* (1995) 105:837–43. doi: 10.1111/1523-1747.ep12326630
30. Li Y, Innocentin S, Withers DR, Roberts NA, Gallagher AR, Grigorieva EF, et al. Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. *Cell.* (2011) 147:629–40. doi: 10.1016/j.cell.2011.09.025
31. Zhang B, Wu J, Jiao Y, Bock C, Dai M, Chen B, et al. Differential requirements of TCR signaling in homeostatic maintenance and function of dendritic epidermal T cells. *J Immunol.* (2015) 195:4282–91. doi: 10.4049/jimmunol.1501220
32. Chodaczek G, Papanna V, Zal MA, Zal T. Body-barrier surveillance by epidermal γδ TCRs. *Nat Immunol.* (2012) 13:272–82. doi: 10.1038/ni.2240
33. Girardi M, Lewis J, Glusac E, Filler RB, Geng L, Hayday AC, et al. Resident skin-specific gammadelta T cells provide local, nonredundant regulation of cutaneous inflammation. *J Exp Med.* (2002) 195:855–67. doi: 10.1084/jem.20012000
34. Jameson JM, Cauvi G, Witherden DA, Havran WL. A keratinocyte-responsive gamma delta TCR is necessary for dendritic epidermal T cell activation by damaged keratinocytes and maintenance in the epidermis. *J Immunol.* (2004) 172:3573–9. doi: 10.4049/jimmunol.172.6.3573
35. Havran WL, Chien YH, Allison JP. Recognition of self antigens by skin-derived T cells with invariant gamma delta antigen receptors. *Science.* (1991) 252:1430–2. doi: 10.1126/science.1828619
36. Shiohara T, Moriya N, Hayakawa J, Itohara S, Ishikawa H. Resistance to cutaneous graft-vs.-host disease is not induced in T cell receptor delta gene-mutant mice. *J Exp Med.* (1996) 183:1483–9. doi: 10.1084/jem.183.4.1483
37. Komori HK, Witherden DA, Kelly R, Sendaydiego K, Jameson JM, Teyton L, et al. Cutting edge: dendritic epidermal gammadelta T cell ligands are rapidly and locally expressed by keratinocytes following cutaneous wounding. *J Immunol.* (2012) 188:2972–6. doi: 10.4049/jimmunol.1100887
38. Witherden DA, Verdino P, Rieder SE, Garijo O, Mills RE, Teyton L, et al. The junctional adhesion molecule JAML is a costimulatory receptor for epithelial gammadelta T cell activation. *Science.* (2010) 329:1205–10. doi: 10.1126/science.1192698
39. Witherden DA, Watanabe M, Garijo O, Rieder SE, Sarkisyan G, Cronin SJ, et al. The CD100 receptor interacts with its plexin B2 ligand to regulate epidermal gammadelta T cell function. *Immunity.* (2012) 37:314–25. doi: 10.1016/j.immuni.2012.05.026
40. Schuhmachers G, Ariizumi K, Mathew PA, Bennett M, Kumar V, Takashima A. 2B4, a new member of the immunoglobulin gene superfamily, is expressed on murine dendritic epidermal T cells and plays a functional role in their killing of skin tumors. *J Invest Dermatol.* (1995) 105:592–6. doi: 10.1111/1523-1747.ep12323533
41. Ibusuki A, Kawai K, Yoshida S, Uchida Y, Nitahara-Takeuchi A, Kuroki K, et al. NKG2D triggers cytotoxicity in murine epidermal gammadelta T cells via PI3K-dependent, Syk/ZAP70-independent signaling pathway. *J Invest Dermatol.* (2014) 134:396–404. doi: 10.1038/jid.2013.353
42. Girardi M, Oppenheim DE, Steele CR, Lewis JM, Glusac E, Filler R, et al. Regulation of cutaneous malignancy by gammadelta T cells. *Science.* (2001) 294:605–9. doi: 10.1126/science.1063916
43. Jung H, Hsiung B, Pestal K, Procyk E, Raulet DH. RAE-1 ligands for the NKG2D receptor are regulated by E2F transcription factors, which control cell cycle entry. *J Exp Med.* (2012) 209:2409–22. doi: 10.1084/jem.20120565
44. Takashima A, Nixon-Fulton JL, Bergstresser PR, Tigelaar RE. Thy-1+ dendritic epidermal cells in mice: precursor frequency analysis and cloning of concanavalin A-reactive cells. *J Invest Dermatol.* (1988) 90:671–8. doi: 10.1111/1523-1747.ep12560835
45. Matsue H, Bergstresser PR, Takashima A. Keratinocyte-derived IL-7 serves as a growth factor for dendritic epidermal T cells in mice. *J Immunol.* (1993) 151:6012–9. doi: 10.1016/0923-1811(93)90782-K
46. Wang Y, Bai Y, Li Y, Liang G, Jiang Y, Liu Z, et al. IL-15 enhances activation and IGF-1 production of dendritic epidermal t cells to promote wound healing in diabetic mice. *Front Immunol.* (2017) 8:1557. doi: 10.3389/fimmu.2017.01557
47. Zelante T, Fric J, Wong AY, Ricciardi-Castagnoli P. Interleukin-2 production by dendritic cells and its immuno-regulatory functions. *Front Immunol.* (2012) 3:161. doi: 10.3389/fimmu.2012.00161
48. Nitahara A, Shimura H, Ito A, Tomiyama K, Ito M, Kawai K. NKG2D ligation without T cell receptor engagement triggers both cytotoxicity and cytokine production in dendritic epidermal T cells. *J Invest Dermatol.* (2006) 126:1052–8. doi: 10.1038/sj.jid.5700112
49. Matsue H, Cruz PD Jr, Bergstresser PR, Takashima A. Profiles of cytokine mRNA expressed by dendritic epidermal T cells in mice. *J Invest Dermatol.* (1993) 101:537–42. doi: 10.1111/1523-1747.ep12365917
50. Ryu TH, Kye H, Choi JE, Ahn HH, Kye YC, Seo SH. Features causing confusion between basal cell carcinoma and squamous cell carcinoma in clinical diagnosis. *Ann Dermatol.* (2018) 30:64–70. doi: 10.5021/ad.2018.30.1.64
51. Ratushny V, Gober MD, Hick R, Ridky TW, Seykora JT. From keratinocyte to cancer: the pathogenesis and modeling of cutaneous squamous cell carcinoma. *J Clin Invest.* (2012) 122:464–72. doi: 10.1172/JCI57415
52. Martinez VD, Becker-Santos DD, Vucic EA, Lam S, Lam WL. Induction of human squamous cell-type carcinomas by arsenic. *J Skin Cancer.* (2011) 2011:454157. doi: 10.1155/2011/454157
53. Pfeifer GP, Besaratinia A. UV wavelength-dependent DNA damage and human non-melanoma and melanoma skin cancer. *Photochem Photobiol Sci.* (2012) 11:90–7. doi: 10.1039/C1PP05144J
54. Stanoszek LM, Wang GY, Harms PW. Histologic mimics of basal cell Carcinoma. *Arch Pathol Lab Med.* (2017) 141:1490–502. doi: 10.5858/arpa.2017-0222-RA
55. Black AP, Ogg GS. The role of p53 in the immunobiology of cutaneous squamous cell carcinoma. *Clin Exp Immunol.* (2003) 132:379–84. doi: 10.1046/j.1365-2249.2003.02159.x
56. Kaminski MJ, Cruz PD Jr, Bergstresser PR, Takashima A. Killing of skin-derived tumor cells by mouse dendritic epidermal T-cells. *Cancer Res.* (1993) 53:4014–9.
57. Cavanagh LL, Barnetson RS, Basten A, Halliday GM. Dendritic epidermal T-cell involvement in induction of CD8+ T cell-mediated immunity against an ultraviolet radiation-induced skin tumor. *Int J Cancer.* (1997) 70:98–105. doi: 10.1002/sici1097-02151997010670:198::aid-ijc153.0.co;2-9
58. Macleod AS, Rudolph R, Corriden R, Ye I, Garijo O, Havran WL. Skin-resident T cells sense ultraviolet radiation-induced injury and contribute to DNA repair. *J Immunol.* (2014) 192:5695–702. doi: 10.4049/jimmunol.1303297
59. Wang L, Yi T, Zhang W, Pardoll DM, Yu H. IL-17 enhances tumor development in carcinogen-induced skin cancer. *Cancer Res.* (2010) 70:10112–20. doi: 10.1158/0008-5472.CAN-10-0775
60. Chen X, Cai G, Liu C, Zhao J, Gu C, Wu L, et al. IL-17R-EGFR axis links wound healing to tumorigenesis in Lrig1(+) stem cells. *J Exp Med.* (2019) 216:195–214. doi: 10.1084/jem.20171849
61. Fabre J, Giustiniani J, Garbar C, Antonicelli F, Merrouche Y, Bensussan A, et al. Targeting the tumor microenvironment: the protumor effects of IL-17 related to Cancer type. *Int J Mol Sci.* (2016) 17:1433. doi: 10.3390/ijms17091433
62. Rei M, Goncalves-Sousa N, Lanca T, Thompson RG, Mensurado S, Balkwill FR, et al. Murine CD27(-) Vγ6(+) γδ T cells producing IL-17A promote ovarian cancer growth via mobilization of protumor small peritoneal macrophages. *Proc Natl Acad Sci USA.* (2014) 111:E3562–70. doi: 10.1073/pnas.1403424111

63. Daley D, Zambirinis CP, Seifert L, Akkad N, Mohan N, Werba G, et al. γδ T cells support pancreatic oncogenesis by restraining αβ T cell activation. *Cell.* (2016) 166:1485–99.e15. doi: 10.1016/j.cell.2016.07.046
64. Hinrichs CS, Kaiser A, Paulos CM, Cassard L, Sanchez-Perez L, Heemskerk B, et al. Type 17 CD8+ T cells display enhanced antitumor immunity. *Blood.* (2009) 114:596–9. doi: 10.1182/blood-2009-02-203935
65. Ma Y, Aymeric L, Locher C, Mattarollo SR, Delahaye NF, Pereira P, et al. Contribution of IL-17-producing gamma delta T cells to the efficacy of anticancer chemotherapy. *J Exp Med.* (2011) 208:491–503. doi: 10.1084/jem.20100269
66. Martin-Orozco N, Muranski P, Chung Y, Yang XO, Yamazaki T, Lu S, et al. T helper 17 cells promote cytotoxic T cell activation in tumor immunity. *Immunity.* (2009) 31:787–98. doi: 10.1016/j.immuni.2009.09.014
67. Cavanagh LL, Halliday GM. Dendritic epidermal T cells in ultraviolet-irradiated skin enhance skin tumor growth by inhibiting CD4+ T-cell-mediated immunity. *Cancer Res.* (1996) 56:2607–15.
68. Dao V, Liu Y, Pandeswara S, Svatek RS, Gelfond JA, Liu A, et al. Immune-stimulatory effects of rapamycin are mediated by stimulation of antitumor γδ T Cells. *Cancer Res.* (2016) 76:5970–82. doi: 10.1158/0008-5472.CAN-16-0091
69. Nielsen MM, Dyring-Andersen B, Schmidt JD, Witherden D, Lovato P, Woetmann A, et al. NKG2D-dependent activation of dendritic epidermal T cells in contact hypersensitivity. *J Invest Dermatol.* (2015) 135:1311–9. doi: 10.1038/jid.2015.23
70. Strid J, Roberts SJ, Filler RB, Lewis JM, Kwong BY, Schpero W, et al. Acute upregulation of an NKG2D ligand promotes rapid reorganization of a local immune compartment with pleiotropic effects on carcinogenesis. *Nat Immunol.* (2008) 9:146–54. doi: 10.1038/ni1556
71. Strid J, Sobolev O, Zafirova B, Polic B, Hayday A. The intraepithelial T cell response to NKG2D-ligands links lymphoid stress surveillance to atopy. *Science.* (2011) 334:1293–7. doi: 10.1126/science.1211250
72. Whang MI, Guerra N, Raulet DH. Costimulation of dendritic epidermal gammadelta T cells by a new NKG2D ligand expressed specifically in the skin. *J Immunol.* (2009) 182:4557–64. doi: 10.4049/jimmunol.0802439
73. Nixon-Fulton JL, Hackett J Jr, Bergstresser PR, Kumar V, Tigelaar RE. Phenotypic heterogeneity and cytotoxic activity of Con A and IL-2-stimulated cultures of mouse Thy-1+ epidermal cells. *J Invest Dermatol.* (1988) 91:62–8. doi: 10.1111/1523-1747.ep12463290
74. Huber H, Descossy P, van Brandwijk R, Knop J. Activation of murine epidermal TCR-gamma delta+ T cells by keratinocytes treated with contact sensitizers. *J Immunol.* (1995) 155:2888–94.
75. Garni-Wagner BA, Purohit A, Mathew PA, Bennett M, Kumar V. A novel function-associated molecule related to non-MHC-restricted cytotoxicity mediated by activated natural killer cells and T cells. *J Immunol.* (1993) 151:60–70.
76. Sentman CL, Hackett J Jr, Moore TA, Tutt MM, Bennett M, Kumar V. Pan natural killer cell monoclonal antibodies and their relationship to the NK1.1 antigen. *Hybridoma.* (1989) 8:605–14. doi: 10.1089/hyb.1989.8.605
77. Love-Schimenti CD, Kripke ML. Inhibitory effect of a dendritic epidermal T cell line on K1735 melanoma cells *in vivo* and *in vitro*. *J Leukoc Biol.* (1994) 55:379–84. doi: 10.1002/jlb.55.3.379
78. Champsaur M, Lanier LL. Effect of NKG2D ligand expression on host immune responses. *Immunol Rev.* (2010) 235:267–85. doi: 10.1111/j.0105-2896.2010.00893.x

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Get in Touch With Dendritic Epithelial T Cells!

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Innate and adaptive immune systems continuously interchange information and orchestrate their immune responses to protect the host. γδT cells play crucial roles, as they incorporate both innate and adaptive immune characteristics. Dendritic epidermal T cells (DETC) are specialized γδT cells, which are uniquely positioned to rapidly respond to skin wounds and infections. Their elongated dendrite morphology allows them to be in continuous contact with multiple neighboring keratinocytes and Langerhans cells. Cellular interactions are fundamental to the formation, activation and maintenance of immune cell functions during steady state and pathology. Recent technological advances, especially in the field of cellular imaging, have contributed greatly to the characterization of complex cellular interactions in a spatiotemporally resolved manner. In this review, we will highlight the often-underappreciated function of DETC and other γδT cells during steady state and an ongoing immune response. More specifically, we discuss how DETC-precursors are shaped in the fetal thymus during embryogenesis as well as how direct cell-cell interactions of DETC with neighboring epidermal cells shape skin homeostasis and effector functions. Furthermore, we will discuss seminal work and recent discoveries made in the γδT cell field, which have highlighted the importance of γδT cells in the skin, both in humans and mice.

Keywords: epithelial, DETC, γδT cell, activation, costimulation, epidermis

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INTRODUCTION

T cell immune responses are tightly connected to their ability to circulate and migrate into tissues, as their priming and effector function is dependent on direct cell-cell interactions. In the last two decades, advancements in imaging techniques, such as two-photon microscopy, have shed light on the complex processes involved in αβT cell priming, effector differentiation and function (1–10). Interestingly, only a handful of studies have used these imaging techniques to study the immune function of γδT cells (11–17). These studies have shown remarkable differences in morphology and migratory behavior of γδT cells residing in different tissues. For example, γδT cells in lymph nodes migrate vigorously (11, 16) in contrast to the slowly migrating γδT cells in the gut parenchyma (14), whereas γδT cells in the epidermis are firmly sessile (15, 18). Moreover, while dermal γδT cells continuously migrate and home to draining lymph node, γδT cells in the epidermis do not recirculate, at least not during steady state conditions (12, 13). Here we describe how γδT cells in the epidermis are formed and, despite their immotile nature, perform their essential guardian function in the biggest barrier tissue. In this review we will focus on the murine epidermal γδT cells, named dendritic epidermal T cells (DETC), which perform essential homeostatic functions and are pivotal for sounding the alarm during an epidermal barrier breach.

DETC SELECTION AND SEEDING OF EPIDERMIS

Although in low numbers in secondary lymphoid organs and circulation, γδT cells in both humans and in rodents are concentrated in peripheral organs, such as the digestive tract, lungs or skin (19–21). Further, γδT cells differ from conventional αβT cells, due to their restricted T cell receptor (TCR) diversity (22). Interestingly, when looking at the potential combinations of the variable (V), diversity (D) and joining (J) segments, the γδTCR diversity is significantly higher than both the B cell receptor (BCR) and αβTCR with 10^{20} potential combinatorial diversities, in comparison to 10^{11} and 10^{15}, for BCR and αβTCR, respectively (23, 24). Remarkably, the TCR repertoire effectively expressed by γδT cells is greatly limited, with some oligoclonal γδT cell subsets dominating in certain organs. Indeed, γδT cells in mouse epidermis express a very distinct TCR, with most if not all expressing Vγ3-Jγ1-Cγ1/Vδ1-Dδ2-Jδ2-Cδ, Garman nomenclature (25–27). It is worth noting that human tissue-resident γδT cells, at least in skin, gut, and liver, also express a restricted TCR, which is characterized by expression of Vδ1 and distinct from their largely Vδ2-expresssing circulatory counterparts (28, 29). Analysis of the γδTCR structure has revealed a close resemblance to the BCR structure, suggesting the possibility that the γδTCR recognizes antigen directly without the need for MHC-processing and presentation (30). In fact, most γδT cells form normally in beta2-microglobulin knockout mice, which lack MHC-I expression. Further, direct γδTCR binding to pathogen-derived antigens, as well as phospho-antigen, have been reported (31–34). Although in this review we will discuss γδTCR ligands and signaling in the context of epidermal γδT cell development and function, we will not further discuss antigen recognition by the γδTCR, as this has been reviewed recently (35).

DETC are specialized murine epidermis-resident T cells that express the monoclonal Vγ3Vδ1 TCR. DETC precursors seed the epidermis during days 14 through 18 of gestation (20, 26). It was initially thought that since DETC are highly restricted in their TCR usage, Vγ3Vδ1 expression was essential for skin homing. This hypothesis was later rejected as multiple studies have reported DETC formation in different transgenic mouse models, which utilize an array of different Vγ and Vδ gene combinations. Indeed, both Vγ3 and Vδ1 knockout mice develop functional DETC in the epidermis (36, 37). Interestingly, in TCRδ knockout mice, which lack all γδT cells, the epidermis is colonized by αβTCR-expressing DETC (αβDETC), at similar numbers and morphology to conventional DETC. However, αβDETC show reduced functionality and their frequency declines in adulthood (38). Importantly, seeding and colonization of the epidermal niche is restricted to the time from late embryogenesis until a few days after birth, as conditional depletion of DETC in adult mice leads to permanent absence of epidermal T cells, both of γδ and αβTCR origins (39).

Early on, it became clear that γδTCR signaling was essential for DETC development and maintenance, as deletion of molecules involved in TCR signaling, such as ZAP-70, LAT, Syk, or Lck resulted in a significant reduction of DETC (40–43). Indeed, strong TCR signaling in combination with a weak Notch signal is essential for γδT cell linage commitment, whereas low TCR signals together with a robust and sustained Notch signal favor αβT cell linage commitment (44–48). It is noteworthy however, that absence of a Notch signal is not enough to drive γδT cell lineage commitment and that other cellular interactions in the thymus guide γδT cell differentiation (49). A curious observation in a specific FVB mouse strain, shed light on additional factors which drive early stages of DETC formation in the fetal thymus. FVB mice purchased from Taconic (FVB-TAC), but not from Jackson (FVB-JAX), lacked the canonical Vγ3Vδ1 DETC in the epidermis of adult mice. Remarkably, Vγ3Vδ1 DETC precursors were still present in the fetal thymus of these mice yet were found to lack key markers for maturation and skin homing, suggesting a defect in the intrathymic differentiation of these cells (50). A later study pinpointed the defect in DETC formation in FVB-TAC mice to a mutation in the Skint1 gene (selection and upkeep of intraepithelial cells 1) (51). Skint1 is highly expressed in the mouse fetal thymus and in keratinocytes (KC) and overexpression of Skint1 in FVB-TAC mice rescues Vγ3Vδ1 DETC progenitor differentiation (51, 52). However, direct binding of Skint1 to the Vγ3Vδ1 TCR has not been demonstrated. Nonetheless it has been shown that interactions between Skint1^{+} thymic cells and Vγ3Vδ1 cells are essential for imprinting a DETC progenitor phenotype. Indeed, these interactions favor a commitment toward an IFNγ-producing γδT cell fate through the Egr-3 pathway, which inhibits expression of transcription factors favoring IL-17-producing γδT cells, commonly found in the gut lamina propria (53, 54). Interestingly, it became clear that direct TCR signaling is essential to induce such DETC progenitor phenotype as well as to promote the expression of skin homing markers. In fact, TCR signaling induces upregulation of S1PR, CCR10, E-P selectins, and CCR4 (55, 56), as well as upregulation of the transcription factor T-bet (53). The upregulation of these surface markers allows DETC progenitors to egress the thymus via S1PR signaling and to home to the epidermis using the adhesion molecules P- and E-selectin and following the CCR10 ligand CCL27 expressed by KC (55–58). In summary, current evidence suggests that DETC selection, in contrast to the well-described negative selection of αβT cells, is poised for strong self-TCR signaling which promotes expression of skin homing molecules. In humans, the Skint1-like gene is inactive due to multiple premature stop codons (51), therefore, it remains still unknown what requirements are necessary for intrathymic selection and imprinting of human epidermal γδT cell populations. Furthermore, members of the butyrophilin family, the gene family in humans most homologous to the Skints, have been shown to play essential roles in activating circulatory and gut resident γδT cells (33, 34, 59, 60). Therefore, it is possible that a butyrophilin family member could play similar functions as Skint1 for skin γδT cells in humans. However, such equivalent has not yet been described and will need further investigation.

Interestingly, formation and seeding of mouse gut resident CCR9$^+$ Vγ5$^+$ γδT cells seems to be remarkably biased toward thymocytes that are phenotypically antigen-naïve (61). The distinct TCR-signaling selection of Vγ5$^+$ and Vγ3$^+$ γδT cells suggest that their TCR-dependent activation in their respective peripheral organs may also be functionally different. Indeed, Vγ5$^+$ gut γδT cells are poised to produce IL-17 upon activation, whereas DETC are potent IFNγ producers. However, precisely how this difference in intrathymic selection affects their effector function remains to be investigated.

TCR SIGNALING IN THE EPIDERMIS

The epidermis is the most outer layer of the skin, protecting the organism from environmental elements. The epidermal tissue architecture is orchestrated by differentiating KC, with the top layer containing enucleated KC (stratum corneum), followed by three layers of KC which express tight junction molecules, such as ZO-1 and Claudin-1 (62–64), referred to as stratum granulosum. Under the stratum granulosum are located the stratum spinosum and the basal layer, which are bound to the thick and complex basement membrane that separates the epidermis from the dermis. Immune cells, such as γδT cells, Langerhans cells (LC) and tissue resident memory (TRM) CD8$^+$ T cells are situated in the basal layer and stratum spinosum. DETC are the exclusive T cell subset in the epidermis of naïve rodents (65), whereas in humans, both αβT cells and γδT cells make up the T cell compartment of the epidermis, and both T cell subsets seem to have somewhat parallel functions to mouse DETC (66). As the name indicates, DETC are characterized by their arborized and elongated cell body (67), which remarkably resembles LC morphology. In contrast, CD8$^+$ TRM, which have elongated cell bodies in comparison to T cells in lymph nodes, form significantly less cellular protrusion than DETC in the epidermis (15). Notably, whereas CD8$^+$ TRM retain migratory capability in the epidermis at steady state, both DETC and LC are mainly sessile and only slowly and seldomly retract and extent their protrusions (15, 18). The elongated dendrites of both DETC and LC are orientated upwards toward the stratum granulosum (18, 64, 68) where they interact with KC tight junctions. Moreover, DETC protrusions are simultaneously in stable contact with multiple KC and LC (**Figure 1**). This is in stark contrast with CD8$^+$ TRM dendrites which are continuously contracting and extending, protruding laterally between KC in the basal layer probing the surrounding cells for cognate antigen (69). The highly stable DETC-KC and LC-KC contacts at steady state elude to the continuous monitoring function of these cells, ensuring barrier integrity. Unexpectedly, it has been shown that the γδTCR, together with the integrins CD103 and LFA1, is highly enriched at the tips of the dendrites and actively engages at tight junctions of KC (**Figure 2**). Indeed, constitutive γδTCR signaling during steady state has been reported in DETC by staining for phospho-tyrosine 142 of the CD3ζ chain (18). This constitutive TCR-activation seen in Vγ3Vδ1 DETC, but not in naturally occurring Vγ3^{neg}- or αβDETC, suggests that self-antigen specific interaction is occurring during steady state. The study from Minagawa et al. (70), further strengthens this idea, showing that during early embryonic development Vγ3$^+$ cells can also be found in the gut epithelia, yet only the skin epidermal Vγ3$^+$ cells persist in newborn mice suggesting Vγ3Vδ1 self-antigen recognition in epidermis promotes DETC persistence. Nevertheless, it is important to highlight that a DETC-specific antigen could not be detected by Vγ3Vδ1-tetramer staining in resting skin, whereas KC-derived antigen was readily detected near skin wound edges (71), suggesting antigen upregulation following KC damage. However, lack of DETC-tetramer staining at steady state, could be explained by low antigen abundance and/or accessibility in non-wounded skin.

Several studies have shown the paramount importance of TCR signaling for DETC formation, maintenance, self-renewal and activation upon barrier disruption (38, 40–43, 50). For instance, deficiency in LAT, a linker protein for TCR signaling, inhibits DETC proliferation during neonatal epidermal colonization (72). Further, LAT conditional depletion in adult mice, reduced DETC homeostatic proliferation, underlining the importance of constant TCR engagement for DETC self-renewal (72). Studies using transgenic KN6 mice, expressing the Vγ2Vδ7, further expand on the hypothesis of a requirement for balanced TCR signaling supporting DETC maintenance and survival. The KN6 γδTCR has been shown to recognize two non-classical MHC-I molecules T10 and T22, which are highly expressed in the skin (73). These mice possess similar numbers of DETC at a young age (74), however their numbers decrease over time (58). The authors argue that the defect in KN6 DETC maintenance is caused by sustained strong TCR stimulation, which leads to excessive DETC activation in the epidermis. Indeed, KN6 DETC numbers were rescued in KN6 mice deficient for interleukin-2-inducible T-cell kinase (ITK), a TEC family kinase involved in TCR signaling. Interestingly, polyclonal ITK knockout mice showed a decrease in DETC numbers, which was linked to shortcomings of positive selection in fetal thymus and failure to upregulate skin homing markers in DETC progenitors (58). In sum, the DETC TCR appears to be constitutively active at low levels during steady state, promoting a basal level of DETC activation essential for their maintenance. Yet, this appears to be below a threshold required for full activation and effector function of DETC.

DETC RESIDENCY AND HOMEOSTATIC FUNCTION IN EPIDERMIS

Lifelong persistence of DETC and their remarkable uniform distribution in the epidermis, indicates their ability to sense and fill empty spaces via proliferation. The self-renewal mechanism is partially regulated by TCR signaling, as discussed above (58, 72, 75), as well as by soluble factors, such as IL-15 produced by surrounding KC (76) (**Figure 1**). Defects in IL-15 downstream signaling in mice lacking JAK3 or STAT5, have clearly shown

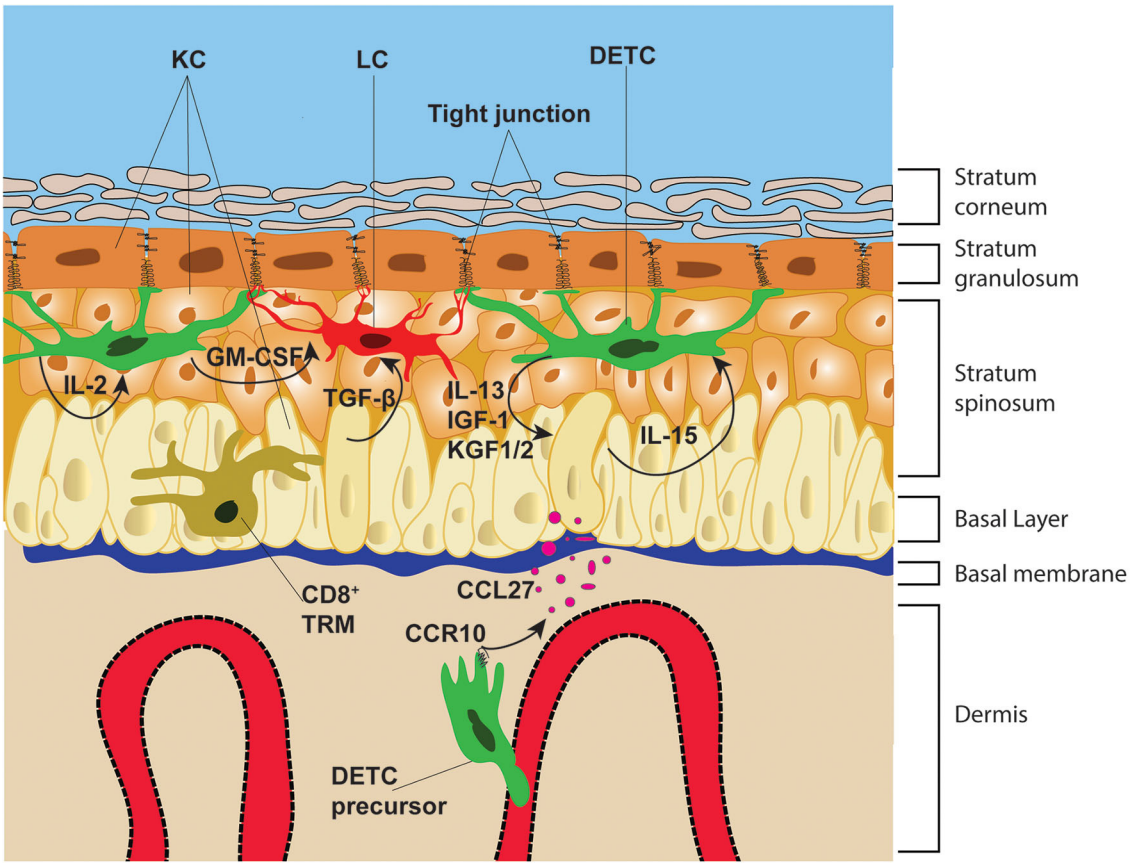

FIGURE 1 | Epithelial architecture and immune cell distribution. The epidermis is divided from the dermis by the basal membrane. The epidermis is composed of four distinct layers: the stratum corneum, stratum granulosum, stratum spinosum and basal layer. These layers are composed mainly of KC that are infiltrated with immune cells, such as DETC, LC, and $CD8^+$ TRM. DETC precursors in neonatal mice, home to the epidermis via the blood vessels and following the chemotactic gradient of KC-derived CCL27. DETC and LC form stable interactions with KC tight junctions, whereas $CD8^+$ TRM migrate along the basal layer and in-between KC. DETC produce multiple soluble factors that promote KC survival and proliferation and receive IL-15 from KC, which promotes their lifelong survival and self-renewal.

the pivotal role of IL-15 in promoting DETC proliferation and survival (77, 78). These signals seem to be particularly important during the early expansion of DETC precursors in newborn mice. Indeed, early studies suggested that DETC precursors quickly proliferate in neonatal epidermis and expand laterally until covering the entire epidermal surface (67). This has been recently further characterized, utilizing multicolor fate mapping, illustrating how founder DETC, which seed the epidermis during embryogenesis, proliferate laterally forming clusters of clones (79). The expression of the stem-cell marker c-Kit has been linked to the early expansion of DETC founders in neonatal skin. Mice lacking the expression of the Aryl hydrocarbon receptor (Ahr) show reduced expression of c-Kit in newborn epidermal DETC. $Ahr^{-/-}$ DETC fail to expand and colonize the epidermis, leading to complete loss of DETC in adult mice. In adult $Ahr^{-/-}$ animals, the epidermis is not occupied by αβDETC likely due to the restricted temporal seeding window as aforementioned (80). Performing longitudinal imaging, the process of self-renewal has been visualized *in vivo*, DETC sense empty spaces in the surrounding tissue, by mechanisms that are not yet fully understood, and quickly proliferate. The daughter cell actively migrates in a directional fashion toward the empty niche and establishes residency (18). Similarly, DETC surrounding a skin graft show slow expansion and directional immigration into grafted tissue (79). It would be interesting to further study the colonization of host DETC into skin grafts that are depleted of donor DETC and see if their immigration is accelerated compared to non-depleted donor skin grafts, indicative of their ability to sense empty niches. Moreover, chemical inhibitors or genetic modification of donor skin could be used to pinpoint factors promoting DETC proliferation and lateral expansion. These findings could reveal new therapeutic approaches for improving treatment of chronic wounds, which are prevalent in diabetic and obese patients.

It has become clear that metabolic alteration, both in humans and mice, can have detrimental effects on skin regeneration, which can lead to chronic non-healing wounds and life-threatening complications (81–84). Interestingly, epidermal γδT

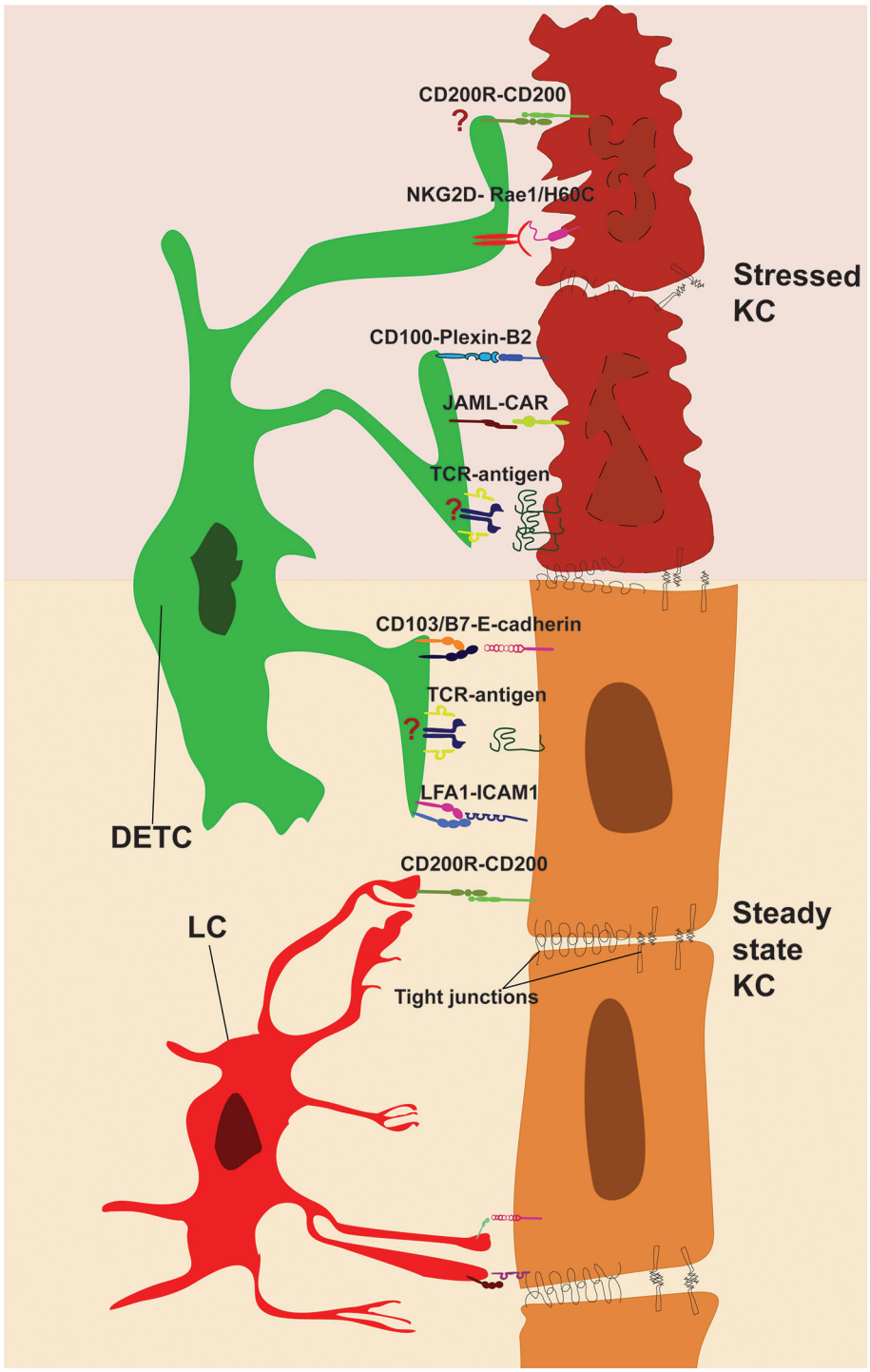

FIGURE 2 | Direct cell-cell contacts regulating immune surveillance of epidermis. DETC and KC form stable interactions during steady state (bottom). TCR and adhesion molecules LFA-1 and CD103 are enriched and engaged with KC-derived ligands at tips of DETC protrusions. LC-KC interaction via CD200R-CD200 suppresses LC activation at steady state. Stressed KC (top) upregulates costimulatory ligands and DETC-specific antigens, which activate DETC via JAML, CD100, NKG2D, and TCR. However, further research is needed to pinpoint the role of antigen presentation and CD200-CD200R signaling in shaping DETC effector functions (indicated by "?").

cell numbers and functions are decreased in these conditions. For example, hyperglycemia inhibits DETC proliferation as well as their responsiveness to skin injuries, which is linked to the reduced activity of the master regulator mTOR as well as the lower level of active phosphorylated STAT5 (85). Similar defects of DETC functions have been described in

mice receiving prolonged treatment with the mTOR inhibitor rapamycin (86). Rapamycin dampens the production of IL-15 in KC, which leads to a decrease in DETC numbers and in their production of the KC growth factor IGF-1. Local administration of either IL-15 or IGF-1 can rescue DETC proliferation and functions, promoting wound healing (86–88). Recent, studies have further shown that nutrition can have a significant impact on γδT cell functions and proliferation. A ketogenic diet, which consists of a low carbohydrate and high fat content diet, boosts γδT cell proliferation in lung, which can be beneficial during influenza infection (89). However, prolonged non-caloric-restricted ketogenic diet causes weight gain and obesity in mice, reversing the beneficial effects on γδT cells and augmenting inflammation (90). Exactly how diet and its metabolites can modulate γδT cell proliferation, and if these factors affect all γδT cell subsets in the body, is still unknown. Further, it will be interesting to investigate if caloric restriction in ketogenic diet could promote γδT cell function in the long term and if this could be beneficial in certain disease models, such as wound healing, inflammatory bowel diseases and cancer.

COSTIMULATION AND DIRECT CELL-CELL INTERACTIONS

Epidermal γδT cell activation during skin injury is important to regulate immune responses and favors rapid wound healing both in humans and mice (66, 91). As mentioned above, DETC-tetramer staining in wounded epidermis detects rapid appearance of a KC-derived DETC-specific antigen (71). Antigen recognition and TCR-induced activation of DETC seem to be important for wound healing processes, since αβDETC in TCRδ$^{-/-}$ animals do not sense or respond to KC-derived antigen, which leads to a delay in wound healing (91). It is noteworthy that, although direct binding to the TCR has not been reported, KC at wound edges express multiple members of the Skint family. Decreased expression of Skint family members in aged mice has been linked to reduced STAT3-phosphorylation compared to young mice. Selective downregulation of STAT3 or Skint genes affects DETC activation and delays wound healing (92), suggesting that Skint family members play not only important roles in regulating DETC selection in fetal thymus but are also involved in DETC activation in epidermis.

It has been suggested that DETC positive selection in the thymus ensures that DETC have a high activation threshold in the epidermis. Indeed, like what has been described for αβT cells, DETC activation is not solely due to TCR signaling but requires synergy with costimulatory receptors. Stressed KC at wound edges upregulate multiple surface molecules that can promote DETC activation. Semaphorin 4D (also known as CD100) is a receptor for Plexin-B2, Plexin-B1, and CD72 and it is expressed by multiple immune cells, including DETC. PlexinB2-CD100 interactions were originally described as providing axon-guidance cues, yet ligation of CD100 and its role outside the nervous system are now evident (93, 94). During skin injury, Plexin-B2 is rapidly upregulated and expressed on the surface of KC at wound edges and its interaction with CD100 on DETC induces bidirectional signaling. Activation via CD100 induces DETC rounding and secretion of IL-2. Indeed, CD100-deficient mice show delays in wound healing (95). Furthermore, subcutaneous injection of soluble CD100 promotes wound healing in a diabetic mouse model (96). On the other end, CD100 interaction with Plexin-B2 promotes the NF-κB signaling pathway in KC, which leads to secretion of pro-inflammatory molecules, such as IL-1β. Knockdown experiments have shown that inhibiting Plexin-B2 expression in KC can dampen inflammation and reduce pathogenesis in a murine psoriasis model (97). Similarly, CD100-induced inflammation has been linked to the promotion of contact hypersensitivity (CHS), caused by excess immune cell infiltration (94). Thus, CD100 is a strong costimulatory receptor that facilitates DETC activation, leads to DETC rounding and promotes inflammatory immune responses.

Similar upregulation of stress molecules in KC have been reported for the NKG2D ligands (**Figure 2**), such as retinoic acid early-inducible 1 (Rae-1) isoforms, murine UL-16 binding protein-like transcript 1 (Mult1) and histocompatibility 60 (H60). These ligands are absent or expressed at low level at steady state and become upregulated in stressed, chemically irritated cells and following DNA damage (98–100). Indeed, skin exposure to carcinogens induces H60C expression in KC and leads to NKG2D-dependent DETC activation (101). Similarly, 2B4, a non-MHC-dependent surveillance receptor on DETC, has been reported to prevent cancer formation by lysis of transformed cells (102). Additionally, Strid et al. (103) found that overexpression of Rae1 specifically on KC, was sufficient to induce NKG2D-dependent DETC activation and secretion of IL-2 and IL-13. Interestingly, LC which do not express NKG2D, were activated during the same timeframe, suggesting a rapid DETC-dependent tissue-wide state of alert. Indeed, activated DETC produce a variety of inflammatory molecules, such as CCL5 (also known as RANTES), which is a potent activator of dendritic cells (104, 105). NKG2D-H60C interaction has been shown to promote wound healing, as blocking antibodies against H60C reduced DETC rounding and delay wound healing (106).

Finally, junctional adhesion molecule JAML which binds to the Coxsackie and adenovirus receptor (CAR) has been identified as a potent costimulatory receptor of DETC and γδ intraepithelial lymphocytes (IEL) in the gut. JAML expression is upregulated in DETC upon activation and its ligation to CAR induces recruitment of PI3K to the intracellular domain of JAML, as delineated for the αβT cell costimulatory receptor CD28 (107). Potent costimulation via JAML could be detected in DETC and γδ IEL but not in circulating γδT cells, further underlining the tissue-specific adaptation of γδT cells. Furthermore, blocking CAR-JAML interactions delays wound healing at a rate comparable to that of TCRδ knockout mice (108). To equilibrate the effects of costimulatory molecules, KC express constitutively CD200. Its receptor CD200R is a potent inhibitor of LC activation, which prevents autoimmune reactions during steady state (109). Interestingly DETC upregulate CD200R expression upon activation, however it is yet to be elucidated what inhibitory function, if any, CD200R may play in the context of wound healing.

As previously mentioned, DETC activation is characterized by rounding and loss of arborized morphology in these cells, which has been suggested to allow DETC migration as evidence suggests they accumulate at the wound edge (91). Supporting this hypothesis, activated DETC downregulate adhesion molecules, such as CD103 and E-cadherin, which have been linked to cell residency both in DETC and TRM, as well as promoting LC egress (38, 69, 110–113). In contrast, Occludin, a transmembrane enzyme normally found at tight junctions, is upregulated in activated DETC. Its expression has been associated with DETC rounding and with the ability of DETC to egress and home to draining lymph nodes (114). Similarly, DETC migration to, and accumulation in, skin draining lymph nodes has been reported in a CHS study (115). However, these studies did not directly visualize DETC migration, therefore it is yet to be directly demonstrated whether, upon activation, DETC gain cell motility and actively migrate toward the draining lymph node or wound edge.

DETC FUNCTION UPON ACTIVATION

As aforementioned, DETC TCRs are continuously engaged. This basal activation allows DETC to secrete low-levels of the IL-2 family member IL-13 at steady state. IL-13 promotes homeostatic KC proliferation and survival, which is disrupted in IL-13-deficient mice, causing increased susceptibility to carcinomas (116). In fact, it has become clear that both in humans and mice, γδT cells play an important part in regulating homeostatic functions of organs as well as in promoting tissue repair upon injury (66, 91, 117, 118). DETC produce a multitude of cytokines and growth factors, that modulate neighboring cell functions as well as stimulating themselves via autocrine signaling. For instance, KGF-1 and KGF-2 (also known as FGF-7 and -10), produced by DETC, promote KC proliferation, maturation and migration. Furthermore, DETC-derived KGF-1 and -2 induce epithelial cell production and secretion of hyaluronan, which significantly augments neutrophil and macrophage infiltration at skin wounds (119). The secretion of these growth hormones by activated DETC is essential for rapid wound healing (91, 120). Insulin-like growth factor (IGF-1) is another important soluble factor produced by DETC during steady state (121) as IGF-1 regulates KC development and maintenance (122). Further, IL-15 secreted by KC promotes IGF-1 production by DETCs, generating a positive feedback loop (88) (**Figure 1**). Interestingly, although in humans there is not a direct counterpart of DETC, human epidermal γδ and αβ T cells also produce IGF-1, supporting wound healing. In chronic wounds this beneficial production of IGF-1 is absent. Indeed, isolated γδ and αβ epidermal T cells from such wounds are completely unresponsive to stimulation, indicative of their essential function in regulating wound-healing and resembling the functions of mouse DETC (66). Finally, DETC are the main source of GM-CSF in the epidermis, which has been shown to promote LC maturation (123). Indeed, in Ahr-deficient mice which lack DETC, GM-CSF levels in the skin were reduced causing defects in LC maturation and activation (80, 124). Interestingly, although DETC are developmentally poised to produce IFNγ (53), a subset of DETC produce IL-17a, which is essential for rapid wound healing (125). Whether this IL-17 production ability is also imprinted in the thymus or is a product of the spatiotemporal activation of this population of DETC is yet to be determined.

CONCLUSIONS

DETC are strategically positioned to survey and rapidly respond to a pathogenic insult or a mechanical disruption of the barrier tissue. Their characteristic morphology, as well as their static migratory behavior, are indicative of their continuous surveillance function. In fact, their importance in sensing and responding to skin injuries has been reported both in humans and mice (66, 91). Although in recent years significant advancements have been made in understanding DETC biology, many aspects on how their direct cell-cell contact with neighboring cells regulates their homeostatic function and allows for rapid activation upon injury, remain to be elucidated.

We would like to dedicate this review to Professor Wendy Havran, who was a pioneer of the DETC research field, and we had the pleasure to work with. The sad and sudden loss of this wonderful immunologist and mentor has shocked the γδT cell field. Her seminal work, such as describing the unique developmental waves of DETC (20) and their importance in mediating wound healing (91), has sparked worldwide interest and research in these unconventional T cells. We know that her legacy has and will further fuel new discoveries in this exciting research field.

AUTHOR CONTRIBUTIONS

FT and DW wrote the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Bousso P, Robey E. Dynamics of $CD8^+$ T cell priming by dendritic cells in intact lymph nodes. *Nat Immunol.* (2003) 4:579–85. doi: 10.1038/ni928
2. Stein JV, Soriano SF, M'rini C, Nombela-Arrieta C, de Buitrago GG, Rodríguez-Frade JM, et al. CCR7-mediated physiological lymphocyte homing involves activation of a tyrosine kinase pathway. *Blood.* (2003) 101:38–44. doi: 10.1182/blood-2002-03-0841
3. Luster AD, Alon R, von Andrian UH. Immune cell migration in inflammation: present and future therapeutic targets. *Nat Immunol.* (2005) 6:1182–90. doi: 10.1038/ni1275

4. Worbs T, Bernhardt G, Förster R. Factors governing the intranodal migration behavior of T lymphocytes. *Immunol Rev*. (2008) 221:44–63. doi: 10.1111/j.1600-065X.2008.00580.x
5. Harris TH, Banigan EJ, Christian DA, Konradt C, Tait Wojno ED, Norose K, et al. Generalized Lévy walks and the role of chemokines in migration of effector CD8$^+$ T cells. *Nature*. (2012) 486:545–8. doi: 10.1038/nature11098
6. Henrickson SE, Perro M, Loughhead SM, Senman B, Stutte S, Quigley M, et al. Antigen availability determines CD8$^+$ T cell-dendritic cell interaction kinetics and memory fate decisions. *Immunity*. (2013) 39:496–507. doi: 10.1016/j.immuni.2013.08.034
7. Lämmermann T, Afonso PV, Angermann BR, Wang JM, Kastenmüller W, Parent CA, et al. Neutrophil swarms require LTB4 and integrins at sites of cell death *in vivo*. *Nature*. (2013) 498:371–5. doi: 10.1038/nature12175
8. Guidotti LG, Inverso D, Sironi L, Di Lucia P, Fioravanti J, Ganzer L, et al. Immunosurveillance of the liver by intravascular effector CD8(+) T cells. *Cell*. (2015) 161:486–500. doi: 10.1016/j.cell.2015.03.005
9. Ozga AJ, Moalli F, Abe J, Swoger J, Sharpe J, Zehn D, et al. pMHC affinity controls duration of CD8$^+$ T cell–DC interactions and imprints timing of effector differentiation versus expansion. *J Exp Med*. (2016) 413:2811–29. doi: 10.1084/jem.20160206
10. Stolp B, Thelen F, Ficht X, Altenburger LM, Ruef N, Inavalli VVGK, et al. Salivary gland macrophages and tissue-resident CD8$^+$ T cells cooperate for homeostatic organ surveillance. *Sci Immunol*. (2020) 5:eaaz4371. doi: 10.1126/sciimmunol.aaz4371
11. Chennupati V, Worbs T, Liu X, Malinarich FH, Schmitz S, Haas JD, et al. Intra- and intercompartmental movement of γδ T cells: intestinal intraepithelial and peripheral γδ T cells represent exclusive nonoverlapping populations with distinct migration characteristics. *J Immunol*. (2010) 185:5160–8. doi: 10.4049/jimmunol.1001652
12. Gray EE, Suzuki K, Cyster JG. Cutting edge: Identification of a motile IL-17-producing gammadelta T cell population in the dermis. *J Immunol*. (2011) 186:6091–5. doi: 10.4049/jimmunol.1100427
13. Sumaria N, Roediger B, Ng LG, Qin J, Pinto R, Cavanagh LL, et al. Cutaneous immunosurveillance by self-renewing dermal γδ T cells. *J Exp Med*. (2011) 208:505–18. doi: 10.1084/jem.20101824
14. Edelblum KL, Shen L, Weber CR, Marchiando AM, Clay BS, Wang Y, et al. Dynamic migration of γδ intraepithelial lymphocytes requires occludin. *Proc Natl Acad Sci USA*. (2012) 109:7097–102. doi: 10.1073/pnas.1112519109
15. Zaid A, Mackay LK, Rahimpour A, Braun A, Veldhoen M, Carbone FR, et al. Persistence of skin-resident memory T cells within an epidermal niche. *Proc Natl Acad Sci USA*. (2014) 111:5307–12. doi: 10.1073/pnas.1322292111
16. Romagnoli PA, Sheridan BS, Pham QM, Lefrançois L, Khanna KM. IL-17A-producing resident memory γδ T cells orchestrate the innate immune response to secondary oral Listeria monocytogenes infection. *Proc Natl Acad Sci USA*. (2016) 113:8502–7. doi: 10.1073/pnas.1600713113
17. Chodaczek G, Toporkiewicz M, Zal MA, Zal T. Epidermal T cell dendrites serve as conduits for bidirectional trafficking of granular cargo. *Front Immunol*. (2018) 9:1430. doi: 10.3389/fimmu.2018.01430
18. Chodaczek G, Papanna V, Zal MA, Zal T. Body-barrier surveillance by epidermal γδ TCRs. *Nat Immunol*. (2012) 13:272–82. doi: 10.1038/ni.2240
19. Goodman T, Lefrancois L. Intraepithelial lymphocytes. Anatomical site, not T cell receptor form, dictates phenotype and function. *J Exp Med*. (1989) 170:1569–81. doi: 10.1084/jem.170.5.1569
20. Havran WL, Allison JP. Origin of Thy-1$^+$ dendritic epidermal cells of adult mice from fetal thymic precursors. *Nature*. (1990) 344:68–70. doi: 10.1038/344068a0
21. Lahn M. The role of gammadelta T cells in the airways. *J Mol Med*. (2000) 78:409–25. doi: 10.1007/s001090000123
22. Vantourout P, Hayday A. Six-of-the-best: unique contributions of γδ T cells to immunology. *Nat Rev Immunol*. (2013) 13:88–100. doi: 10.1038/nri3384
23. Carding SR, Egan PJ. γδ T cells: Functional plasticity and heterogeneity. *Nat Rev Immunol*. (2002) 2:336–45. doi: 10.1038/nri797
24. Hahn AM, Winkler TH. Resolving the mystery—how TCR transgenic mouse models shed light on the elusive case of gamma delta T cells. *J Leukoc Biol*. (2020) 107:993–1007. doi: 10.1002/JLB.1MR0120-237R
25. Garman RD, Doherty PJ, Raulet DH. Diversity, rearrangement, and expression of murine T cell gamma genes. *Cell*. (1986) 45:733–42. doi: 10.1016/0092-8674(86)90787-7
26. Havran WL, Allison JP. Developmentally ordered appearance of thymocytes expressing different T-cell antigen receptors. *Nature*. (1988) 335:443–5. doi: 10.1038/335443a0
27. Havran WL, Grell S, Duwe G, Kimura J, Wilson A, Kruisbeek AM, et al. Limited diversity of T-cell receptor γ-chain expression of murine Thy-1$^+$ dendritic epidermal cells revealed by V(γ)3-specific monoclonal antibody. *Proc Natl Acad Sci USA*. (1989) 86:4185–9. doi: 10.1073/pnas.86.11.4185
28. Holtmeier W, Pfänder M, Hennemann A, Zollner TM, Kaufmann R, Caspary WF. The TCR δ repertoire in normal human skin is restricted and distinct from the TCR δ repertoire in the peripheral blood. *J Invest Dermatol*. (2001) 116:275–80. doi: 10.1046/j.1523-1747.2001.01250.x
29. Bonneville M, O'Brien RL, Born WK. γδ T cell effector functions: a blend of innate programming and acquired plasticity. *Nat Rev Immunol*. (2010) 10:467–78. doi: 10.1038/nri2781
30. Chien Y, Meyer C, Bonneville M. γδ T cells: first line of defense and beyond. *Annu Rev Immunol*. (2014) 32:121–55. doi: 10.1146/annurev-immunol-032713-120216
31. Correa I, Bix M, Liao NS, Zijlstra M, Jaenisch R, Raulet D. Most γδ T cells develop normally in β2-microglobulin-deficient mice. *Proc Natl Acad Sci USA*. (1992) 89:653–7. doi: 10.1073/pnas.89.2.653
32. Morita CT, Jin C, Sarikonda G, Wang H. Nonpeptide antigens, presentation mechanisms, and immunological memory of human Vγ2Vδ2 T cells: discriminating friend from foe through the recognition of prenyl pyrophosphate antigens. *Immunol Rev*. (2007) 215:59–76. doi: 10.1111/j.1600-065X.2006.00479.x
33. Harly C, Guillaume Y, Nedellec S, Peigné CM, Mönkkönen H, Mönkkönen J, et al. Key implication of CD277/butyrophilin-3 (BTN3A) in cellular stress sensing by a major human γδ T-cell subset. *Blood*. (2012) 120:2269–79. doi: 10.1182/blood-2012-05-430470
34. Willcox CR, Vantourout P, Salim M, Zlatareva I, Melandri D, Zanardo L, et al. Butyrophilin-like 3 directly binds a human Vγ4$^+$ T cell receptor using a modality distinct from clonally-restricted antigen. *Immunity*. (2019) 51:813–25.e4. doi: 10.1016/j.immuni.2019.09.006
35. Hayday AC, Vantourout P. The innate biologies of adaptive antigen receptors. *Annu Rev Immunol*. (2020) 38:487–510. doi: 10.1146/annurev-immunol-102819-023144
36. Mallick-Wood CA, Lewis JM, Richie LI, Owen MJ, Tigelaar RE, Hayday AC. Conservation of T cell receptor conformation in epidermal γδ cells with disrupted primary V(γ) gene usage. *Science*. (1998) 279:1729–33. doi: 10.1126/science.279.5357.1729
37. Hara H, Kishihara K, Matsuzaki G, Takimoto H, Tsukiyama T, Tigelaar RE, et al. Development of dendritic epidermal T Cells With A Skewed Diversity of γδTCRs in Vδ1-deficient mice. *J Immunol*. (2000) 165:3695–705. doi: 10.4049/jimmunol.165.7.3695
38. Jameson JM, Cauvi G, Witherden DA, Havran WL. A keratinocyte-responsive gamma delta TCR is necessary for dendritic epidermal T cell activation by damaged keratinocytes and maintenance in the epidermis. *J Immunol*. (2004) 172:3573–9. doi: 10.4049/jimmunol.172.6.3573
39. Sandrock I, Reinhardt A, Ravens S, Binz C, Wilharm A, Martins J, et al. Genetic models reveal origin, persistence and nonredundant functions of IL-17-producing γδ T cells. *J Exp Med*. (2018) 215:3006–18. doi: 10.1084/jem.20181439
40. Kawai K, Kishihara K, Molina TJ, Wallace VA, Mak TW, Ohashi PS. Impaired development of Vγ3 dendritic epidermal T cells in p56lck protein tyrosine kinase-deficient and CD45 protein tyrosine phosphatase-deficient mice. *J Exp Med*. (1995) 181:345–9. doi: 10.1084/jem.181.1.345
41. Mallick-Wood CA, Pao W, Cheng AM, Lewis JM, Kulkarni S, Bolen JB, et al. Disruption of epithelial γδ T cell repertoires by mutation of the Syk tyrosine kinase. *Proc Natl Acad Sci USA*. (1996) 93:9704–9. doi: 10.1073/pnas.93.18.9704
42. Van Oers NSC, Lowin-Kropf B, Finlay D, Connolly K, Weiss A. αβ T cell development is abolished in mice lacking both Lck and Fyn protein tyrosine kinases. *Immunity*. (1996) 5:429–36. doi: 10.1016/S1074-7613(00)80499-9
43. Endo Y, Ishikawa O, Negishi I. Zeta-chain-associated protein-70 molecule is essential for the proliferation and the final maturation of dendritic epidermal T cells. *Exp Dermatol*. (2005) 14:188–93. doi: 10.1111/j.0906-6705.2005.00264.x

44. Haks MC, Lefebvre JM, Lauritsen JPH, Carleton M, Rhodes M, Miyazaki T, et al. Attenuation of γδTCR signaling efficiently diverts thymocytes to the αβ lineage. *Immunity*. (2005) 22:595–606. doi: 10.1016/j.immuni.2005.04.003
45. Hayes SM, Li LQ, Love PE. TCR signal strength influences αβ/γδ lineage fate. *Immunity*. (2005) 22:583–93. doi: 10.1016/j.immuni.2005.03.014
46. Garbe AI, Krueger A, Gounari F, Zúñiga-Pflücker JC, Von Boehmer H. Differential synergy of Notch and T cell receptor signaling determines αβ versus γδ lineage fate. *J Exp Med*. (2006) 203:1579–90. doi: 10.1084/jem.20060474
47. Hayes SM, Love PE. A retrospective on the requirements for γδ T-cell development. *Immunol Rev*. (2007) 215:8–14. doi: 10.1111/j.1600-065X.2006.00476.x
48. Ciofani M, Zúñiga-Pflücker JC. Determining γδ versus αβ T cell development. *Nat Rev Immunol*. (2010) 10:657–63. doi: 10.1038/nri2820
49. Ciofani M, Knowles GC, Wiest DL, von Boehmer H, Zúñiga-Pflücker JC. Stage-specific and differential notch dependency at the αβ and γδ T lineage bifurcation. *Immunity*. (2006) 25:105–16. doi: 10.1016/j.immuni.2006.05.010
50. Lewis JM, Girardi M, Roberts SJ, Barbee SD, Hayday AC, Tigelaar RE. Selection of the cutaneous intraepithelial $\gamma\delta^+$ T cell repertoire by a thymic stromal determinant. *Nat Immunol*. (2006) 7:843–50. doi: 10.1038/ni1363
51. Boyden LM, Lewis JM, Barbee SD, Bas A, Girardi M, Hayday AC, et al. Skint1, the prototype of a newly identified immunoglobulin superfamily gene cluster, positively selects epidermal γδ T cells. *Nat Genet*. (2008) 40:656–62. doi: 10.1038/ng.108
52. Barbee SD, Woodward MJ, Turchinovich G, Mention JJ, Lewis JM, Boyden LM, et al. Skint-1 is a highly specific, unique selecting component for epidermal T cells. *Proc Natl Acad Sci USA*. (2011) 108:3330–5. doi: 10.1073/pnas.1010890108
53. Turchinovich G, Hayday AC. Skint-1 identifies a common molecular mechanism for the development of interferon-γ-secreting versus interleukin-17-secreting γδ T cells. *Immunity*. (2011) 35:59–68. doi: 10.1016/j.immuni.2011.04.018
54. Patil RS, Bhat SA, Dar AA, Chiplunkar SV. The Jekyll and Hyde story of IL17-producing γδT cells. *Front Immunol*. (2015) 6:37. doi: 10.3389/fimmu.2015.00037
55. Xiong N, Kang C, Raulet DH. Positive selection of dendritic epidermal γδ T cell precursors in the fetal thymus determines expression of skin-homing receptors. *Immunity*. (2004) 21:121–31. doi: 10.1016/j.immuni.2004.06.008
56. Jiang X, Campbell JJ, Kupper TS. Embryonic trafficking of γδ T cells to skin is dependent on E/P selectin ligands and CCR4. *Proc Natl Acad Sci USA*. (2010) 107:7443–8. doi: 10.1073/pnas.0912943107
57. Jin Y, Xia M, Sun A, Saylor CM, Xiong N. CCR10 is important for the development of skin-specific γδT cells by regulating their migration and location. *J Immunol*. (2010) 185:5723–31. doi: 10.4049/jimmunol.1001612
58. Xia M, Qi Q, Jin Y, Wiest DL, August A, Xiong N. Differential roles of IL-2–inducible T cell kinase-mediated TCR signals in tissue-specific localization and maintenance of skin intraepithelial T cells. *J Immunol*. (2010) 184:6807–14. doi: 10.4049/jimmunol.1000453
59. O'Sullivan D, van der Windt GJW, Huang SC-C, Curtis JD, Chang C-H, Buck MD, et al. Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. *Immunity*. (2014) 41:75–88. doi: 10.1016/j.immuni.2014.06.005
60. Mayassi T, Ladell K, Gudjonson H, McLaren JE, Shaw DG, Tran MT, et al. Chronic inflammation permanently reshapes tissue-resident immunity in celiac disease. *Cell*. (2019) 176:967–81.e19. doi: 10.1016/j.cell.2018.12.039
61. Jensen KDC, Su X, Shin S, Li L, Youssef S, Yamasaki S, et al. Thymic selection determines γδ T cell effector fate: antigen-naive cells make interleukin-17 and antigen-experienced cells make interferon γ. *Immunity*. (2008) 29:90–100. doi: 10.1016/j.immuni.2008.04.022
62. Furuse M, Hata M, Furuse K, Yoshida Y, Haratake A, Sugitani Y, et al. Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. *J Cell Biol*. (2002) 156:1099–111. doi: 10.1083/jcb.200110122
63. Tsuruta D, Green KJ, Getsios S, Jones JCR. The barrier function of skin: how to keep a tight lid on water loss. *Trends Cell Biol*. (2002) 12:355–7. doi: 10.1016/S0962-8924(02)02316-4
64. Kubo A, Nagao K, Yokouchi M, Sasaki H, Amagai M. External antigen uptake by Langerhans cells with reorganization of epidermal tight junction barriers. *J Exp Med*. (2009) 206:2937–46. doi: 10.1084/jem.20091527
65. Macleod AS, Havran WL. Functions of skin-resident γδ T cells. *Cell Mol Life Sci*. (2011) 68:2399–408. doi: 10.1007/s00018-011-0702-x
66. Toulon A, Breton L, Taylor KR, Tenenhaus M, Bhavsar D, Lanigan C, et al. A role for human skin–resident T cells in wound healing. *J Exp Med*. (2009) 206:743–50. doi: 10.1084/jem.20081787
67. Payer E, Elbe A, Stingl G. Circulating $CD3^+$/T cell receptor V gamma 3^+ fetal murine thymocytes home to the skin and give rise to proliferating dendritic epidermal T cells. *J Immunol*. (1991) 146:2536–43.
68. Ariotti S, Beltman JB, Chodaczek G, Hoekstra ME, van Beek AE, Gomez-Eerland R, et al. Tissue-resident memory $CD8^+$ T cells continuously patrol skin epithelia to quickly recognize local antigen. *Proc Natl Acad Sci USA*. (2012) 109:19739–44. doi: 10.1073/pnas.1208927109
69. Zaid A, Hor JL, Christo SN, Groom JR, Heath WR, Mackay LK, et al. Chemokine receptor-dependent control of skin tissue-resident memory T cell formation. *J Immunol*. (2017) 199:2451–9. doi: 10.4049/jimmunol.1700571
70. Minagawa M, Ito A, Shimura H, Tomiyama K, Ito M, Kawai K. Homogeneous epithelial γδ T cell repertoire of the skin is shaped through peripheral selection. *J Dermatol Sci*. (2001) 25:150–5. doi: 10.1016/S0923-1811(00)00119-5
71. Komori HK, Witherden DA, Kelly R, Sendaydiego K, Jameson JM, Teyton L, et al. Cutting edge: dendritic epidermal γδ T cell ligands are rapidly and locally expressed by keratinocytes following cutaneous wounding. *J Immunol*. (2012) 188:2972–6. doi: 10.4049/jimmunol.1100887
72. Zhang B, Wu J, Jiao Y, Bock C, Dai M, Chen B, et al. Differential requirements of TCR signaling in homeostatic maintenance and function of dendritic epidermal T cells. *J Immunol*. (2015) 195:4282–91. doi: 10.4049/jimmunol.1501220
73. Van Kaer L, Wu M, Ichikawa Y, Ito K, Bonneville M, Ostrand-Rosenberg S, et al. Recognition of MHC TL gene products by gamma delta T cells. *Immunol Rev*. (1991) 120:89–115. doi: 10.1111/j.1600-065X.1991.tb00589.x
74. Bonneville M, Itohara S, Krecko EG, Mombaerts P, Ishida I, Katsuki M, et al. Transgenic mice demonstrate that epithelial homing of γ/δ T cells is determined by cell lineages independent of T cell receptor specificity. *J Exp Med*. (1990) 171:1015–26. doi: 10.1084/jem.171.4.1015
75. Ye S-K, Maki K, Lee H-C, Ito A, Kawai K, Suzuki H, et al. Differential roles of cytokine receptors in the development of epidermal γδ T cells. *J Immunol*. (2001) 167:1929–34. doi: 10.4049/jimmunol.167.4.1929
76. De Creus A, Van Beneden K, Stevenaert F, Debacker V, Plum J, Leclercq G. Developmental and functional defects of thymic and epidermal Vγ3 cells in IL-15-deficient and IFN regulatory factor-1-deficient mice. *J Immunol*. (2002) 168:6486–93. doi: 10.4049/jimmunol.168.12.6486
77. Park SY, Saijo K, Takahashi T, Osawa M, Areas H, Hirayama N, et al. Developmental defects of lymphoid cells in Jak3 kinase-deficient mice. *Immunity*. (1995) 3:771–82. doi: 10.1016/1074-7613(95)90066-7
78. Kang J, DiBenedetto B, Narayan K, Zhao H, Der SD, Chambers CA. STAT5 is required for thymopoiesis in a development stage-specific manner. *J Immunol*. (2004) 173:2307–14. doi: 10.4049/jimmunol.173.4.2307
79. Gentek R, Ghigo C, Hoeffel G, Jorquera A, Msallam R, Wienert S, et al. Epidermal γδ T cells originate from yolk sac hematopoiesis and clonally self-renew in the adult. *J Exp Med*. (2018) 215:2994–3005. doi: 10.1084/jem.20181206
80. Kadow S, Jux B, Zahner SP, Wingerath B, Chmill S, Clausen BE, et al. Aryl hydrocarbon receptor is critical for homeostasis of invariant γδ T cells in the murine epidermis. *J Immunol*. (2011) 187:3104–10. doi: 10.4049/jimmunol.1100912
81. Taylor KR, Costanzo AE, Jameson JM. Dysfunctional γ T cells contribute to impaired keratinocyte homeostasis in mouse models of obesity. *J Invest Dermatol*. (2011) 131:2409–18. doi: 10.1038/jid.2011.241
82. Fay NS, Larson EC, Jameson JM. Chronic inflammation and γδ T cells. *Front Immunol*. (2016) 7:210. doi: 10.3389/fimmu.2016.00210
83. Järbrink K, Ni G, Sönnergren H, Schmidtchen A, Pang C, Bajpai R, et al. The humanistic and economic burden of chronic wounds: a protocol for a systematic review. *Syst Rev*. (2017) 6:15. doi: 10.1186/s13643-016-0400-8

84. Saltiel AR, Olefsky JM. Inflammatory mechanisms linking obesity and metabolic disease. *J Clin Invest*. (2017) 127:1–4. doi: 10.1172/JCI92035
85. Taylor KR, Mills RE, Costanzo AE, Jameson JM. γδ T cells are reduced and rendered unresponsive by hyperglycemia and chronic TNFα in mouse models of obesity and metabolic disease. *PLoS ONE*. (2010) 5:e11422. doi: 10.1371/journal.pone.0011422
86. Mills RE, Taylor KR, Podshivalova K, McKay DB, Jameson JM. Defects in skin γδ T cell function contribute to delayed wound repair in rapamycin-treated mice. *J Immunol*. (2008) 181:3974–83. doi: 10.4049/jimmunol.181.6.3974
87. Liu Z, Liang G, Gui L, Li Y, Liu M, Bai Y, et al. Weakened IL-15 production and impaired mTOR activation alter dendritic epidermal T cell homeostasis in diabetic mice. *Sci Rep*. (2017) 7:6028. doi: 10.1038/s41598-017-05950-5
88. Wang Y, Bai Y, Li Y, Liang G, Jiang Y, Liu Z, et al. IL-15 enhances activation and IGF-1 production of dendritic epidermal T cells to promote wound healing in diabetic mice. *Front Immunol*. (2017) 8:1557. doi: 10.3389/fimmu.2017.01557
89. Goldberg EL, Molony RD, Kudo E, Sidorov S, Kong Y, Dixit VD, et al. Ketogenic diet activates protective γδ T cell responses against influenza virus infection. *Sci Immunol*. (2019) 4:eaav2026. doi: 10.1126/sciimmunol.aav2026
90. Goldberg EL, Shchukina I, Asher JL, Sidorov S, Artyomov MN, Dixit VD. Ketogenesis activates metabolically protective γδ T cells in visceral adipose tissue. *Nat Metab*. (2020) 2:50–61. doi: 10.1038/s42255-019-0160-6
91. Jameson J, Ugarte K, Chen N, Yachi P, Fuchs E, Boismenu R, et al. A role for skin gammadelta T cells in wound repair. *Science*. (2002) 296:747–9. doi: 10.1126/science.1069639
92. Keyes BE, Liu S, Asare A, Naik S, Levorse J, Polak L, et al. Impaired epidermal to dendritic T cell signaling slows wound repair in aged skin. *Cell*. (2016) 167:1323–38.e14. doi: 10.1016/j.cell.2016.10.052
93. Kikutani H, Kumanogoh A. Semaphorins in interactions between T cells and antigen-presenting cells. *Nat Rev Immunol*. (2003) 3:159–67. doi: 10.1038/nri1003
94. Zhu Z, Luo Y, Yu J, Gao J, Zhang Y, Xiao C, et al. Sema4D is required in both the adaptive and innate immune responses of contact hypersensitivity. *Mol Immunol*. (2016) 78:98–104. doi: 10.1016/j.molimm.2016.09.003
95. Witherden DA, Watanabe M, Garijo O, Rieder SE, Sarkisyan G, Cronin SJF, et al. The CD100 receptor interacts with its plexin B2 ligand to regulate epidermal γδ T cell function. *Immunity*. (2012) 37:314–25. doi: 10.1016/j.immuni.2012.05.026
96. Wang F, Liu B, Yu Z, Wang T, Song Y, Zhuang R, et al. Effects of CD100 promote wound healing in diabetic mice. *J Mol Histol*. (2018) 49:277–87. doi: 10.1007/s10735-018-9767-2
97. Zhang C, Xiao C, Dang E, Cao J, Zhu Z, Fu M, et al. CD100–Plexin-B2 promotes the inflammation in psoriasis by activating NF-κB and the inflammasome in keratinocytes. *J Invest Dermatol*. (2018) 138:375–83. doi: 10.1016/j.jid.2017.09.005
98. Whang MI, Guerra N, Raulet DH. Costimulation of dendritic epidermal γδ T cells by a new NKG2D ligand expressed specifically in the skin. *J Immunol*. (2009) 182:4557–64. doi: 10.4049/jimmunol.0802439
99. Raulet DH, Gasser S, Gowen BG, Deng W, Jung H. Regulation of ligands for the NKG2D activating receptor. *Annu Rev Immunol*. (2013) 31:413–41. doi: 10.1146/annurev-immunol-032712-095951
100. Nielsen MM, Dyring-Andersen B, Schmidt JD, Witherden D, Lovato P, Woetmann A, et al. NKG2D-dependent activation of dendritic epidermal T cells in contact hypersensitivity. *J Invest Dermatol*. (2015) 135:1311–9. doi: 10.1038/jid.2015.23
101. Girardi M, Oppenheim DE, Steele CR, Lewis JM, Glusac E, Filler R, et al. Regulation of cutaneous malignancy by γδ T cells. *J Immunol*. (2001) 294:605–9. doi: 10.1126/science.1063916
102. Schuhmachers G, Ariizumi K, Mathew PA, Bennett M, Kumar V, Takashima A. 2B4, a new member of the immunoglobulin gene superfamily, is expressed on murine dendritic epidermal T cells and plays a functional role in their killing of skin tumors. *J Invest Dermatol*. (1995) 105:592–6. doi: 10.1111/1523-1747.ep12323533
103. Strid J, Sobolev O, Zafirova B, Polic B, Hayday A. The intraepithelial T cell response to NKG2D-ligands links lymphoid stress surveillance to atopy. *Science*. (2011) 334:1293–7. doi: 10.1126/science.1211250
104. Boismenu R, Feng L, Xia YY, Chang JC, Havran WL. Chemokine expression by intraepithelial gamma delta T cells. Implications for the recruitment of inflammatory cells to damaged epithelia. *J Immunol*. (1996) 157:985–92.
105. Fischer FR, Luo Y, Luo M, Santambrogio L, Dorf ME. RANTES-induced chemokine cascade in dendritic cells. *J Immunol*. (2001) 167:1637–43. doi: 10.4049/jimmunol.167.3.1637
106. Yoshida S, Mohamed RH, Kajikawa M, Koizumi J, Tanaka M, Fugo K, et al. Involvement of an NKG2D ligand H60c in epidermal dendritic T cell-mediated wound repair. *J Immunol*. (2012) 188:3972–9. doi: 10.4049/jimmunol.1102886
107. Verdino P, Witherden DA, Havran WL, Wilson IA. The molecular interaction of CAR and JAML recruits the central cell signal transducer PI3K. *Science*. (2010) 329:1210–4. doi: 10.1126/science.1187996
108. Witherden DA, Verdino P, Rieder SE, Garijo O, Mills RE, Teyton L, et al. The junctional adhesion molecule JAML is a costimulatory receptor for epithelial γδ T cell activation. *Science*. (2010) 329:1205–10. doi: 10.1126/science.1192698
109. Rosenblum MD, Woodliff JE, Madsen NA, McOlash LJ, Keller MR, Truitt RL. Characterization of CD200-receptor expression in the murine epidermis. *J Invest Dermatol*. (2005) 125:1130–8. doi: 10.1111/j.0022-202X.2005.23948.x
110. Jakob T, Udey MC. Regulation of E-cadherin-mediated adhesion in Langerhans cell-like dendritic cells by inflammatory mediators that mobilize Langerhans cells *in vivo*. *J Immunol*. (1998) 160:4067–73.
111. Schön MP, Schön M, Parker CM, Williams IR. Dendritic epidermal T cells (DETC) are diminished in integrin αE(CD103)-deficient mice. *J Invest Dermatol*. (2002) 119:190–3. doi: 10.1046/j.1523-1747.2002.17973.x
112. Schlickum S, Sennefelder H, Friedrich M, Harms G, Lohse MJ, Kilshaw P, et al. Integrin alpha E(CD103)beta 7 influences cellular shape and motility in a ligand-dependent fashion. *Blood*. (2008) 112:619–25. doi: 10.1182/blood-2008-01-134833
113. Uchida Y, Kawai K, Ibusuki A, Kanekura T. Role for E-cadherin as an inhibitory receptor on epidermal gammadelta T cells. *J Immunol*. (2011) 186:6945–54. doi: 10.4049/jimmunol.1003853
114. Saito T, Yano M, Ohki Y, Tomura M, Nakano N. Occludin expression in epidermal γδ T cells in response to epidermal stress causes them to migrate into draining lymph nodes. *J Immunol*. (2017) 199:62–71. doi: 10.4049/jimmunol.1600848
115. Nielsen MM, Lovato P, MacLeod AS, Witherden DA, Skov L, Dyring-Andersen B, et al. IL-1β-dependent activation of dendritic epidermal T cells in contact hypersensitivity. *J Immunol*. (2014) 192:2975–83. doi: 10.4049/jimmunol.1301689
116. Dalessandri T, Crawford G, Hayes M, Castro Seoane R, Strid J. IL-13 from intraepithelial lymphocytes regulates tissue homeostasis and protects against carcinogenesis in the skin. *Nat Commun*. (2016) 7:1–12. doi: 10.1038/ncomms12080
117. Nielsen MM, Witherden DA, Havran WL. γδ T cells in homeostasis and host defence of epithelial barrier tissues. *Nat Rev Immunol*. (2017) 17:733–45. doi: 10.1038/nri.2017.101
118. Cruz MS, Diamond A, Russell A, Jameson JM. Human αβ and γδ T cells in skin immunity and disease. *Front Immunol*. (2018) 9:1304. doi: 10.3389/fimmu.2018.01304
119. Jameson JM, Cauvi G, Sharp LL, Witherden DA, Havran WL. γδ T cell-induced hyaluronan production by epithelial cells regulates inflammation. *J Exp Med*. (2005) 201:1269–79. doi: 10.1084/jem.20042057
120. Werner S, Smola H, Liao X, Longaker MT, Krieg T, Hofschneider PH, et al. The function of KGF in morphogenesis of epithelium and reepithelialization of wounds. *Science*. (1994) 266:819–22. doi: 10.1126/science.7973639
121. Sharp LL, Jameson JM, Cauvi G, Havran WL. Dendritic epidermal T cells regulate skin homeostasis through local production of insulin-like growth factor 1. *Nat Immunol*. (2005) 6:73–9. doi: 10.1038/ni1152
122. Edmondson SR, Thumiger SP, Werther GA, Wraight CJ. Epidermal homeostasis: the role of the growth hormone and insulin-like growth factor systems. *Endocr Rev*. (2003) 24:737–64. doi: 10.1210/er.2002-0021

123. Yokota K, Ariizumi K, Kitajima T, Bergstresser PR, Street NE, Takashima A. Cytokine-mediated communication between dendritic epidermal T cells and Langerhans cells. *In vitro* studies using cell lines. *J Immunol*. (1996) 157:1529–37.
124. Jux B, Kadow S, Esser C. Langerhans cell maturation and contact hypersensitivity are impaired in aryl hydrocarbon receptor-null mice. *J Immunol*. (2009) 182:6709–17. doi: 10.4049/jimmunol.0713344
125. Macleod AS, Hemmers S, Garijo O, Chabod M, Mowen K, Witherden DA, et al. Dendritic epidermal T cells regulate skin antimicrobial barrier function. *J Clin Invest*. (2013) 123:4364–74. doi: 10.1172/JCI70064

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High Abundance of Intratumoral γδ T Cells Favors a Better Prognosis in Head and Neck Squamous Cell Carcinoma: A Bioinformatic Analysis

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γδ T cells are a small subset of unconventional T cells that are enriched in the mucosal areas, and are responsible for pathogen clearance and maintaining integrity. However, the role of γδ T cells in head and neck squamous cell carcinoma (HNSCC) is largely unknown. Here, by using RNA-seq data from The Cancer Genome Atlas (TCGA), we discovered that HNSCC patients with higher levels of γδ T cells were positively associated with lower clinical stages and better overall survival, and high abundance of γδ T cells was positively correlated with CD8+/CD4+ T cell infiltration. Gene ontology and pathway analyses showed that genes associated with T cell activation, proliferation, effector functions, cytotoxicity, and chemokine production were enriched in the group with a higher γδ T cell abundance. Furthermore, we found that the abundance of γδ T cells was positively associated with the expression of the butyrophilin (BTN) family proteins BTN3A1/BTN3A2/BTN3A3 and BTN2A1, but only MICB, one of the ligands of NKG2D, was involved in the activation of γδ T cells, indicating that the BTN family proteins might be involved in the activation and proliferation of γδ T cells in the tumor microenvironment of HNSCC. Our results indicated that γδ T cells, along with their ligands, are promising targets in HNSCC with great prognostic values and treatment potentials.

Keywords: head and neck squamous cell carcinoma (HNSCC), γδ T cells, butyrophilin, BTN3A1, MICB, TCGA, ImmuCellAI, CIBERSORTx

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INTRODUCTION

Head and neck cancer is a group of cancers that occurs in the head and neck region, including the oral cavity, pharynx and larynx, of which more than 90% cases are squamous cell carcinoma (1). Head and neck squamous cell carcinoma (HNSCC) is the seventh most common cancer globally in 2018, accounting for 3% of all cases of cancer incidence and 1.5% of all cancer deaths annually (2). Although the treatment of HNSCC has evolved from surgery to a multidisciplinary treatment including surgery, radiotherapy, chemotherapy and molecular targeted therapy in the past few decades, the 5-year overall survival (OS) of HNSCC patients has not been significantly improved, remaining at ∼60% (3). In addition, more than 50% of HNSCC patients have a locoregional recurrence or distant metastasis within 3 years, which results in a poorer prognosis (4). In recent years, with the advancement of cancer immunotherapy, some patients with advanced cancer have

benefited from it, but the response rate in HNSCC patients is only ∼13–20% (4, 5). Therefore, it is urgent to find novel molecular markers or cell types with therapeutic and prognostic values in HNSCC.

T cells are a group of immune cells that play the key role in antitumor immune response. Based on the composition of T cell receptors (TCR), T cells are mainly divided into αβ T cells and γδ T cells (6). αβ T cells, including CD4+ T cells and CD8+ T cells, have been extensively studied in tumor immunity, but the role of γδ T cells in the tumor microenvironment (TME) is largely unknown. γδ T cells account for only 1–5% of total T cells in the peripheral blood, but they mainly reside in mucosal areas, such as the intestinal epithelia and oral mucosa, accounting for 10–100% of intraepithelial lymphocytes (7). Generally, γδ T cells are responsible for pathogen clearance and maintaining integrity of the epithelium (7). According to the arrangement of the Vδ chain, human γδ T cells are classified into three different subsets, namely Vδ1 T cells, Vδ2 T cells, and Vδ3 T cells which are only found in liver (8). Vδ2 T cells are the main subset of circulating γδ T cells in the peripheral blood, accounting for about 50 ∼ 90% γδ T cells (9).In addition, Vδ2 T cells also accumulate in tumor tissues and exert potent cytotoxic activity, indicating that these cells possess a potential antitumor activity. In contrast, Vδ1 T cells are mainly distributed in mucosal areas, playing an important role in killing bacteria or viruses and maintaining tissue homeostasis, in addition, they are also involved in antitumor immunity (10).

Recent studies have reported that the infiltration level of cytotoxic T cells or NK cells in the TME is usually correlated with an improved prognosis in various kinds of cancers (11). γδ T cells, as one of the cytotoxic T cells that infiltrates tumor tissue, are reported to possess a high cytotoxic activity in lung cancer, breast cancer, colon cancer and gastric cancer, and are related to the improvement of overall survival (12). However, recent studies showed that γδ T cells may also promote tumor progression (13). However, until now, the functions and prognostic value of γδ T cells in HNSCC have been rarely studied. A previous study explored the γδ T cells in the peripheral blood of HNSCC patients and found that there was no correlation between γδ T cell abundance and T stages (14), but the abundance of γδ T cells in the TME has not been studied.

Unlike conventional T cells, which recognize antigens presented by tumor cells or antigen presenting cells (APC) through major histocompatibility complex (MHC) molecules, γδ T cells recognize various types of antigens without MHC restriction (15), but the exact mechanisms that trigger γδ T cell activation and proliferation are largely unknown. Notably, recent studies have found that Vδ2 T cells are activated by phosphoantigens (P-Ags) produced by malignant cells or infected cells through the presentation of butyrophilin family proteins (BTNs) to TCRγδ on Vδ2 T cells (16). BTNs belong to the type I transmembrane proteins of the immunoglobulin superfamily. In humans, these proteins can be classified into BTN1, BTN2, and BTN3 subfamilies, including BTN1A1, BTN2A1-2, and BTN3A1-3 (17). Among them, BTN2A1 and BTN3A1-3 have been reported to take up and present P-Ags to the TCR of γδ T cells (18–20). Studies have shown that through the binding to P-Ags presented by BTN3A1 and BTN2A1 on infected or malignant cells, γδ T cells could be activated, proliferate rapidly, and release cytokines to induce anti-infection or antitumor responses (20, 21). Recent studies have found that a higher expression of BTN3A2 in ovarian cancer and triple negative breast cancer is positively correlated with increased T cell infiltration and a better prognosis (22, 23), but other studies have reported that BTN3A2 is associated with poor prognosis in gastric cancer and pancreatic ductal adenocarcinoma (PDAC) (22, 24). In addition, the engagement of natural killer group 2 member D (NKG2D) with its ligands provides the costimulatory signaling pathway that activates γδ T cells (25). The NKG2D ligands (NKG2DLs) include MHC class I polypeptide-related sequence A and B (MICA/MICB) and the UL16 binding protein 1-6 (ULBP1-6) (26). However, the potential correlation of γδ T cells and BTN families in the TME of HNSCC is still unclear, and whether the NKG2DL-NKG2D pathway participates in the antitumor immunity of HNSCC remains to be discovered.

In this study, by using the patient cohorts from TCGA database, we discovered that HNSCC patients with a higher abundance of γδ T cells had prolonged overall survival, suggesting that γδ T cells were of great prognostic values for HNSCC, and were highly correlated with CD8+ T cell and CD4+ T cell infiltration in the TME. We further found that activation of γδ T cells in the TME was associated with the BTN family proteins. This study revealed the prognostic value of γδ T cells in head and neck cancer, and revealed the possible activation mechanisms of γδ T cells in HNSCC.

MATERIALS AND METHODS

HNSCC and CESC Datasets From TCGA Database

Data on the HNSCC patient cohort was downloaded from The Cancer Genome Atlas (TCGA). Patients without RNA-seq data were excluded. A total of 537 samples were included in this study, including 494 tumor tissue samples and 43 adjacent normal tissue samples, all of the 43 adjacent normal tissue samples were classified into normal group. The phenotype information and survival data of HNSCC patients (version: 08-07-2019) and RNA-seq data (version: 07-19-2019) were downloaded from UCSC Xena (https://xenabrowser.net/datapages/). In addition, the RNA-seq data (version: 07-19-2019), phenotype (version: 08-07-2019) data and survival data (version: 07-19-2019) of a cervical squamous cell carcinoma (CESC) patient cohort were also downloaded from UCSC Xena. Patients without RNA-seq data were excluded. A total of 281 samples were included in this study, including 278 tumor tissue samples and 3 adjacent normal tissue samples. The classification of T stage, N stage and clinical stage of each patient was based on American Joint Committee of Cancer (AJCC, 7th edition) (27). The RNA-seq data included Fragments per Kilobase Million (FPKM) matrix and a counts matrix and to reduce biases among different samples, the FPKM format was converted into a Transcripts Per Million (TPM) format for further analysis (28). The function for the conversion of FPKM to TPM is listed below, and this process was calculated by R language:

TPM(i) = (FPKM(i)/sum(FPKM all transcripts)) × 10^6 (where i refers to the specific gene of specific sample in the expression matrix).

After conversion, the sum of all transcripts in each sample was on the order of 10^6, which makes it more convincing to compare the expression levels of specific genes across different samples.

Gene Expression Comparison and Correlation Analysis

γδ T cell abundance is defined by the geometric mean of the TPM values of TRDC/TRGC1/TRGC2, where TRDC encodes the constant chain of TCRδ, and TRGC1 and TRGC2 encode the constant chain of TCRγ. Based on the median expression of the geometric mean, the HNSCC patients were dichotomized into γδT-hi and γδT-lo groups (247 patients in each group, **Table 1**). Furthermore, to explore the effects of the BTN family proteins on the prognosis of HNSCC patients, the cohort was dichotomize based on the median expression levels of BTN3A1/BTN3A2/BTN3A3/BTN2A1, respectively. The effector and cytotoxic functions of γδ T cells were assessed by the expression levels of IFNG (interferon-γ), GZMA (granzyme A), GZMB (granzyme B), and GNLY (granulysin). The activation degree of cytotoxic γδ T cells is reflected by transcription factor Hobit (encoded by ZNF683) and activation receptor NKG2D (encoded by KLRK1). The difference in the ability to recruit CD8+ T cells between the γδT-hi and γδT-lo groups was shown by the expression levels of the chemokines CCL5 and CXCL9, which was reported previously (29). The expression levels of γδ T cell activation ligands among the γδT-hi, γδT-lo and normal groups are shown by the expression levels of ligands that can be recognized and bound by TCRγδ (BTN2A1, BTN3A1-3), and ligands that bind to NKG2D and activate γδ T cells (MICA, MICB, and ULBP1-6). Boxplots, heatmap and scatter plots were drawn by using R packages ggplot2, ggpubr and pheatmap. In the boxplots, the center line of box represents the median value, the upper and lower edges of box indicate the 75th and 25th percentiles, respectively. The whiskers extend 1.5 times the interquartile range (IQR) beyond the 75th and 25th percentiles of the box, respectively. Student's *t*-test was used to compare the difference in gene expression values between the two groups. Linear regression analysis was used to compare the correlation between each ligand and the geometric mean of the γδ T cell markers. For the linear regression analysis, the gene expression levels were first converted to $\log_{10}$ (TPM+1), and Pearson correlation coefficient *R*-values and *P*-values were obtained. Among these, the absolute value of R (|R|) > 0.7 was considered as a strong correlation, 0.4<|R|<0.7 was considered as a moderate correlation, and |R|<0.4 was considered as a weak correlation. $P < 0.05$ is considered to be statistically significant.

Estimation of the Relative Abundance of Immune Cells in the RNA-seq Data

The relative abundance of immune cells in the RNA-seq data of HNSCC and CESC patients was estimated by using the newly developed online tool ImmuCellAI (Immune Cell Abundance Identifier), which is comprised of 24 immune cells, including 18 T cell subpopulations and another 6 immune cells (30). The relative abundances of 24 immune cells in tumors or normal tissues of HNSCC or CESC were downloaded from http://bioinfo.life.hust.edu.cn/ImmuCellAI#!/resource. In addition, the relative proportions of immune cells in the tumor or normal tissues of HNSCC were also calculated by using another online immune cell analytical tool, CIBERSORTx, which supports a deconvolution algorithm to evaluate the relative proportion of immune cells in tumor tissues (31). The composition of immune cells was calculated online (https://cibersortx.stanford.edu/).

TABLE 1 | Baseline and clinical information of HNSCC patients in TCGA database.

Factor		γδT-hi number(%)	γδT-lo number(%)	*P*-value
γδT cell marker expression (TPM)		0.60–19.03	0.00–0.59	
Gender	Male	179 (36.2%)	183 (37.0%)	0.684
	female	68 (13.8%)	64 (13.0%)	
Age	≥60y	148 (30.0%)	130 (26.3%)	0.103
	< 60y	99 (20.0%)	117 (23.7%)	
Smoking history	Yes	154 (31.2%)	149 (30.2%)	0.644
	No	93 (18.8%)	98 (19.8%)	
Alcohol history	Yes	167 (33.8%)	160 (32.4%)	0.506
	No	80 (16.2%)	87 (17.6%)	
Tumor site	Oral cavity	140 (28.3%)	165 (33.4%)	0.003**
	Pharynx	53 (10.7%)	26 (5.3%)	
	Larynx	54 (10.9%)	56 (11.3%)	
T stage	1	25 (5.1%)	10 (2.0%)	0.016*
	2	80 (16.2%)	67 (13.6%)	
	3	59 (11.9%)	75 (15.2%)	
	4	83 (16.8%)	95 (19.2%)	
N stage	0	115 (23.5%)	129 (26.3%)	0.211
	1	38 (7.8%)	47 (9.6%)	
	2	88 (18.0%)	68 (13.9%)	
	3	3 (0.6%)	2 (0.4%)	
Clinical stage	1	15 (3.0%)	5 (1.0%)	0.020*
	2	46 (9.3%)	49 (9.9%)	
	3	44 (8.9%)	65 (13.2%)	
	4	142 (28.7%)	128 (25.9%)	
Perineural invasion	Yes	71 (14.4%)	93 (18.8%)	0.109
	No	98 (19.8%)	87 (17.6%)	
	Unknown	78 (15.8%)	67 (13.6%)	
HPV status	Yes	25 (5.1%)	5 (1.0%)	0.001**
	No	33 (6.7%)	39 (7.9%)	
	Unknown	189 (38.3%)	203 (41.1%)	

*$P < 0.05$, **$P < 0.01$.

Analysis of Differentially Expressed Genes

The differentially expressed genes (DEG) in tumor tissues between the γδT-hi and γδT-lo groups were analyzed by R package DESeq2, and the selection criteria for DEGs were an absolute value of log2FoldChange > 1 and adjusted *P*-value (Padj) <0.05. Biological process (BP) in Gene Ontology (GO) and Kyoto Gene and Genome Encyclopedia (KEGG) term

enrichment analyses were performed by R package clusterProfiler (version: 3.16.1) using the DEGs in the γδT-hi and γδT-lo group, respectively, the significance levels of enrichment results (adjusted *P*-values) were calculated by hypergeometric distribution. The Gene Set Enrichment Analysis (GSEA) was performed using the REACTOME database in MSigDB (version 7.1), and *P*-values for GSEA analyses were calculated by permutation tests. The normalized enrichment score (NES) >1, $P < 0.05$, and False Discovery Rates (FDR) <0.25 were considered statistically significant in GSEA analyses.

Statistical Analyses

SPSS software version 25.0 was used for statistical analysis. The clinical parameters in this study included gender, age, smoking history, drinking history, tumor location, T stage, N stage, clinical stage, perineural invasion (PNI) and human papillomavirus (HPV) status. A Chi-square test was used to compare the clinical parameters between the two groups. OS was calculated and described by the Kaplan–Meier method. The difference of survival curves was tested by log-rank test. Univariate Cox proportional models were used to analyze the associations between clinical parameters and OS, and the factors with statistical significance were further included into multivariate Cox regression analysis. $P < 0.05$ was considered to be statistically significant (Wald test). GraphPad Prism version 7.0 was used to draw stacked histograms and survival curves of γδT-hi and γδT-lo groups. ns, not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

RESULTS

A High Abundance of γδ T Cells Is Significantly Correlated With Improved Survival of HNSCC Patients

First, we compared the clinical parameters and the OS of HNSCC patients between the γδT-hi and γδT-lo groups. The results showed that patients in the γδT-hi group accounted for a higher proportion in the T1/T2 stages (**Figure 1A**, **Table 1**) and in phase I/II clinical stages (**Figure 1B**, **Table 1**), whereas patients in the γδT-lo group were more aggregated in the T3/T4 stages and phase III/IV stages. In addition, there was a negative correlation between T stages and γδT cell marker expression levels ($R = -0.17$, $P < 0.05$, **Figure 1C**). The overall survival curve of the two groups showed that the survival time of the γδT-hi group was significantly prolonged ($P < 0.05$, **Figure 1D**). Except for the tumor site and HPV status, the clinical parameters showed no significant differences between the two groups (**Table 1**). However, when these factors were analyzed for univariate analysis, only γδ T cell abundance, gender and PNI were significantly associated with the OS (**Table 2**). Furthermore, multivariate Cox regression analysis showed that only γδ T cell abundance and gender were statistically significant between the

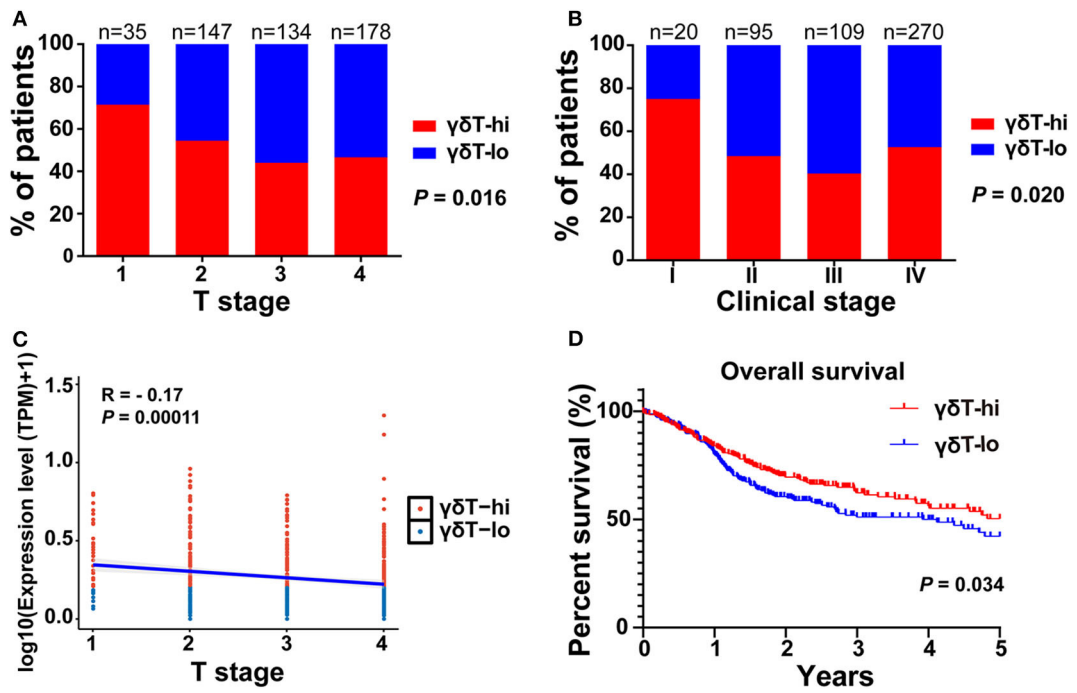

FIGURE 1 | The distribution of T stages, clinical stages, and overall survival curves between the γδT-hi ($n = 247$) and γδT-lo ($n = 247$) groups in the HNSCC dataset. **(A)** Proportions of patients in each T stage (1–4) in the γδT-hi and γδT-lo groups. **(B)** Proportions of patients in each clinical stage (I-IV) in the γδT-hi and γδT-lo groups, the *P*-values in **(A,B)** were obtained by Chi-square test. **(C)** Correlation between T stages and γδ T cell markers in HNSCC, each point represents the tumor sample in each patient, the expression levels of γδ T cell markers were calculated by the geometric mean of TPM values of TRGC1, TRGC2 and TRDC, and were converted by $\log_{10}$ (TPM + 1). **(D)** Five-year overall survival curves in the γδT-hi and γδT-lo groups.

two groups (**Table 2**). These results showed that the abundance of γδ T cells was significantly correlated with the lower clinical stages and prolonged survival of HNSCC patients. To validate whether our findings could be replicated to other types of cancer, we studied an additional squamous cell carcinoma, CESC. The results showed that similar to our findings in HNSCC, CESC patients with a higher γδ T cell infiltration were positively correlated with a better OS (**Supplementary Figure 1A**) and the proportions of the γδT-hi group decreased in T3/4 stages, although there was no statistical significance among the T stages ($P > 0.05$, **Supplementary Figure 1B**).

γδ T Cell Abundance Is Positively Correlated With CD4+ and CD8+ T Cell Abundance in the HNSCC Samples

Since the γδ T cell abundance was positively correlated with improved survival of HNSCC patients, we next tried to explore the potential reasons for these new findings. We used the new online immune cell proportion estimation tool ImmuCellAI to analyze the abundance of 24 kinds of immune cells in the RNA-seq results of 537 samples. This tool was reported to possess a high accuracy in predicting the composition of immune cells in tumor tissues, especially T cells (30). The results showed that the γδ T cell proportion in the γδT-hi group was significantly increased ($P < 0.0001$, **Figure 2A**), which was consistent with our grouping method. Moreover, both CD4+ T cell and CD8+ T cell abundances in the γδT-hi group increased significantly ($P < 0.0001$, **Figures 2B,C**). Among CD4+ T cells, T helper cells type 1 (Th1), T helper cells type 2(Th2), and follicular helper T cells (Tfh) were significantly increased in the γδT-hi group ($P < 0.0001$, **Figure 2B**), while the proportion of naïve CD4+ T cells and T helper cell type 17 (Th17) was lower than those in γδT-lo or normal groups (**Figure 2B**). However, the increase in CD4+ T cell infiltration was also accompanied by the increase of regulatory T cell abundance in the γδT-hi group (**Figure 2B**). In CD8+ T cells, central memory T cells, cytotoxic T cells, and exhausted T cells accumulated in the γδT-hi group, while the proportion of naïve CD8+ T cells decreased significantly, but the proportion of effector memory T cells was not increased when compared to the γδT-lo or normal groups (**Figure 2C**). In addition, the other cell abundances showed that the relative abundances of B cells, natural killer (NK) cells and macrophages increased significantly in the γδT-hi group, and the proportion of natural killer T (NKT) cells, monocytes and neutrophils decreased significantly (**Figure 2D**). To verify these results, we used another online immune cell fraction estimation method, CIBERSORTx, to calculate the relative abundance of immune cells in the HNSCC samples. The results, consistent with those of ImmuCellAI algorithm, showed that the CD8+ T cells, activated CD4+ T cells, Tfh and NK cells in the γδT-hi group were significantly increased (**Supplementary Figure 2A**), while the monocytes and neutrophils were decreased (**Supplementary Figure 2B**). It has been reported that tumor-infiltratingTh17, neutrophils, and monocytes are able to promote tumor progression (32–34), and thus the relatively low abundance of Th17 cells, neutrophils and monocytes also contributed to the better OS in the γδT-hi group. Furthermore, M1 macrophages with an antitumor effect were increased in the TME of the γδT-hi group, while the proportion of M2 macrophages, which promote tumor development, was significantly reduced (**Supplementary Figure 2B**). Furthermore, we have found a similar immune cell distribution pattern between the γδT-hi and γδT-lo groups in the CESC dataset calculated by ImmuCellAI algorithm (**Supplementary Figures 1C–F**), indicating the immune cell infiltration may be affected by γδ T cells in the TME across different types of cancer.

Genes Related to T Cell Effector Functions and Chemokine Production Are Highly Expressed in the γδT-hi Group

We further analyzed the differential genes between γδT-hi and γδT-lo groups by using R package DESeq2. As shown in the **Figures 3A,B**, a total of 1,113 genes were upregulatedand 329 genes were down-regulated in the γδT-hi group ($|\log2FoldChange| > 1$, $Padj < 0.05$, **Supplementary Table 1**). Among these DEGs, genes encoding CD3 (CD3E/CD3G/CD3D), and genes related to Th1 cells (CD4/TBX21), CD8+ T cells (CD8A/CD8B), B cells (CD19/MS4A1), and NK cells (NCR1/NKG7) were significantly upregulated (**Figure 3A**), which was in line with the increased proportion of Th1/CD8+ T cells/B cells/NK cells in the γδT-hi group. In contrast, the gene KRT1 related to keratinocytes, the tumor marker AFP (alpha-fetoprotein), and ARG1 (encoding arginae1) related to inhibiting CD8+ T cell function were upregulated in the γδT-lo group (**Figure 3B**). NKG2D, the surface marker of activated cytotoxic γδ T cells, and Hobit, the transcription factor that enhance the cytotoxicity of γδ T cells (35), were both upregulated in the γδT-hi group compared to those in the γδT-lo or normal group (**Figure 3C**). At the same time, the expression level of cytokines and granzymes (IFNG/GZMA/GZMB/GNLY) were also significantly increased in the γδT-hi group (**Figure 3D**). These results revealed the enhanced effector functions of tumor-infiltrating T cells of the γδT-hi group. Previous studies have reported that γδ T cells could release chemokines CCL5 and CXCL9 to attract other T cells to fight against pathogens or tumors (26, 36). In the γδT-hi group, we found that these two chemokines were also significantly upregulated, and they are reported to be essential for the recruitment of CD8+ T cells to the TME for tumor cell killing (**Figure 3E**). Therefore, γδ T cells may also recruit CD8+ T cells via chemokine release.

Gene Sets and Cell Pathways Related to T Cell Activation, Proliferation, Chemokine Production and Cytotoxicity Are Enriched in the γδT-hi Group

We then performed GO and KEGG term enrichment on the DEGs in the γδT-hi group and the γδT-lo group, respectively. The results showed that the genes upregulated in the γδT-hi group enriched the functions related to T cell activation, proliferation, differentiation, cytokine production, and cell-cell adhesion in the GO biological process (**Figure 4A**). In the KEGG results, the γδT-hi group was enriched for terms including cytokine-cytokine

TABLE 2 | Univariate and multivariate survival analysis of HNSCC patients in TCGA database.

Factor	*P*-value (Univariate)	Hazard ratio (Multivariate)	*P*-value (Multivariate)
γδ T cell abundance	0.047*	0.734	0.036*
gender	0.023*	0.702	0.023*
age	0.295		
Smoking history	0.906		
Alcohol history	1.000		
tumor site	0.153		
T stage	0.297		
N stage	0.360		
Clinical stage	0.962		
Perineural invasion	<0.001*	1.066	0.058
HPV status	0.212		

*$P < 0.05$.

receptor interaction, chemokine signaling pathway, T cell and B cell receptor pathway, Th1 and Th2 cell differentiation, and others (**Figure 4B**). Furthermore, it was noted that chemokine bind chemokine receptors, complement cascade, interferon-γ pathway, and MHC-II antigen presentation were significantly enriched in the γδT-hi group through the GSEA analysis ($P < 0.05$, FDR < 0.25, **Figure 4C**). The genes that participate in the complement pathway and antigen presentation, including C7/CR2/HLA-DRA, were also found in the γδT-hi group (**Figures 3A,B**), indicating that the complement cascade and antigen presentation were also involved in the γδ T cell-mediated immune response. In contrast, only biological processes such as cell keratinization and hormone metabolism were enriched in the γδT-lo group (**Figure 4D**). It was reported that γδ T cells could also serve as antigen-presenting cells to activate conventional T cells in infectious disease or the TME (37), our results, consistent with these previous studies, showed that γδ T cells may exert antitumor responses by activating and recruiting effector T cells or NK cells to the TME and through MHC-II antigen presentation, but the exact mechanism is still unknown.

The Abundance of γδ T Cells Is Positively Associated With the Expression of Butyrophilin Family Proteins in Tumor Cells

We have explored the possible antitumor mechanism of γδ T cells in HNSCC, but the factors that contribute to the increase in γδ T cells in the TME are still unclear. Thus, we explored the expression levels of BTNs and NKG2DLs among the three groups and performed the linear correlation analysis between the expression levels of BTNs or NKG2DLs and the γδ T cell markers. We found that the expression levels of BTN2A1/BTN3A1/BTN3A2/BTN3A3 in the γδT-hi group were significantly higher than those in the γδT-lo group or normal group ($P < 0.0001$), and the expression levels of BTNs in the normal control group were the lowest (**Figure 5A**). Through linear regression analysis, we found that there was a relatively strong correlation between BTN3A1/BTN3A2/BTN3A3 and the γδ T cell markers, while the correlation between BTN2A1 and the γδ T cell markers was relatively weak (**Figure 5C**). Furthermore, the results showed that only MICB expression in the γδT-hi group was significantly higher than that in the γδT-lo or normal groups, while MICA expression was not significantly higher than that in the γδT-lo group (**Figure 5B**). Linear regression analysis showed that MICB expression had moderate correlation with γδ T cell markers, while MICA showed nearly no correlation (**Figure 5C**). In contrast, the expression of ULBP family proteins in γδT-hi was lower than that in the γδT-lo group, suggesting that activation of the γδ T cells in HNSCC might not be related to the ULBP family proteins (**Supplementary Figure 3A**). However, when we dichotomized the HNSCC cohort based on the median expression levels of BTN3A1/BTN3A2/BTN3A3/BTN2A1, we found that although there was no statistical significance between the high and low groups, the patients with higher expression levels of the BTN family proteins showed better overall survival than patients whose expression levels were lower than the median expression levels ($P > 0.05$, **Supplementary Figure 3B**).

DISCUSSION

In the present study, we used TCGA dataset to demonstrate that the high abundance of γδ T cells in HNSCC was positively associated with an improved prognosis of patients, possibly due to the enhanced antitumor effect of γδ T cells and the recruitment of CD8+ T cells to the TME. Finally, we found that the increased γδ T cell abundance in the TME was associated with upregulation of BTN family proteins and the NKG2D ligand MICB in tumor cells, indicating that the activation of γδ T cells may be associated with BTN family proteins and MICB.

Tumor-infiltrating lymphocytes play a vital role in the control of tumor progression, with different clinical effects (38). Similar to αβ T cells, γδ T cells are also found in various types of human tumors. Both Vδ1 T and Vδ2 T cells have been found in various epithelial tumors, such as lung cancer, renal cancer, melanoma, and colorectal cancer (12). By applying the CIBERSORT analysis in a pan-cancer analysis of over 18,000 human tumors in TCGA data, γδ T cells were found to be the most favorable prognostic populations among all types of tumor-infiltrating leukocytes across all types of tumors (39). However, whether γδ T cells

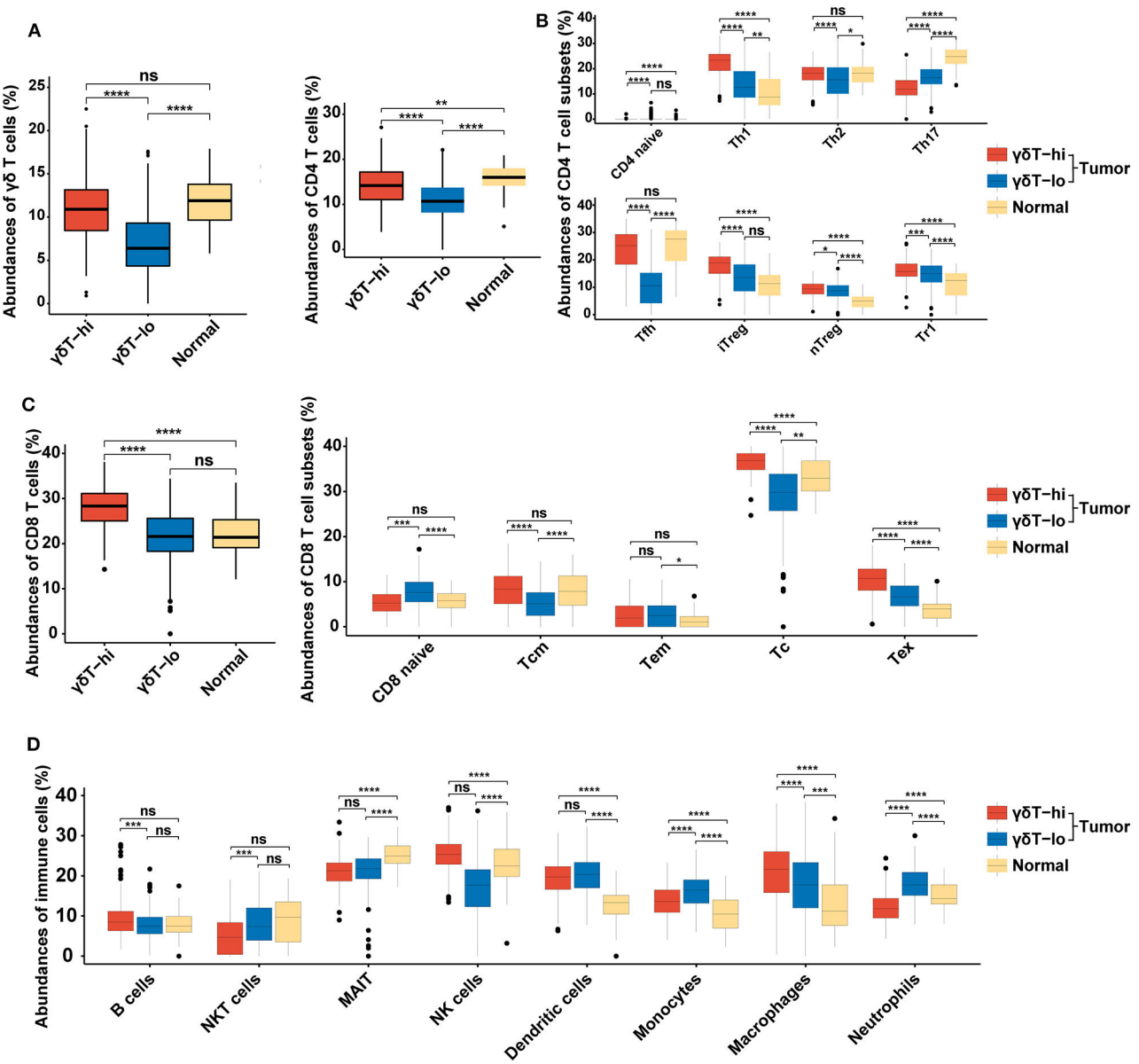

FIGURE 2 | Boxplots showing the relative abundances of 24 cell types among the γδT-hi, γδT-lo and normal groups in HNSCC dataset using ImmuCellAI algorithm. **(A)** The relative abundances of γδ T cells among the γδT-hi, γδT-lo and normal groups. **(B)** The relative abundances of CD4+ T cells and their subsets among the γδT-hi, γδT-lo and normal groups. **(C)** The relative abundances of CD8+ T cells and their subsets among the γδT-hi, γδT-lo and normal groups. **(D)** The relative proportions of B cells, NKT cells, MAIT, NK cells, dendritic cells, monocytes, macrophages and neutrophils among the γδT-hi, γδT-lo and normal groups. *P*-values were calculated by student's *t*-test. CD4 naïve, naïve CD4+ T cells; Th1, T helper cells type 1; Th2, T helper cells type 2; Th17, T helper cells type 17; Tfh, follicular helper T cells; iTreg, induced regulatory T cells; nTreg, natural regulatory T cells; Tr1, type 1 regulatory T cellss; CD8 naïve, naïve CD8+ T cell; Tcm, central memory T cells; Tem, effector memory T cells; Tc, cytotoxic T cells; Tex, exhausted T cells; NKT cells, natural killer T cells; MAIT, mucosal-associated invariant T cells; NK cells, natural killer cells. ns, not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

exert antitumor or protumor effects in the TME has not been validated. Vδ2 T cells activated by non-peptide phosphoantigens *in vitro* have been proven to inhibit tumor growth both *in vitro* and *in vivo* (40, 41). Currently, the results of phase I clinical trials on advanced lung cancer, renal cancer and melanoma have revealed that adoptive Vδ2 T cell transfer therapies have shown certain antitumor effects (42). However, other studies have reported that Vδ2 T cells can also promote tumor progression (43). Under specific stimulation, Vδ2 T cells can be differentiated into subsets endowed with Th17 or Treg characteristics, exerting protumor and immunosuppressive effects by producing IL-17 and IL-10, respectively (44). In addition, Vδ1 T cells have also been found to have dual effects in tumor immunity. In patients with hepatocellular carcinoma (45) and gastric cancer (46),

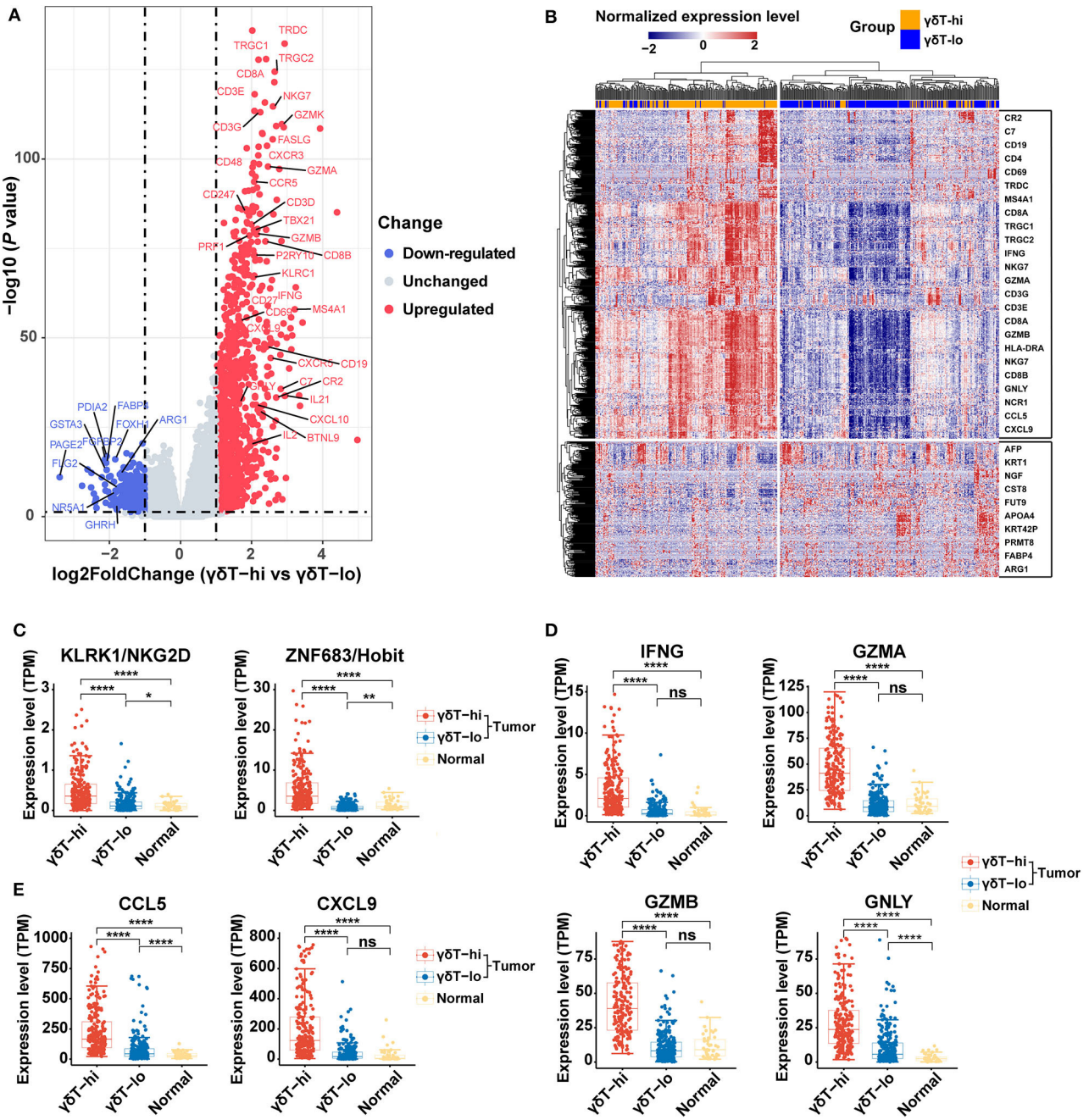

FIGURE 3 | Differentially expressed genes among the γδT-hi, γδT-lo and normal groups in HNSCC using R package DESeq2. **(A)** Volcano plot of $\log_2$ (Fold Change) vs. $-\log_{10}$ (*P*-value) shows the differentially expressed genes between the γδT-hi and γδT-lo groups. Red points represent the significantly upregulated genes in the γδT-hi group, blue points represent the significantly down-regulated genes in the γδT-hi group (upregulated in γδT-lo group), and gray points represent the genes without significant differences (unchanged). Dotted lines show a *P*-value of 0.05 (horizontal) and a fold change of ±2 (vertical). **(B)** Cluster heatmap showing the differential expressed genes between the γδT-hi (orange) and γδT-lo (light blue) groups. All of the expression values of each gene were rescaled to the range from −2 to 2 by Z-Score normalization. The red color in the heatmap indicates the genes that are upregulated, blue color indicates the genes that are down-regulated. Some representative genes were labeled in the boxes on the right side, the upper box contains some representative genes that are upregulated in the γδT-hi group, whereas the lower box contains some representative genes that are down-regulated in the γδT-hi group. **(C)** NKG2D (KLRK1) and Hobit (ZNF683) expression levels among the γδT-hi, γδT-lo and normal groups. **(D)** IFNG/GZMA/GZMB/GNLY expression levels among the three groups. **(E)** Expression levels of chemokines CCL5/CXCL9 among the three groups. Each dot represents the expression value (TPM) of the specific gene in each sample. *P*-values were calculated by student's *t*-test. ns, not significant; $^*P < 0.05$; $^{**}P < 0.01$; $^{****}P < 0.0001$.

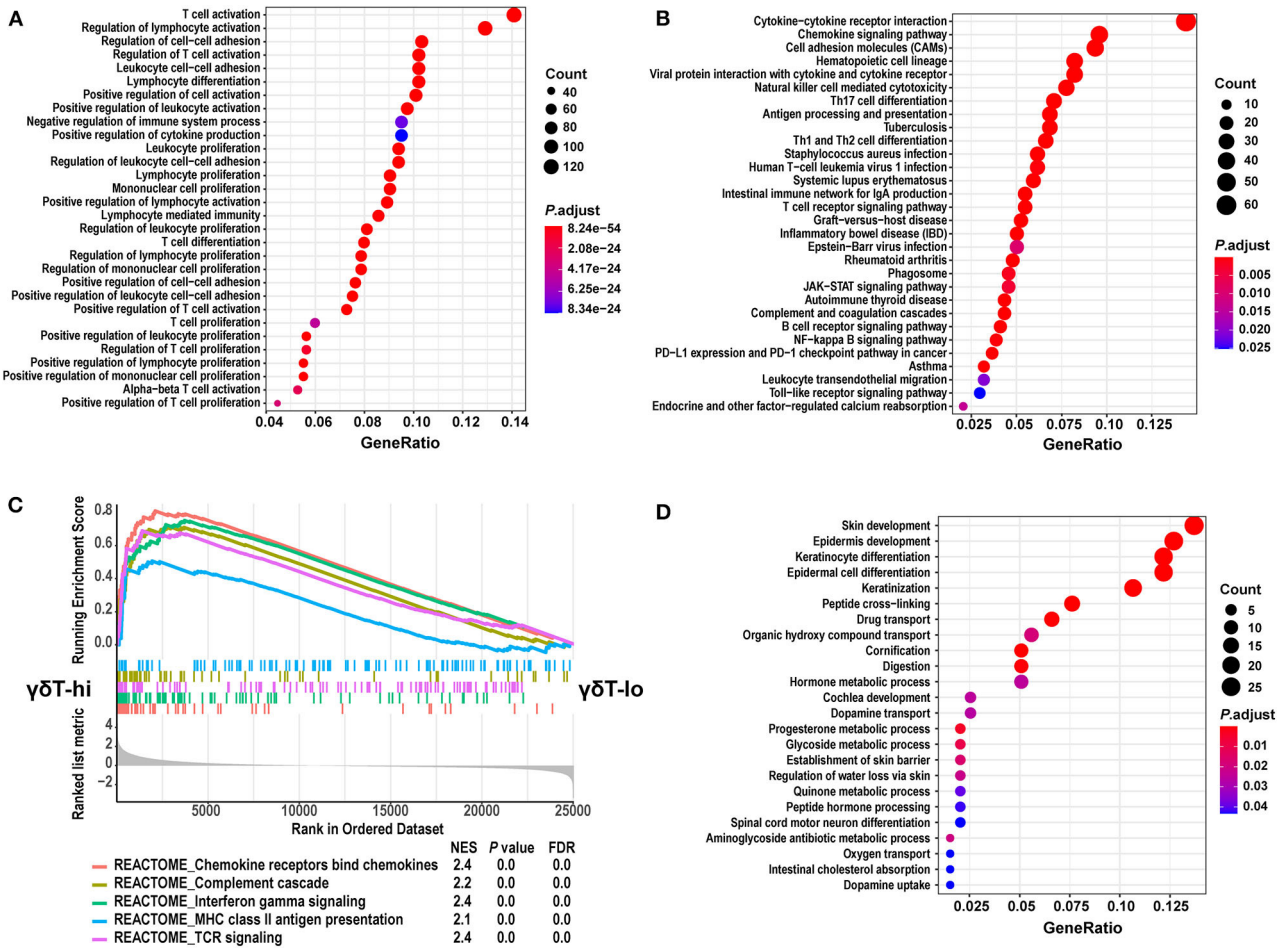

FIGURE 4 | GO and KEGG term enrichment analyses for the differentially expressed genes and GSEA analysis between the γδT-hi and γδT-lo groups. **(A)** The top 30 enrichment results from GO biological process terms in γδT-hi group. **(B)** The top 30 enrichment results from KEGG pathway terms in the γδT-hi group. **(C)** GSEA analysis shows the 5 REACTOME pathways that are enriched in the γδT-hi group. **(D)** The enrichment results from GO biological process terms in the γδT-lo group. GO and KEGG enrichment analyses were calculated by R package clusterProfiler (version: 3.16.1), the terms were not mutual excluded, adjusted *P*-values (P. adjust) were calculated by hypergeometric distribution. The size of dots indicates the count of significantly upregulated genes in γδT-hi or γδT-lo group that overlap with the specific geneset in GO or KEGG database, the color of dots indicates the adjusted *P*-values, red color indicates the smaller adjusted *P*-values. GeneRatio means the relative proportion of the overlapped genes in the specific dataset in GO or KEGG database. GSEA analysis was performed using the REACTOME database in MSigDB (version 7.1), and *P*-values for GSEA analyses were calculated by permutation tests. The lower part of **(C)** shows the genes that are ranked from the most upregulated in γδT-hi group (left side) to the most upregulated in γδT-lo group (right side). The middle part shows the distribution of genes in specific dataset in the ranked list. The upper part shows the running enrichment score of each dataset. NES, normalized enrichment score; FDR, false discovery rate.

a high infiltration of Vδ1 T cells is associated with a longer survival time, but in patients with breast cancer (47) or colorectal cancer (13), high infiltration of γδT cells is associated with poor prognosis. Our results have showed that patients with higher γδ T cell proportions were correlated with lower T stages, and a longer overall survival of HNSCC patients both in univariate and multivariate analyses, suggesting that γδ T cells may be involved in the antitumor immunity in HNSCC. However, a previous study has shown that there was no correlation between the proportions of γδ T cells and tumor stages in HNSCC patients (14), which is seemingly paradoxical with our results. The possible explanation is that the previous study only compared the γδ T cell abundance in peripheral blood, whereas γδ T cells in the TME were not analyzed. The composition of lymphocytes in the peripheral blood and the TME may be different, and due to the lack of RNA-seq data from the peripheral blood in HNSCC patients of TCGA dataset, it is not possible to explore whether γδ T cells in peripheral blood reflect those in the TME.

γδ T cells are considered as a bridge between innate and adaptive immune responses. Apart from direct tumor cell killing, γδ T cells interact with other innate and adaptive immune cells in the TME to exert indirect antitumor responses. IFN-γ secreted by γδ T cells promotes the upregulation of MHC-I molecules on tumor cells and positively regulates the antitumor function of CD8+ T cells (48). In addition, γδ T cells activate NK cells through the CD137/CD137L axis,

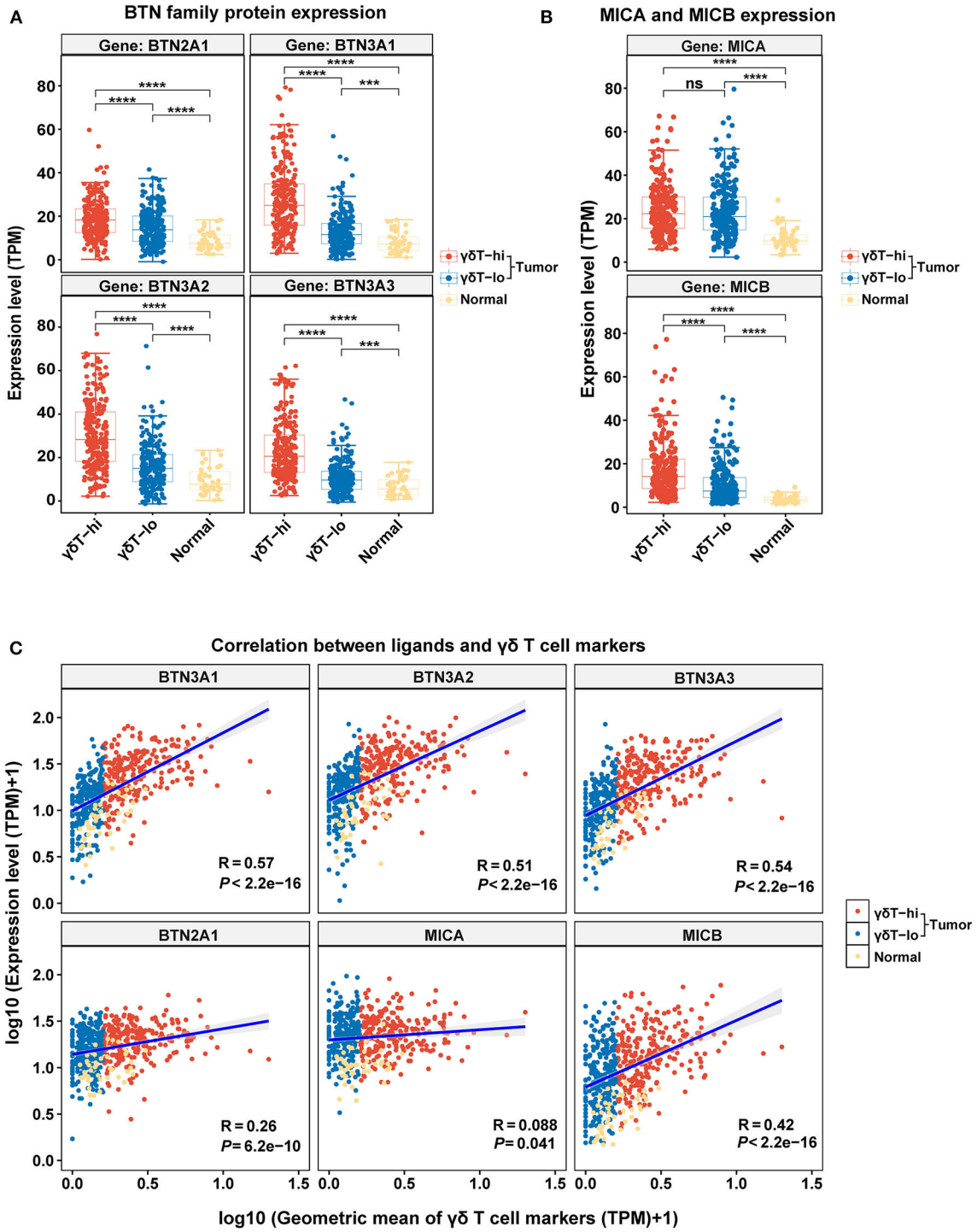

FIGURE 5 | The boxplots showing the expression levels of γδ T cell ligands among the γδT-hi, γδT-lo and normal groups and the correlation analysis between the γδT markers and ligands. **(A)** BTN2A1/BTN3A1/BTN3A2/BTN3A3 expression levels among the γδT-hi, γδT-lo and normal groups. **(B)** MICA/MICB expression levels among the γδT-hi, γδT-lo and normal groups. Each point represents the expression value (TPM) of the specific gene in each sample. *P*-values were calculated by student's *t*-test in **(A,B)**. **(C)** Linear regression analyses show the correlation between the ligands and the geometric mean of the γδT markers. For the linear regression analysis, all the expression data were transformed into $\log_{10}$ (TPM+1). Each point represents the expression value [$\log_{10}$ (TPM + 1)] of the specific gene in each sample. *R*-values and *P*-values were calculated by linear regression analyses. ns, not significant; ***$P < 0.001$; ****$P < 0.0001$.

promoting cytotoxicity in solid tumors (49). In the presence of P-Ags and interleukin 21, Vδ2 T cells can also express Tfh cell-related markers, such as ICOS, CD40L and CXCR5 and these Tfh-like γδ T cells (γδ Tfh) secrete cytokines, such as IL-4 and CXCL13, to increase antibody production of B cells (48). In addition, γδ T cells also serve as antigen presenting cells to activate CD8+ T cells and Th1 cells (37). Consistent with the previous research, our study found that the high abundance of γδ T cells was accompanied by the increase in CD8+ T cells, Th1 cells, Tfh cells, B cells, and other cell subsets. Moreover, through gene ontology and pathway enrichment analyses, it was indicated that γδ T cells might be involved in the activation of the IFN-γ signaling pathway, antigen presentation, chemokine secretion and other biological processes.

The unexpected discoveries in our study are that regulatory T cells, which are known as negative regulators of the immune response and contribute to tumor progression, accumulated in the γδT-hi group. Previous studies have revealed that higher Treg abundance was associated with shorter overall survival in renal cancer, breast cancer or melanoma (50), while high infiltration of Foxp3+ Treg cells in HNSCC was associated with an improved overall survival (51), and studies have revealed that higher CD8+ T cell or NK cell infiltration was usually accompanied by higher infiltration of Tregs in HNSCC (51); therefore it is hypothesized that Tregs are trafficked into the TME after CD8+ T and NK cells as a negative feedback to prevent the excessive inflammation mediated by CD8+ T cells and NK cells. In addition, we also discovered that the proportion of exhausted CD8+ T cells accumulated but that NKT cells were decreased in the γδT-hi group, which was seemingly paradoxical with our results. Although it is known that T cell exhaustion is a state in which antigen-specific CD8+ T cells undergo a progressive and hierarchical loss of effector functions during chronic antigen stimulation in the tumor microenvironment (52), this process does not occur in other bystander CD8+ T cells (53), and recent studies have also revealed that it is the exhausted T cells that exert the antitumor effect in an antigen-specific manner (53); previous studies have also discovered this phenomenon that HNSCC patients with higher expression of exhausted markers, including PDCD1, TIM3, and CD39, were associated with a better OS (54). Therefore, the more antigen-specific T cells that enter the TME, the greater chance they may terminally differentiate into exhausted T cells. For NKT cell, although it is an innate-like lymphocyte with invariant TCR that exerts potent antitumor immunity (55), the activation and expansion of NKT cells requires the presentation of α-galactosylceramide (α-GalCer) (56), a specific glycolipid antigen that is almost absent in the TME, and thus the expansion capacity of NKT cells in the TME is restrained. Therefore, compared to other T cell subsets that proliferate rapidly in the TME in the γδT-hi group, including CD8+ T cells, CD4+ T cells or γδ T cells, the number of NKT cells remains unchanged, resulting in the relatively lower proportions compared to the γδT-lo group.

As a novel group of type I transmembrane proteins of the immunoglobulin superfamily, the butyrophilin family of proteins shares a high homology with the B7 family proteins of the extracellular domains, suggesting that BTNs may also possess immunoregulatory functions (57). Among the BTN families, BTN1A1 and BTN2A2 have been reported to inhibit the activation and immune response of conventional T cells (58, 59). However, BTN3A1, along with BTN3A2 and BTN3A3, was required for P-Ag presentation and activation of Vδ2 T cells (18, 19). In addition, recent studies have revealed that BTN2A1 was another ligand that cooperated with BTN3A1 to activate Vδ2 T cells (20, 60).Studies have shown that a TP53 gene mutation would lead to the activation of the mevalonate pathway in cancer cells, resulting in the accumulation of isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP) in tumor cells (61). These metabolites would be taken up by BTNs and presented to Vδ2 T cells, leading to the activation of γδ T cells and an enhanced antitumor immune response (62, 63). Studies have shown that through binding to P-Ags presented by BTN3A1 and BTN2A1 on infected or malignant cells, γδ T cells could be activated, proliferate rapidly, and induce anti-infection or antitumor responses through IFN-γ production (20). Notably, it has been demonstrated that the B30.2 domain of BTN3As can bind P-Ag and drive the activation of Vδ2 T cells through conformational changes of the extracellular domains (64, 65), and periplakin and RhoB are the key proteins that play important roles in spatial rearrangement of BTN3As following intracellular P-Ag sensing (66–68). But how then do P-Ag enter the cells to initiate γδ T cell activation following binding to cytosolic B30.2 is needed to be clarified. Recent studies have found that a higher expression of BTN3A2 in ovarian cancer or triple negative breast cancer is positively correlated with an increased T cell infiltration and a better prognosis (23, 69). Our results, in line with previous studies, showed that γδ T cells in the TME might be activated in a butyrophilin-dependent manner, and mediated an antitumor response against HNSCC.

Apart from the interaction of the BTN family proteins and TCRγδ, the binding of NKG2D with NKG2DLs is the costimulatory signaling pathway that activates γδ T cells. It was reported that tumor cells expressing NKG2DLs (both MIC proteins and ULBP proteins) were more susceptible to γδ T cell-mediated lysis (70). However, although the expression level of NKG2D was much higher in the γδT-hi group, only MICB expression was upregulated in the γδT-hi group among the NKG2DLs, while MICA expression was not upregulated, with the ULBP family proteins showing the opposite results. The possible explanation for these results is that the activation of γδ T cells is not primarily through the NKG2DL-NKG2D pathway, and another possible explanation is that apart from the NKG2DLs expressed on tumor cells, the soluble NKG2DLs can also suppress the antitumor response of γδ T cells (71). The exact mechanisms of how NKG2DLs activate γδ T cells in head and neck cancer still need further exploration. Collectively, our results showed that γδ T cells might be activated and exert antitumor effects mainly through the recognition of BTN family proteins, which might be promising targets for γδ T-cell mediated immunotherapy.

In conclusion, our results showed that the abundance of γδ T cells in the tumors was positively associated with the improvement of prognosis in HNSCC patients. This antitumor effect might be attributed to the enhancement of γδ T cell-mediated cytotoxicity and the recruitment and activation of other antitumor lymphocytes. BTN2A1 and BTN3As might be the direct ligands that activate γδ T cells in head and neck cancers. Our results provide a new perspective of the HNSCC microenvironment, and provide potential targets for immunotherapy of HNSCC, which deserve further exploration.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

ZW contributed to conception and design of the study. HL contributed to the design of the study and acquisition and analysis of the data. WD, JG, and DW contributed to the analysis and interpretation of the data. SW, LY, DL, WX, LW, and JF contributed to the interpretation of the data. HL, WD, and JG wrote the first draft of the manuscript. DW, SW, LY, DL, and WX wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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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.2020.573920/full#supplementary-material

Supplementary Figure 1 | The distribution of T stages and overall survival curves between γδT-hi ($n = 139$) and γδT-lo ($n = 139$) groups and the immune cell abundances among the γδT-hi, γδT-lo and normal groups ($n = 3$) in the CESC dataset. **(A)** Five-year overall survival curves between the γδT-hi and γδT-lo groups. **(B)** The proportions of patients in each T stage (1–4) in the γδT-hi and γδT-lo groups. **(C)** The relative abundances of γδ T cells among the γδT-hi, γδT-lo and normal groups calculated by ImmuCellAI algorithm. **(D)** The relative abundances of CD4+ T cells and their subsets among the γδT-hi, γδT-lo and normal groups calculated by ImmuCellAI algorithm. **(E)** The relative abundances of CD8+ T cells and their subsets among the γδT-hi, γδT-lo and normal groups calculated by ImmuCellAI algorithm. **(F)** The relative abundances of B cells, NKT cells, MAIT, NK cells, dendritic cells, monocytes, macrophages and neutrophils among the γδT-hi, γδT-lo and normal groups calculated by ImmuCellAI algorithm. *P*-values were calculated by student's *t*-test. CD4 naïve, naïve CD4+ T cells; Th1, T helper cells type 1; Th2, T helper cells type 2; Th17, T helper cells type 17; Tfh, follicular helper T cells; iTreg, induced regulatory T cells; nTreg, natural regulatory T cells; Tr1, type 1 regulatory T cellss; CD8 naïve, naïve CD8+ T cell; Tcm, central memory T cells; Tem, effector memory T cells; Tc, cytotoxic T cells; Tex, exhausted T cells; NKT cells, natural killer T cells; MAIT, mucosal-associated invariant T cells; NK cells, natural killer cells. ns, not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

Supplementary Figure 2 | Relative abundance of cell types among the γδT-hi, γδT-lo and normal groups in HNSCC by using CIBERSORTx. **(A)** The proportions of lymphocyte subsets (B cells, T cells and NK cells) among the γδT-hi, γδT-lo and normal groups. **(B)** The relative proportions of myeloid cell subsets (monocytes, macrophages dendritic cells, mast cells, eosinophils and neutrophils) among the γδT-hi, γδT-lo and normal groups. *P*-values were calculated by student's *t*-test. ns, not significant; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

Supplementary Figure 3 | The expression levels of ULBP family proteins among the γδT-hi, γδT-lo and normal groups, and the association between BTN family proteins and 5-year OS. **(A)** The expression levels of ULBP1/ULBP2/ULBP3/RAET1E/RAET1G/RAET1L among the γδT-hi, γδT-lo and normal groups. Each point represents the expression value (TPM) of the specific gene in each sample. *P* values were calculated by student's *t*-test. **(B)** HNSCC patients were dichotomized into high and low group based on the median expression of BTN3A1/BTN3A2/BTN3A3/BTN2A1, and the OS curves were drawn for the high and low group, respectively. ns, not significant; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

REFERENCES

1. Peres MA, Macpherson LMD, Weyant RJ, Daly B, Venturelli R, Mathur MR, et al. Oral diseases: a global public health challenge. *Lancet.* (2019) 394:249–60. doi: 10.1016/S0140-6736(19)31146-8
2. Chow LQM. Head and neck cancer. *N Engl J Med.* (2020) 382:60–72. doi: 10.1056/NEJMra1715715
3. Chi AC, Day TA, Neville BW. Oral cavity and oropharyngeal squamous cell carcinoma–an update. *CA Cancer J Clin.* (2015) 65:401–21. doi: 10.3322/caac.21293
4. Ferris RL, Blumenschein G Jr, Fayette J, Guigay J, Colevas AD, et al. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. *N Engl J Med.* (2016) 375:1856–67. doi: 10.1056/NEJMoa1602252
5. Seiwert TY, Burtness B, Mehra R, Weiss J, Berger R, Eder JP, et al. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial. *Lancet Oncol.* (2016) 17:956–65. doi: 10.1016/S1470-2045(16)30066-3
6. Silva-Santos B, Mensurado S, Coffelt SB. gammadelta T cells: pleiotropic immune effectors with therapeutic potential in cancer. *Nat Rev Cancer.* (2019) 19:392–404. doi: 10.1038/s41568-019-0153-5
7. Nielsen MM, Witherden DA, Havran WL. gammadelta T cells in homeostasis and host defence of epithelial barrier tissues. *Nat Rev Immunol.* (2017) 17:733–45. doi: 10.1038/nri.2017.101
8. Zhao Y, Lin L, Xiao Z, Li M, Wu X, Li W, et al. Protective role of gammadelta t cells in different pathogen infections and its potential clinical application. *J Immunol Res.* (2018) 2018:5081634. doi: 10.1155/2018/5081634
9. Tanaka Y, Morita CT, Tanaka Y, Nieves E, Brenner MB, Bloom BR. Natural and synthetic non-peptide antigens recognized by human gamma delta T cells. *Nature.* (1995) 375:155–8. doi: 10.1038/375155a0
10. Poggi A, Venturino C, Catellani S, Clavio M, Miglino M, Gobbi M, et al. Vdelta1 T lymphocytes from B-CLL patients recognize ULBP3 expressed on leukemic B cells and up-regulated by trans-retinoic acid. *Cancer Res.* (2004) 64:9172–9. doi: 10.1158/0008-5472.CAN-04-2417
11. Gooden MJ, de Bock GH, Leffers N, Daemen T, Nijman HW. The prognostic influence of tumour-infiltrating lymphocytes in cancer: a systematic review with meta-analysis. *Br J Cancer.* (2011) 105:93–103. doi: 10.1038/bjc.2011.189

12. Paul S, Lal G. Regulatory and effector functions of gamma-delta (γδ) T cells and their therapeutic potential in adoptive cellular therapy for cancer. *Int J Cancer.* (2016) 139:976–85. doi: 10.1002/ijc.30109
13. Wu P, Wu D, Ni C, Ye J, Chen W, Hu G, et al. γδT17 cells promote the accumulation and expansion of myeloid-derived suppressor cells in human colorectal cancer. *Immunity.* (2014) 40:785–800. doi: 10.1016/j.immuni.2014.03.013
14. Bas M, Bier H, Schirlau K, Friebe-Hoffmann U, Scheckenbach K, Balz V, et al. Gamma-delta T-cells in patients with squamous cell carcinoma of the head and neck. *Oral Oncol.* (2006) 42:691–7. doi: 10.1016/j.oraloncology.2005.11.008
15. Vermijlen D, Gatti D, Kouzeli A, Rus T, Eberl M. gammadelta T cell responses: how many ligands will it take till we know? *Semin Cell Dev Biol.* (2018) 84:75–86. doi: 10.1016/j.semcdb.2017.10.009
16. Blazquez JL, Benyamine A, Pasero C, Olive D. New insights into the regulation of gammadelta T cells by BTN3A and other BTN/BTNL in tumor immunity. *Front Immunol.* (2018) 9:1601. doi: 10.3389/fimmu.2018.01601
17. Abeler-Dorner L, Swamy M, Williams G, Hayday AC, Bas A. Butyrophilins: an emerging family of immune regulators. *Trends Immunol.* (2012) 33:34–41. doi: 10.1016/j.it.2011.09.007
18. Vavassori S, Kumar A, Wan GS, Ramanjaneyulu GS, Cavallari M, El Daker S, et al. Butyrophilin 3A1 binds phosphorylated antigens and stimulates human gammadelta T cells. *Nat Immunol.* (2013) 14:908–16. doi: 10.1038/ni.2665
19. Vantourout P, Laing A, Woodward MJ, Zlatareva I, Apolonia L, Jones AW, et al. Heteromeric interactions regulate butyrophilin (BTN) and BTN-like molecules governing gammadelta T cell biology. *Proc Natl Acad Sci USA.* (2018) 115:1039–44. doi: 10.1073/pnas.1701237115
20. Rigau M, Ostrouska S, Fulford TS, Johnson DN, Woods K, Ruan Z, et al. Butyrophilin 2A1 is essential for phosphoantigen reactivity by gammadelta T cells. *Science.* (2020) 367:aay5516. doi: 10.1126/science.aay5516
21. Decaup E, Duault C, Bezombes C, Poupot M, Savina A, Olive D, et al. Phosphoantigens and butyrophilin 3A1 induce similar intracellular activation signaling in human TCRVγ9+ γδ T lymphocytes. *Immunol Lett.* (2014) 161:133–7. doi: 10.1016/j.imlet.2014.05.011
22. Benyamine A, Loncle C, Foucher E, Blazquez JL, Castanier C, Chrétien AS, et al. BTN3A is a prognosis marker and a promising target for Vγ9Vδ2 T cells based-immunotherapy in pancreatic ductal adenocarcinoma (PDAC). *Oncoimmunology.* (2017) 7:e1372080. doi: 10.1080/2162402X.2017.1372080
23. Cai P, Lu Z, Wu J, Qin X, Wang Z, Zhang Z, et al. BTN3A2 serves as a prognostic marker and favors immune infiltration in triple-negative breast cancer. *J Cell Biochem.* (2020) 121:2643–54. doi: 10.1002/jcb.29485
24. Zhu M, Yan C, Ren C, Huang X, Zhu X, Gu H, et al. Exome array analysis identifies variants in SPOCD1 and BTN3A2 that affect risk for gastric cancer. *Gastroenterology.* (2017) 152:2011–21. doi: 10.1053/j.gastro.2017.02.017
25. Chitadze G, Lettau M, Luecke S, Wang T, Janssen O, Furst D, et al. NKG2D- and T-cell receptor-dependent lysis of malignant glioma cell lines by human gammadelta T cells: modulation by temozolomide and A disintegrin and metalloproteases 10 and 17 inhibitors. *Oncoimmunology.* (2016) 5:e1093276. doi: 10.1080/2162402X.2015.1093276
26. Simoes AE, Di Lorenzo B, Silva-Santos B. Molecular determinants of target cell recognition by human gammadelta T Cells. *Front Immunol.* (2018) 9:929. doi: 10.3389/fimmu.2018.00929
27. Edge SB, Compton CC. The american joint committee on cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol.* (2010) 17:1471–4. doi: 10.1245/s10434-010-0985-4
28. Wagner GP, Kin K, Lynch VJ. Measurement of mRNA abundance using RNA-seq data: RPKM measure is inconsistent among samples. *Theory Biosci.* (2012) 131:281–5. doi: 10.1007/s12064-012-0162-3
29. Dangaj D, Bruand M, Grimm AJ, Ronet C, Barras D, Duttagupta PA, et al. Cooperation between constitutive and inducible chemokines enables t cell engraftment and immune attack in solid tumors. *Cancer Cell.* (2019) 35:885–900 e10. doi: 10.1016/j.ccell.2019.05.004
30. Miao YR, Zhang Q, Lei Q, Luo M, Xie GY, Wang H, et al. ImmuCellAI: a unique method for comprehensive t-cell subsets abundance prediction and its application in cancer immunotherapy. *Adv Sci.* (2020) 7:1902880. doi: 10.1002/advs.201902880
31. Newman AM, Steen CB, Liu CL, Gentles AJ, Chaudhuri AA, Scherer F, et al. Determining cell type abundance and expression from bulk tissues with digital cytometry. *Nat Biotechnol.* (2019) 37:773–82. doi: 10.1038/s41587-019-0114-2
32. Qian BZ, Li J, Zhang H, Kitamura T, Zhang J, Campion LR, et al. CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. *Nature.* (2011) 475:222–5. doi: 10.1038/nature10138
33. Zhou SL, Zhou ZJ, Hu ZQ, Huang XW, Wang Z, Chen EB, et al. Tumor-Associated neutrophils recruit macrophages and t-regulatory cells to promote progression of hepatocellular carcinoma and resistance to sorafenib. *Gastroenterology.* (2016) 150:1646–58.e17. doi: 10.1053/j.gastro.2016.02.040
34. Chung AS, Wu X, Zhuang G, Ngu H, Kasman I, Zhang J, et al. An interleukin-17-mediated paracrine network promotes tumor resistance to anti-angiogenic therapy. *Nat Med.* (2013) 19:1114–23. doi: 10.1038/nm.3291
35. Oja AE, Vieira Braga FA, Remmerswaal EB, Kragten NA, Hertoghs KM, Zuo J, et al. The transcription factor hobit identifies human cytotoxic CD4(+) T Cells. *Front Immunol.* (2017) 8:325. doi: 10.3389/fimmu.2017.00325
36. Ajuebor MN, Jin Y, Gremillion GL, Strieter RM, Chen Q, Adegboyega PA. GammadeltaT cells initiate acute inflammation and injury in adenovirus-infected liver via cytokine-chemokine cross talk. *J Virol.* (2008) 82:9564–76. doi: 10.1128/JVI.00927-08
37. Moser B, Eberl M. gammadelta T-APCs: a novel tool for immunotherapy? *Cell Mol Life Sci.* (2011) 68:2443–52. doi: 10.1007/s00018-011-0706-6
38. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol.* (2013) 14:1014–22. doi: 10.1038/ni.2703
39. Gentles AJ, Newman AM, Liu CL, Bratman SV, Feng W, Kim D, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nat Med.* (2015) 21:938–45. doi: 10.1038/nm.3909
40. Meraviglia S, Eberl M, Vermijlen D, Todaro M, Buccheri S, Cicero G, et al. *In vivo* manipulation of Vgamma9Vdelta2 T cells with zoledronate and low-dose interleukin-2 for immunotherapy of advanced breast cancer patients. *Clin Exp Immunol.* (2010) 161:290–7. doi: 10.1111/j.1365-2249.2010.04167.x
41. Li H, Pauza CD. Rapamycin increases the yield and effector function of human gammadelta T cells stimulated *in vitro*. *Cancer Immunol Immunother.* (2011) 60:361–70. doi: 10.1007/s00262-010-0945-7
42. Xiang Z, Tu W. Dual face of Vγ9Vδ2-T cells in tumor immunology: anti- versus pro-tumoral activities. *Front Immunol.* (2017) 8:1041. doi: 10.3389/fimmu.2017.01041
43. Caccamo N, Todaro M, Sireci G, Meraviglia S, Stassi G, Dieli F. Mechanisms underlying lineage commitment and plasticity of human γδ T cells. *Cell Mol Immunol.* (2013) 10:30–4. doi: 10.1038/cmi.2012.42
44. Wesch D, Peters C, Siegers GM. Human gamma delta T regulatory cells in cancer: fact or fiction? *Front Immunol.* (2014) 5:598. doi: 10.3389/fimmu.2014.00598
45. Cai XY, Wang JX, Yi Y, He HW, Ni XC, Zhou J, et al. Low counts of γδ T cells in peritumoral liver tissue are related to more frequent recurrence in patients with hepatocellular carcinoma after curative resection. *APJCP.* (2014) 15:775–80. doi: 10.7314/APJCP.2014.15.2.775
46. Wang J, Lin C, Li H, Li R, Wu Y, Liu H, et al. Tumor-infiltrating γδT cells predict prognosis and adjuvant chemotherapeutic benefit in patients with gastric cancer. *Oncoimmunology.* (2017) 6:e1353858. doi: 10.1080/2162402X.2017.1353858
47. Ma C, Zhang Q, Ye J, Wang F, Zhang Y, Wevers E, et al. Tumor-infiltrating γδ T lymphocytes predict clinical outcome in human breast cancer. *J Immunol.* (2012) 189:5029–36. doi: 10.4049/jimmunol.1201892
48. Paul S, Singh AK, Shilpi, Lal G. Phenotypic and functional plasticity of gamma-delta (γδ) T cells in inflammation and tolerance. *Int Rev Immunol.* (2014) 33:537–58. doi: 10.3109/08830185.2013.863306
49. Maniar A, Zhang X, Lin W, Gastman BR, Pauza CD, Strome SE, et al. Human gammadelta T lymphocytes induce robust NK cell-mediated antitumor cytotoxicity through CD137 engagement. *Blood.* (2010) 116:1726–33. doi: 10.1182/blood-2009-07-234211
50. Shang B, Liu Y, Jiang SJ, Liu Y. Prognostic value of tumor-infiltrating FoxP3+ regulatory T cells in cancers: a systematic review and meta-analysis. *Sci Rep.* (2015) 5:15179. doi: 10.1038/srep15179
51. Mandal R, Senbabaoglu Y, Desrichard A, Havel JJ, Dalin MG, Riaz N, et al. The head and neck cancer immune landscape and its immunotherapeutic implications. *JCI Insight.* (2016) 1:e89829. doi: 10.1172/jci.insight.89829

52. Wherry EJ. T cell exhaustion. *Nat Immunol.* (2011) 12:492–9. doi: 10.1038/ni.2035
53. Simoni Y, Becht E, Fehlings M, Loh CY, Koo SL, Teng KWW, et al. Bystander CD8(+) T cells are abundant and phenotypically distinct in human tumour infiltrates. *Nature.* (2018) 557:575–9. doi: 10.1038/s41586-018-0130-2
54. Gameiro SF, Ghasemi F, Barrett JW, Koropatnick J, Nichols AC, Mymryk JS, et al. Treatment-naive HPV+ head and neck cancers display a T-cell-inflamed phenotype distinct from their HPV- counterparts that has implications for immunotherapy. *Oncoimmunology.* (2018) 7:e1498439. doi: 10.1080/2162402X.2018.1498439
55. Fujii SI, Shimizu K. Immune networks and therapeutic targeting of iNKT cells in cancer. *Trends Immunol.* (2019) 40:984–97. doi: 10.1016/j.it.2019.09.008
56. Zhang Y, Springfield R, Chen S, Li X, Feng X, Moshirian R, et al. alpha-GalCer and iNKT cell-based cancer immunotherapy: realizing the therapeutic potentials. *Front Immunol.* (2019) 10:1126. doi: 10.3389/fimmu.2019.01126
57. Arnett HA, Viney JL. Immune modulation by butyrophilins. *Nat Rev Immunol.* (2014) 14:559–69. doi: 10.1038/nri3715
58. Smith IA, Knezevic BR, Ammann JU, Rhodes DA, Aw D, Palmer DB, et al. BTN1A1, the mammary gland butyrophilin, and BTN2A2 are both inhibitors of T cell activation. *J Immunol.* (2010) 184:3514–25. doi: 10.4049/jimmunol.0900416
59. Ammann JU, Cooke A, Trowsdale J. Butyrophilin Btn2a2 inhibits TCR activation and phosphatidylinositol 3-kinase/Akt pathway signaling and induces Foxp3 expression in T lymphocytes. *J Immunol.* (2013) 190:5030–6. doi: 10.4049/jimmunol.1203325
60. Karunakaran MM, Willcox CR, Salim M, Paletta D, Fichtner AS, Noll A, et al. Butyrophilin-2A1 directly binds germline-encoded regions of the Vgamma9Vdelta2 TCR and is essential for phosphoantigen sensing. *Immunity.* (2020) 52:487–98.e6. doi: 10.1016/j.immuni.2020.02.014
61. Freed-Pastor WA, Mizuno H, Zhao X, Langerod A, Moon SH, Rodriguez-Barrueco R, et al. Mutant p53 disrupts mammary tissue architecture via the mevalonate pathway. *Cell.* (2012) 148:244–58. doi: 10.1016/j.cell.2011.12.017
62. Gober HJ, Kistowska M, Angman L, Jeno P, Mori L, De Libero G. Human T cell receptor gammadelta cells recognize endogenous mevalonate metabolites in tumor cells. *J Exp Med.* (2003) 197:163–8. doi: 10.1084/jem.20021500
63. Gruenbacher G, Nussbaumer O, Gander H, Steiner B, Leonhartsberger N, Thurnher M. Stress-related and homeostatic cytokines regulate Vgamma9Vdelta2 T-cell surveillance of mevalonate metabolism. *Oncoimmunology.* (2014) 3:e953410. doi: 10.4161/21624011.2014.953410
64. Harly C, Guillaume Y, Nedellec S, Peigne CM, Monkkonen H, Monkkonen J, et al. Key implication of CD277/butyrophilin-3 (BTN3A) in cellular stress sensing by a major human gammadelta T-cell subset. *Blood.* (2012) 120:2269–79. doi: 10.1182/blood-2012-05-430470
65. Sandstrom A, Peigne CM, Leger A, Crooks JE, Konczak F, Gesnel MC, et al. The intracellular B30.2 domain of butyrophilin 3A1 binds phosphoantigens to mediate activation of human Vgamma9Vdelta2 T cells. *Immunity.* (2014) 40:490–500. doi: 10.1016/j.immuni.2014.03.003
66. Yang Y, Li L, Yuan L, Zhou X, Duan J, Xiao H, et al. A structural change in butyrophilin upon phosphoantigen binding underlies phosphoantigen-mediated Vgamma9Vdelta2 T Cell Activation. *Immunity.* (2019) 50:1043–53 e5. doi: 10.1016/j.immuni.2019.02.016
67. Sebestyen Z, Scheper W, Vyborova A, Gu S, Rychnavska Z, Schiffler M, et al. RhoB mediates phosphoantigen recognition by Vgamma9Vdelta2 T Cell Receptor. *Cell Rep.* (2016) 15:1973–85. doi: 10.1016/j.celrep.2016.04.081
68. Rhodes DA, Chen HC, Price AJ, Keeble AH, Davey MS, James LC, et al. Activation of human gammadelta T cells by cytosolic interactions of BTN3A1 with soluble phosphoantigens and the cytoskeletal adaptor periplakin. *J Immunol.* (2015) 194:2390–8. doi: 10.4049/jimmunol.1401064
69. Le Page C, Marineau A, Bonza PK, Rahimi K, Cyr L, Labouba I, et al. BTN3A2 expression in epithelial ovarian cancer is associated with higher tumor infiltrating T cells and a better prognosis. *PLoS ONE.* (2012) 7:e38541. doi: 10.1371/journal.pone.0038541
70. Wrobel P, Shojaei H, Schittek B, Gieseler F, Wollenberg B, Kalthoff H, et al. Lysis of a broad range of epithelial tumour cells by human gamma delta T cells: involvement of NKG2D ligands and T-cell receptor- versus NKG2D-dependent recognition. *Scand J Immunol.* (2007) 66:320–8. doi: 10.1111/j.1365-3083.2007.01963.x
71. Marten A, von Lilienfeld-Toal M, Buchler MW, Schmidt J. Soluble MIC is elevated in the serum of patients with pancreatic carcinoma diminishing gammadelta T cell cytotoxicity. *Int J Cancer.* (2006) 119:2359–65. doi: 10.1002/ijc.22186

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MINI REVIEW
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The Role of Gamma-Delta T Cells in Diseases of the Central Nervous System

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Gamma-delta (γδ) T cells are a subset of T cells that promote the inflammatory responses of lymphoid and myeloid lineages, and are especially vital to the initial inflammatory and immune responses. Given the capability to connect crux inflammations of adaptive and innate immunity, γδ T cells are responsive to multiple molecular cues and can acquire the capacity to induce various cytokines, such as GM-CSF, IL-4, IL-17, IL-21, IL-22, and IFN-γ. Nevertheless, the exact mechanisms responsible for γδ T cell proinflammatory functions remain poorly understood, particularly in the context of the central nervous system (CNS) diseases. CNS disease, usually leading to irreversible cognitive and physical disability, is becoming a worldwide public health problem. Here, we offer a review of the neuro-inflammatory and immune functions of γδ T cells, intending to understand their roles in CNS diseases, which may be crucial for the development of novel clinical applications.

Keywords: γδ T cell, Th17 cell, cytokines, inflammation, central nervous system, IL-17

INTRODUCTION

Together, gamma-delta (γδ) and alpha-beta (αβ) T cells represent two different T cell lineages that have been defined by their expression of αβ or γδ T cell receptors (TCRs) (1). Although γδ T cells share many effector capabilities with αβ T cells (for example, cytotoxicity and cytokine production), the lineages exhibit different biological properties, such as thymic-dependent or -independent development, major histocompatibility complex (MHC) restriction, and recognition of soluble protein and non-protein antigens of endogenous origin (2–5).

Unlike αβ T cells, γδ T cells are a relatively minor subset of T lymphocytes in the peripheral blood (PB), comprising only 1–5% of lymphocytes circulating (6). However, γδ T cells are abundant at barrier sites such as the skin, gut, lung, and reproductive tract; up to 20% of intraepithelial lymphocytes in the human colon express the γδ TCRs (7).

γδ T cells are divided according to the type of Vγ and Vδ chain they express at the TCRs. Concerning the Vγ chains, a unique feature of murine γδ T cells is the preferential expression of different Vγ segments in different tissues. For example, $V\gamma5^+$ γδ T cells are present in the skin, $V\gamma7^+$ γδ T cells lie in the intestinal, $V\gamma6^+$ γδ T cells localize to the reproductive mucosa, and $V\gamma1^+$ or $V\gamma4^+$ γδ T cells are found in secondary lymphoid organs (8, 9). The previous studies related to human γδ T cells have identified Vγ9 as the most frequently used Vγ chain in the PB (10). Vγ9 chain associates

with Vδ2 in most cases, defining a Vγ9Vδ2 T cell population (account for 50–95% of γδ T cells in the PB) that is unique to humans and other primates (11, 12).

Vγ9Vδ2 T cells are known to identify microbe-derived [HMBPP, (E)-4-hydroxy-3methyl-but-2-enyl pyrophosphate] and host-derived (IPP, isopentenyl pyrophosphate) phosphorylated metabolites originating from the isoprenoid metabolic mevalonate and non-mevalonate pathways, through association with butyrophilin 3A1 (BTN3A1) and BTN3A2 (13–16). Moreover, $V\delta1^+$ γδ T cells frequently coexpress functional receptors of innate immune cells, such as activating natural killer (NK) receptors such as NKG2D (17–20). It includes MHC class I polypeptide-related chains (MIC) A and B, and UL16 binding proteins (ULBP) (21–24). Although first described for $V\delta1^+$ γδ T cells, interactions of the ULBP and MIC-A/B molecules with NKG2D are now recognized to stimulate $V\delta2^+$ γδ T cells (21, 22). Besides, $V\delta1^+$ γδ T cells recognize lipids and glycolipids presented by CD1 molecules (25, 26). Furthermore, both $V\delta1^+$ and $V\delta2^+$ γδ T cells are activated by heat shock proteins (HSP) (27–29).

Recently, some discrete population of T cells that coexpressed αβ-γδ TCRs and Vγ-Cβ TCRs have been identified (30, 31). Among them, the αβ-γδ T cells protected against infection by licensing encephalitogenic Th17 cells, triggered inflammatory and immune in the central nervous system (CNS). Moreover, our research group found that, in addition to diseases of the CNS, such as multiple sclerosis (MS) and stroke, immune responses induced by γδ T cells are also critically implicated in neuroinflammation associated with spinal cord injury (SCI) (32–34). These findings raise significant questions concerning the inflammatory and immune functions of γδ T cells in CNS disease that have yet to be addressed (35–37). CNS disease, which can result in irreversible sensory, motor, and autonomic impairments, is a severe health problem worldwide. As a central pathological process in CNS diseases, the inflammatory response is vital to clinical prognosis. Here, we provide a review of recent advances in the understanding of γδ T cells with relevance to their inflammatory and immune roles in CNS disease, which suggest potential approaches for future treatment of CNS diseases (**Table 1**).

PROINFLAMMATORY CYTOKINES INDUCED BY γδ T CELLS IN THE CNS

Activation and development of γδ T cells promoting CNS inflammation are chiefly mediated by dendritic cells (DCs). The immunostimulatory component induces IL-1β, IL-6, IL-18, and IL-23 by DCs *via* caspase-1 and inflammasome complex. γδ T cells secrete IL-17 in response to IL-1β, IL-18, and IL-23 in the absence of TCR (38–40). During this process, the retinoid-related orphan receptor (ROR) -γt and IL-7 coordinate the B and T lymphocyte attenuator (BTLA) expression, thus regulating γδ T cell inflammatory responses (41–44). Moreover, Shibata et al. demonstrated that signal transducer and activator of transcription 3 (STAT3) is dispensable for the development of IL-17-producing γδ T (γδT17) cells (45). Also, IL-23-activated γδ T cells suppress the factor forkhead box $P3^+$ (Foxp3) -expressing Treg cells conversion, as well as promoting effector T (Te) cells response (46, 47). The capacity of γδ T cells to produce a burst of IL-17 in the absence of activated αβ T cells is crucial for the initiation of CNS inflammation (48).

Activated DCs also promotes the induction of other proinflammatory cytokines from γδ T cells, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-21, and IL-22 (30, 40) (**Figure 1**). While IL-17A, IL-17F, and IL-22 are prominently expressed in CNS inflammation, they may only marginally contribute to disease development (49–51); however, McGinley et al. recently demonstrated that IL-17 might recruit IL-1β-secreting myeloid cells that prime pathogenic γδ T cells in CNS inflammation (52).

Different from γδ T cells, which can produce IL-17 in response to cytokine (IL-1β, IL-18, and IL-23) signals alone, in the absence of primary (TCR) and secondary (costimulation) signals, IL-17-producing T helper (Th17) cells require primary, secondary, and cytokine (IL-6 and TGF-β) signals to generate IL-17 (40) (**Figure 1**). Seminal studies demonstrated that IL-6 and TGF-β induce Th17 cell differentiation, in which TGF-β is critical for T cells to differentiate into $Foxp3^+$ Treg or Th17 cells (53–58). Moreover, TGF-β is also critical to γδT17 cells (59). Besides, IL-21 is induced by IL-6 in Th17 cells, which establishes a feed-forward loop to support Th17 cell amplification, in which STAT3 and ROR-γt mediate lineage specification (54, 55, 60–63).

During this process, IL-23 acts as a maturation factor for Th17 cells, and both IL-23 and IL-21 can induce IL-17 expression independently of IL-6 (55, 64–66). Therefore, mice lacking IL-23 are resistant to Th17-mediated CNS inflammation (46). To demonstrate the role of IL-23, Awasthi et al. substituted the green fluorescent protein for the intracellular domain of IL-23R, to generate a "knock-in" mouse, which demonstrated that IL-23 is crucial for Th17 cell function (67). IL-23 created a positive feedback loop, whereby GM-CSF secreted by Th17 cells induced the generation of IL-23 (68, 69).

Alongside IL-17, GM-CSF is also essential for CNS inflammation. Further, the activation of the microglial cell, but not macrophage in the periphery, is a GM-CSF-dependent process (70). El-Behi et al. demonstrated that GM-CSF neutralization attenuated CNS inflammation (68). Although both IL-12 and IL-23 can induce Te cells to generate GM-CSF, IL-23 is crucially required for GM-SCF generation (69, 71). In addition to DCs and Th17 cells, γδ T cells generate large amounts of GM-CSF, resulting in neuroinflammation (72).

γδ T CELLS IN CNS DISEASES

Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis

MS is a chronic inflammatory demyelinating CNS disease, resulting in progressive cognitive, sensory, and motor disorders. Experimental autoimmune encephalomyelitis (EAE), a murine MS model, is used to research the proinflammatory

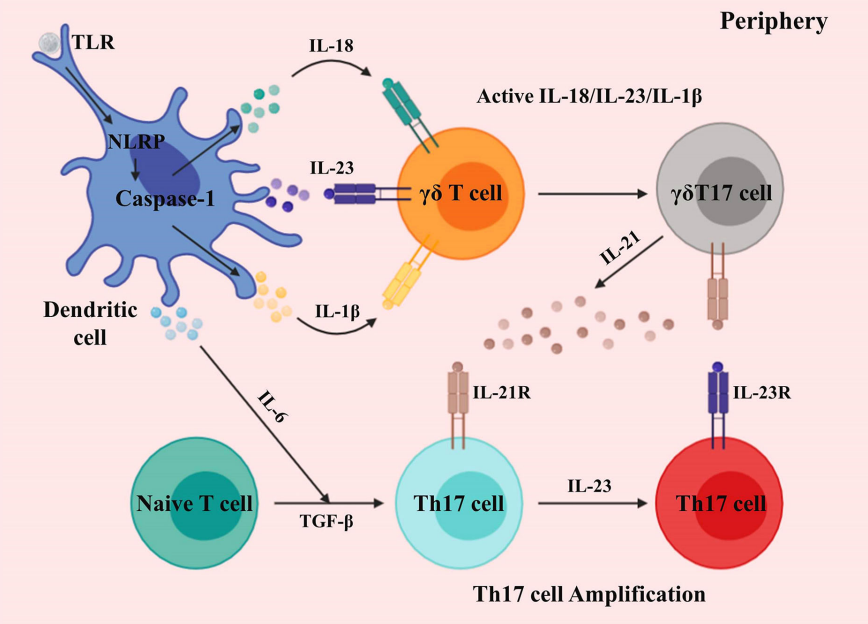

FIGURE 1 | Activation and development of γδ T cells in the periphery. Differentiated dendritic cells and macrophages generate proinflammatory cytokines *via* toll-like and NOD-like receptors. γδ T cells sense IL-1β, IL-18, and IL-23, producing an initial burst of IL-17. The differentiation of Th17 cells is induced by IL-6 and TGF-β. γδT17 cells secrete IL-21, which further amplifies their proliferation, and also that of Th17 cells.

mechanism underlying CNS (73). Before the discovery of Th17 cells, IFN-γ-producing Th1 cells were considered the primary pathogenic cell inducing MS and EAE, which puzzled immunologists for many years, since both IFN-$\gamma^{-/-}$ and IFN-$\gamma R^{-/-}$ mice enhanced EAE development (74–77). Besides, deficiencies of IL-12 and IL-12R, which are critical to the development of Th1 cells, also exhibited exacerbated EAE (64). Together, findings to date indicate that Th1 cells are not the initial T cell involved in EAE. IL-12 and IFN-γ (Th1-associated molecules) negatively regulate tissue inflammation in EAE (78). Nonetheless, Th1 cells are vital to EAE, as they are detected in active EAE.

Subsequently, the identification of IL-23 and Th17 cells partly worked out this issue (61, 65) (**Figure 2**). IL-23p40$^{-/-}$ and IL-23p19$^{-/-}$ mouse strains are both resistant to EAE (64). The depletion of Th17 cells or IL-17 resulted in reduced EAE severity (78). Although Th17 cells are thought to be the major mediators of EAE, γδT cells can also be a potent producer of IL-17, and are dominant over Th17 cells in CNS inflammation (79). Several researchers demonstrated that γδ T cells are frequently present in the peripheral blood (PB) and cerebrospinal fluid (CSF) of MS patients, as well as in the brains of mice with EAE (27, 28, 80–82). During the chronic and acute phases of EAE, the absence of γδ T cells notably reduces the CNS inflammation, suggesting that γδ T cells are significant in EAE, and their inflammatory mobilization is related to the pathogenesis of CNS autoimmunity (83–87). Indeed, an enormous population of CD4$^+$ T cells (IL-17 and IFN-γ double-positive) is observed at the peak of EAE (88). Using a fate-tracking system, 5–10% of γδT17 cells were shown to express IFN-γ in the CNS, indicating that IL-17-IFN-γ-γδ T cells might be consequential intermediates in EAE pathogenesis (89).

Moreover, Vγ4$^+$ γδ T cells were identified as the major γδT17 cells in EAE, while Vγ5$^+$ and Vδ6$^+$ γδ T cells were present (40). Vδ1$^+$, Vδ2$^+$, and Vγ9$^+$ γδ T cells were also observed in acute demyelinating plaques of MS patients (27, 90). Besides, the biological drugs designed to suppress the activity of γδ T cells, such as fingolimod (FTY720) and Natalizumab, partly contribute to the clinical therapeutic effects of MS. (90–92). Further, HSP60 and HSP90 compared with normal CNS tissues are overexpressed in MS plaques, while Selmaj et al. showed the colocalization of HSP65 and γδ T cells in immature oligodendrocytes in MS lesions (27, 80, 93). The multitude of γδ T cells collected from MS patients proliferated in response to HSP70, but not to HSP65, revealing that HSPs may be the antigens responsible for promoting the γδ T cells proliferation (28). CNS inflammation is associated with altered expression of HSPs, which may function as targets in the development of the chronic disease. Interestingly, IL-15-producing γδ T (γδT15) cells, another subset of γδ T cell, were recently discovered; however, whether these cells produce other proinflammatory cytokines in EAE is not well elucidated (94).

In contrast to the above observations, γδ T cells are also reported have a protective function in EAE. Ponomarev et al. reported that γδ T cells of wild-type (WT) reconstitute γδ T cell$^{-/-}$ mice, but not FasL dysfunctional γδ T cells, diminishing inflammation in EAE (95). These findings suggest that the γδ T cell-mediated Fas/FasL-induced T cells apoptosis regulates CNS

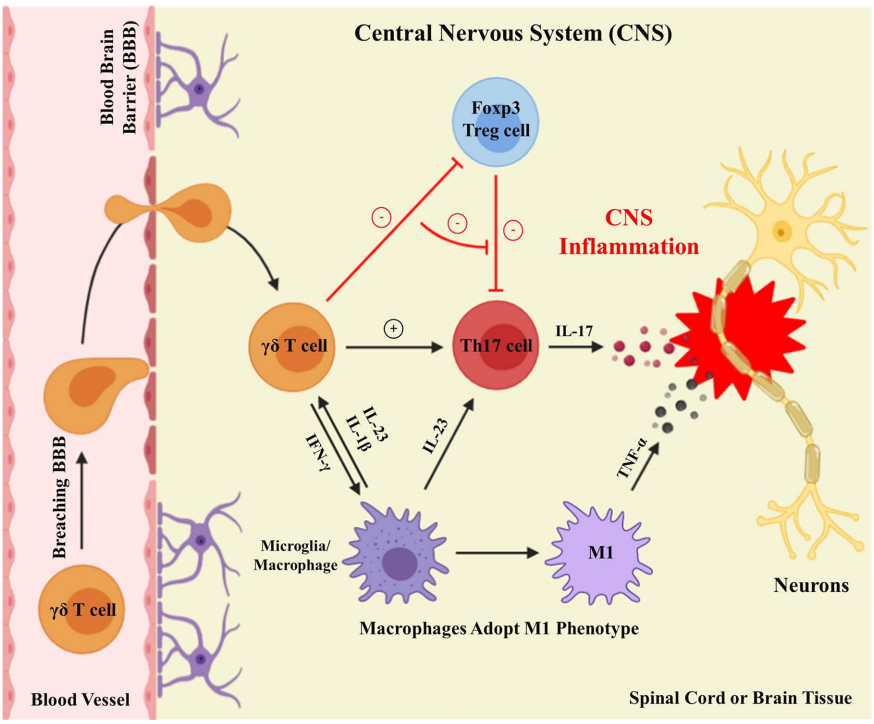

FIGURE 2 | γδ T cells execute proinflammatory functions in the CNS. Activated γδ T cells breach the blood-brain barrier to carry out proinflammatory functions in the CNS. Differentiated microglia/macrophages secrete IL-23 within the CNS to facilitate the production of γδ T cells and Th17 cells. γδ T cells result in CNS inflammation by improving Th17 cell effector functions, restraining Tregs cell suppressive functions, and generating IFN-γ, to induce M1 phenotype macrophages secreting TNF-α.

inflammation. Indeed, the mechanism by which γδ T cells regulate proinflammatory chemokine and cytokine expression in CNS, as well as infiltrating cell heterogeneity, warrant detailed investigation.

Ischemic Brain Injury

The main consequence of ischemic brain injury is manifested as the CNS tissue necrosis, due to the loss of nutrition. The tissue necrosis leads to a secondary inflammation, involving the accumulation of specific immune cells, especially neutrophils, macrophages, and T cells, which is a critical factor to the entire pathophysiology (96, 97).

IL-17 has a specific role in the delayed phase of the ischemic brain injury inflammatory cascade (98). Shichita et al. demonstrated that γδT17 cells play a significant role during late-stage ischemic brain injury, and that they, rather Th17 cells, are (surprisingly) the major origin of IL-17 (99). Moreover, increased IL-17 levels are present in the PB of patients who have suffered a stroke, relative to healthy individuals (100). IL-23, generated by macrophages or monocytes from stroke initiation, is an essential contributor for inducing IL-17 by γδ T cells during the delayed phase of encephalic ischemia. Thereby, IL-23p19$^{-/-}$ mice illustrated a diminishment in infarct extent only 1 day after the ischemic injury, whereas IL-17 deficiency led to reduced infarct size after 4 days. Long-term, deficiencies of IL-17 and IL23 demonstrated obviously diminished CNS injury, relative to WT, or even IFN-γ$^{-/-}$, mice (99). Gelderblom et al. demonstrated that injection of IL-17-neutralizing antibody to mice within the post-stroke 3h could reduce infarct volume and improve disease prognosis after 3 days (101).

Overall, the available evidence demonstrates that γδ T cells are the main source of IL-17. The mechanism of antigen-independent T cell activation post-stroke remains unclear; however, it has been owed chiefly to γδ T cells. Nevertheless, Kleinschnitz et al. demonstrated that γδ T cell-deficient mice remain susceptible to ischemic insult, indicating an extra function for other immune cells in ischemic brain injury. Furthermore, the fact that transgenic-TCR mice are susceptible to stroke implies that, besides γδ T cells, Th17 cells may also have a prominent role in stroke, whereas the precise function of Th17 cells in inducing stroke is not exact (102).

In addition, astrocytes can respond to IL-17 and promote stroke induction and development (101). For instance, IL-17 produced by γδ T cells and TNF-α secreted by macrophages act synergistically on astrocytes, by inducing the expression of CXCL1, a neutrophil chemoattractant (101, 103). Recently, periventricular leukomalacia (PVL), a distinctive form of brain injury in premature infants, was demonstrated to be caused by developmental immaturity of the cerebral vasculature in mid to late gestational age, and large numbers of γδ T cells were observed in postmortem brains from preterm infants (104). Although there were increased IL-17 and IL-22 in mouse

brains after injury, neither cytokine contributes to preterm brain injury (104).

In summary, γδ T cells and IL-17 have essential roles in ischemic brain injury. Hence, γδ T cells and IL-17 should be considered potential therapeutic targets to decrease secondary inflammation after ischemic brain injury (105, 106).

Central Nervous System Infection

The CNS infections commonly lead to the disruption of the blood-brain barrier (BBB) protectiveness and subsequent tissue inflammation; however, inflammation is also crucial to CNS immunity, as reduced γδ T cell expansion leads to increased host vulnerability to viral infection (107, 108). For example, MS patients treated with Natalizumab, a monoclonal antibody against α4-integrin, undergo fatal viral infections, due to the immune cells fail to infiltrate the CNS and eliminate the infection (109).

In contrast to viral infection, there are some (although limited) researches involving the function of γδ T cells in models of CNS bacterial infection (110). For example, children with bacterial meningitis exhibit high γδ T cell fractions in the CSF (111). Nichols et al. suggested that the γδ T cell was an alternative pathway available to respond to Grampositive bacteria CNS infection. They found that $TLR2^{-/-}$ brain abscess mice (TLR2 is a critical receptor for eliciting responses to Grampositive bacteria) were detected elevated IL-17, and γδ T cells were the source of IL-17 (112–114). Similarly, IL-17R signaling regulates γδ T cell infiltration, as well as bacterial clearance, during S. aureus-induced brain abscess formation (115). Also, IL-17 expression is augmented in the CNS of mice infected with Toxoplasma gondii (116). An increased percentage of γδT17 cells was observed in the PB and lesion in children with bacterial meningitis, and the condition was reversed after antibacterial therapy (111). High levels of IL-17 can also be detected in the abscess formation of humans; however, such researches are only associated, since no direct evidence can be demonstrated (117). Nevertheless, evidence for the involvement of γδ T cells in any CNS infection is sparse, and more studies are needed to establish a relation between γδ T cells and CNS infections.

Central Nervous System Traumatic Diseases

Immune responses and neuroinflammation involving γδ T cells are also critically involved in CNS traumatic diseases. Diseases resulting from CNS trauma usually involve irreversible sensory, motor, and autonomic impairments (118). Peripheral immune mechanisms establishment is related to the pathological processes of traumatic brain injury (TBI). Richard et al. found that CD4+ and CD8+ T cells, Tregs, and γδ T cells, increased in number within 24 h after TBI (119).

TABLE 1 | The role of γδ T cells in CNS diseases.

Disease	Species	γδ T subset	Tissue/organ	Cytokine/antigen	Conclusion	References
MS	Human	Vγ9/Vδ1/Vδ2	Brain	HSP60/HSP90	Detrimental	(27)
MS	Human	Vγ2/Vδ1/Vδ2	PB/CSF	HSP70	Detrimental	(28)
MS	Human	Vδ1/Vδ2	PB/CSF	–	Detrimental	(81)
MS	Human	–	PB/CSF	IL-17	Detrimental	(82)
MS	Human	–	Brain/CSF	HSP72	Detrimental	(87)
MS	Human	Vδ1	PB	IFN-γ	Detrimental	(90)
MS	Human	–	Brain	HSP65/HSP90	Detrimental	(93)
EAE	Mouse	–	Spinal cord	HSP60	Detrimental	(80)
EAE	Mouse	–	Brain/Spinal cord	IL-12	Detrimental	(83)
EAE	Mouse	–	Spinal cord	–	Detrimental	(84)
EAE	Mouse	–	Spinal cord	MIP-1α/MCP-1	Detrimental	(86)
EAE	Mouse	Vγ4/Vγ5/Vδ6	Brain	IL-17/IL-21/IL-22	Detrimental	(40)
EAE	Mouse	Vγ4	PB	IL-17	Detrimental	(92)
EAE	Mouse	–	Spleen	IL-15	Detrimental	(94)
EAE	Mouse	–	Brain/Spinal cord	–	Protective	(95)
Stroke	Mouse	–	Brain	IL-17	Detrimental	(99)
Stroke	Human/Mouse	–	Brain	IL-17	Detrimental	(101)
Stroke	Mouse	Vγ6	Brain	IL-17/TNF-α	Detrimental	(103)
Stroke	Mouse	–	Brain/PB	IL-17	Protective	(124)
PVL	Human/Mouse	–	Brain	IL-17F/IL-22	Detrimental	(104)
WNV infection	Mouse	–	Brain/Spleen/PB	–	Protective	(107)
HSV-1 infection	Mouse	–	Brain/Trigeminal ganglia	–	Protective	(108)
Bacterial meningitis	Human	Vγ9/Vδ2	PB/CSF	IL-17	Protective	(111)
Brain abscess	Mouse	–	Brain	IL-17	Protective	(112)
Brain abscess	Mouse	–	Brain	IL-17	Protective	(115)
TBI	Mouse	–	Brain/PB	–	Detrimental	(119)
SCI	Mouse	Vγ4	Spinal cord/CSF	IFN-γ/TNF-α	Detrimental	(34)
Epilepsy	Human	–	Brain	IL-17/GM-CSF	Detrimental	(125)
RE	Human	Vδ1	Brain	–	Detrimental	(126)

MS, multiple sclerosis; HSP, heat shock protein; PB, peripheral blood; CSF, cerebrospinal fluid; EAE, experimental autoimmune encephalomyelitis; MIP, macrophage-inflammatory protein; MCP, monocyte chemoattractant protein; PVL, periventricular leukomalacia; WNV, west nile virus; HSV, herpes simplex virus; TBI, traumatic brain injury; SCI, spinal cord injury; RE, rasmussen encephalitis.

Further, recent results from our laboratory found that γδ T cells, particularly $V\gamma4^+$ γδ T cells, exert a detrimental role in SCI, probably by providing an important origin of IFN-γ, which induces macrophages to adopt the M1 phenotype, with increased secretion of inflammatory cytokines, such as TNF-α (34) (**Figure 2**). Moreover, one significant discovery from our studies was that bone marrow-derived macrophages (BMDMs) respond to IFN-γ. This was supported by two sets of findings. First, IFN-$\gamma R^{-/-}$ mice, chimeras with IFN-$\gamma R^{-/-}$ bone marrow, and mice receiving adoptively transferred IFN-$\gamma R^{-/-}$ peritoneal macrophages, all showed similar recovery following SCI. Second, numbers of M1 macrophages and proinflammatory cytokines are reduced in IFN-$\gamma R^{-/-}$ compared with WT controls (34). Besides, the treatment of SCI with anti-Vγ4 antibodies has a beneficial effect, similar to that obtained with anti-TNF-α (34). In conclusion, manipulation of γδ T cell functions may be a potential treatment approach for future CNS traumatic diseases.

Other Central Nervous System Diseases

The pathological and clinical outcome of CNS diseases can also be affected by the intestinal microflora in the context of autoimmunity (120–124). This relationship has been particularly well established for the response to bacteria, including pathogens and commensals, within the intestinal compartment and its effects on the CNS, a connection that was recently termed the gut-brain axis. The gut environment has been found to significantly influence CNS diseases such as MS, EAE, and ischemic brain injury; however, immune cell mechanisms are unclear. In addition, the pathogenesis of intractable epilepsy is related to γδ T cells, where proinflammatory γδ T cells were concentrated in epileptogenic lesions, and their numbers positively associated with disease severity (125–127).

CONCLUSION

Since the identification of γδ T cells, there has been a boom in related studies and discoveries. Equipped with functions of both innate and adaptive immune cells, γδ T cells can provide consequential functions in the development of CNS diseases, such as recognizing a diverse array of antigens, rapid production of inflammatory mediators, and influencing the differentiation of their αβ counterparts. Recently, understanding the inflammatory and immune roles of γδ T cells has resulted in the development of many prospective therapies for CNS diseases. However, the exact mechanisms behind their contributions are yet to be fully elucidated.

The pandemic of 2019 coronavirus disease COVID-19, caused by the SARS-CoV-2 virus infection, has caused worldwide mortality (128). Past pandemics have demonstrated that COVID-19 is accompanied by diverse neuropsychiatric symptoms, such as encephalopathy, neuromuscular dysfunction, or demyelinating processes (129). Whether recovered SARS-CoV-2 patients will exhibit an increased incidence of MS symptomatology or other delayed neurologic sequelae, is an important, yet unanswered. Nevertheless, more substantial shreds of evidence are required on different subtypes of γδ T cells for defining their opposing roles in CNS inflammation and explaining the confounding findings on their pathogenic or protective role in CNS diseases. In summary, this review discusses recent notable studies of the neuro-inflammatory and immune functions of γδ T cells, intending to understand their roles in CNS disease, which may be crucial for the effective immunotherapies.

AUTHOR CONTRIBUTIONS

JW and FZ contributed to editing the manuscript. ZL and CS provided administrative support. GS and WZ helped the manuscript editing and discussions. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Nanno M, Shiohara T, Yamamoto H, Kawakami K, Ishikawa H. gamma delta T cells: firefighters or fire boosters in the front lines of inflammatory responses. *Immunol Rev* (2007) 215:103–13. doi: 10.1111/j.1600-065X.2006.00474.x
2. Correa I, Bix M, Liao NS, Zijlstra M, Jaenisch R, Raulet D. Most gamma delta T cells develop normally in beta 2-microglobulin-deficient mice. *Proc Natl Acad Sci U S A* (1992) 89:653–7. doi: 10.1073/pnas.89.2.653
3. Bigby M, Markowitz JS, Bleicher PA, Grusby MJ, Simha S, Siebrecht M, et al. Most gamma delta T cells develop normally in the absence of MHC class II molecules. *J Immunol* (1993) 151:4465–75.
4. Martin B, Hirota K, Cua DJ, Stockinger B, Veldhoen M. Interleukin-17-producing gammadelta T cells selectively expand in response to pathogen products and environmental signals. *Immunity* (2009) 31:321–30. doi: 10.1016/j.immuni.2009.06.020
5. Dieli F, Poccia F, Lipp M, Sireci G, Caccamo N, Di Sano C, et al. Differentiation of effector/memory Vdelta2 T cells and migratory routes in lymph nodes or inflammatory sites. *J Exp Med* (2003) 198:391–7. doi: 10.1084/jem.20030235
6. Carding SR, Egan PJ. gamma delta T cells: Functional plasticity and heterogeneity. *Nat Rev Immunol* (2002) 2:336–45. doi: 10.1038/nri797
7. Mayassi T, Jabri B. Human intraepithelial lymphocytes. *Mucosal Immunol* (2018) 11:1281–9. doi: 10.1038/s41385-018-0016-5

8. Heilig JS, Tonegawa S. Diversity of murine gamma genes and expression in fetal and adult T lymphocytes. *Nature* (1986) 322:836–40. doi: 10.1038/322836a0
9. Melandri D, Zlatareva I, Chaleil RAG, Dart RJ, Chancellor A, Nussbaumer O, et al. The gamma delta TCR combines innate immunity with adaptive immunity by utilizing spatially distinct regions for agonist selection and antigen responsiveness. *Nat Immunol* (2018) 19:1352–65. doi: 10.1038/s41590-018-0253-5
10. Andreu-Ballester JC, Garcia-Ballesteros C, Benet-Campos C, Amigo V, Almela-Quilis A, Mayans J, et al. Values for alphabeta and gammadelta T-lymphocytes and CD4+, CD8+, and CD56+ subsets in healthy adult subjects: assessment by age and gender. *Cytometry B Clin Cytom* (2012) 82:238–44. doi: 10.1002/cyto.b.21020
11. Kalyan S, Kabelitz D. Defining the nature of human gamma delta T cells: a biographical sketch of the highly empathetic. *Cell Mol Immunol* (2013) 10:21–9. doi: 10.1038/cmi.2012.44
12. Poggi A, Zocchi MR. gamma delta T lymphocytes as a firs line of immune defense: old and new ways of antigen recognition and implications for cancer immunotherapy. *Front Immunol* (2014) 5:1–6. doi: 10.3389/fimmu.2014.00575
13. Gu SY, Nawrocka W, Adams EJ. Sensing of pyrophosphate metabolites by V gamma 9 delta 2T cells. *Front Immunol* (2015) 5:688. doi: 10.3389/fimmu.2014.00688
14. De Libero G, Lau SY, Mori L. Phosphoantigen Presentation to TCR gammadelta Cells, a Conundrum Getting Less Gray Zones. *Front Immunol* (2014) 5:679. doi: 10.3389/fimmu.2014.00679
15. Vantourout P, Laing A, Woodward MJ, Zlatareva I, Apolonia L, Jones AW, et al. Heteromeric interactions regulate butyrophilin (BTN) and BTN-like molecules governing gamma delta T cell biology. *Proc Natl Acad Sci U S A* (2018) 115:1039–44. doi: 10.1073/pnas.1701237115
16. Harly C, Guillaume Y, Nedellec S, Peigne CM, Monkkonen H, Monkkonen J, et al. Key implication of CD277/butyrophilin-3 (BTN3A) in cellular stress sensing by a major human gamma delta T-cell subset. *Blood* (2012) 120:2269–79. doi: 10.1182/blood-2012-05-430470
17. Hudspeth K, Silva-Santos B, Mavilio D. Natural cytotoxicity receptors: broader expression patterns and functions in innate and adaptive immune cells. *Front Immunol* (2013) 4:1–15. doi: 10.3389/fimmu.2013.00069
18. Correia DV, Lopes A, Silva-Santos B. Tumor cell recognition by gamma delta T lymphocytes T-cell receptor vs. NK-cell receptors. *Oncoimmunology* (2013) 2:e22892. doi: 10.4161/onci.22892
19. Simoes AE, Di Lorenzo B, Silva-Santos B. Molecular Determinants of Target Cell Recognition by Human gamma delta T Cells. *Front Immunol* (2018) 9:929. doi: 10.3389/fimmu.2018.00929
20. Bauer S, Groh V, Wu J, Steinle A, Phillips JH, Lanier LL, et al. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* (1999) 285:727–9. doi: 10.1126/science.285.5428.727
21. Raulet DH. Roles of the NKG2D immunoreceptor and its ligands. *Nat Rev Immunol* (2003) 3:781–90. doi: 10.1038/nri1199
22. Bahram S, Inoko H, Shiina T, Radosavljevic M. MIC and other NKG2D ligands: from none to too many. *Curr Opin Immunol* (2005) 17:505–9. doi: 10.1016/j.coi.2005.07.016
23. Crowley MP, Fahrer AM, Baumgarth N, Hampl J, Gutgemann I, Teyton L, et al. A population of murine gamma delta T cells that recognize an inducible MHC class lb molecule. *Science* (2000) 287:314–6. doi: 10.1126/science.287.5451.314
24. Bonneville M, O'Brien RL, Born WK. Gamma delta T cell effector functions: a blend of innate programming and acquired plasticity. *Nat Rev Immunol* (2010) 10:467–78. doi: 10.1038/nri2781
25. Luoma AM, Castro CD, Adams EJ. gamma delta T cell surveillance via CD1 molecules. *Trends Immunol* (2014) 35:613–21. doi: 10.1016/j.it.2014.09.003
26. de Jong A. Activation of human T cells by CD1 and self-lipids. *Immunol Rev* (2015) 267:16–29. doi: 10.1111/imr.12322
27. Wucherpfennig KW, Newcombe J, Li H, Keddy C, Cuzner ML, Hafler DA. Gamma delta T-cell receptor repertoire in acute multiple sclerosis lesions. *Proc Natl Acad Sci U S A* (1992) 89:4588–92. doi: 10.1073/pnas.89.10.4588
28. Stinissen P, Vandevyver C, Medaer R, Vandegaer L, Nies J, Tuyls L, et al. Increased frequency of gamma delta T cells in cerebrospinal fluid and peripheral blood of patients with multiple sclerosis. Reactivity, cytotoxicity, and T cell receptor V gene rearrangements. *J Immunol* (1995) 154:4883–94.
29. Hirsh MI, Junger WG. Roles of Heat Shock Proteins and gamma delta T Cells in Inflammation. *Am J Resp Cell Mol* (2008) 39:509–13. doi: 10.1165/rcmb.2008-0090TR
30. Edwards SC, Sutton CE, Ladell K, Grant EJ, McLaren JE, Roche F, et al. A population of proinflammatory T cells coexpresses alphabeta and gammadelta T cell receptors in mice and humans. *J Exp Med* (2020) 217:1–16. doi: 10.1084/jem.20190834
31. Bowen S, Sun P, Livak F, Sharrow S, Hodes RJ. A Novel T Cell Subset with Trans-Rearranged V gamma-C beta b TCRs Shows V beta Expression Is Dispensable for Lineage Choice and MHC Restriction. *J Immunol* (2014) 192:169–77. doi: 10.4049/jimmunol.1302398
32. Billiau A, Heremans H, Vandekerckhove F, Dijkmans R, Sobis H, Meulepas E, et al. Enhancement of experimental allergic encephalomyelitis in mice by antibodies against IFN-gamma. *J Immunol* (1988) 140:1506–10.
33. Kroenke MA, Carlson TJ, Andjelkovic AV, Segal BM. IL-12- and IL-23-modulated T cells induce distinct types of EAE based on histology, CNS chemokine profile, and response to cytokine inhibition. *J Exp Med* (2008) 205:1535–41. doi: 10.1084/jem.20080159
34. Sun GD, Yang SX, Cao GC, Wang QH, Hao JL, Wen Q, et al. Gamma delta T cells provide the early source of IFN-gamma to aggravate lesions in spinal cord injury. *J Exp Med* (2018) 215:521–35. doi: 10.1084/jem.20170686
35. Jensen KDC, Su X, Shin S, Li L, Youssef S, Yarnasaki S, et al. Thymic selection determines gamma delta T cell effector fate: Antigen-naive cells make interleukin-17 and antigen-experienced cells make interferon gamma. *Immunity* (2008) 29:90–100. doi: 10.1016/j.immuni.2008.04.022
36. Muñoz-Ruiz M, Ribot JC, Grosso AR, Gonçalves-SoU S A N, Pamplona A, Pennington DJ, et al. TCR signal strength controls thymic differentiation of discrete proinflammatory γδ T cell subsets. *Nat Immunol* (2016) 17:721–7. doi: 10.1038/ni.3424
37. Prinz I, Sansoni A, Kissenpfennig A, Ardouin L, Malissen M, Malissen B. Visualization of the earliest steps of gammadelta T cell development in the adult thymus. *Nat Immunol* (2006) 7:995–1003. doi: 10.1038/ni1371
38. Lalor SJ, Dungan LS, Sutton CE, Basdeo SA, Fletcher JM, Mills KHG. Caspase-1-processed cytokines IL-1 beta and IL-18 promote IL-17 production by gamma delta and CD4 T cells that mediate autoimmunity. *J Immunol* (2011) 186:5738–48. doi: 10.4049/jimmunol.1003597
39. Ribot JC, deBarros A, Pang DJ, Neves JF, Peperzak V, Roberts SJ, et al. CD27 is a thymic determinant of the balance between interferon-gamma-and interleukin 17-producing gamma delta T cell subsets. *Nat Immunol* (2009) 10:427–36. doi: 10.1038/ni.1717
40. Sutton CE, Lalor SJ, Sweeney CM, Brereton CF, Lavelle EC, Mills KH. Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. *Immunity* (2009) 31:331–41. doi: 10.1016/j.immuni.2009.08.001
41. Cua DJ, Tato CM. Innate IL-17-producing cells: the sentinels of the immune system. *Nat Rev Immunol* (2010) 10:479–89. doi: 10.1038/nri2800
42. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* (2006) 126:1121–33. doi: 10.1016/j.cell.2006.07.035
43. Marks BR, Nowyhed HN, Choi JY, Poholek AC, Odegard JM, Flavell RA, et al. Thymic self-reactivity selects natural interleukin 17-producing T cells that can regulate peripheral inflammation. *Nat Immunol* (2009) 10:1125–U108. doi: 10.1038/ni.1783
44. Bekiaris V, Šedý JR, Macauley MG, Rhode-Kurnow A, Ware CF. The inhibitory receptor BTLA controls γδ T cell homeostasis and inflammatory responses. *Immunity* (2013) 39:1082–94. doi: 10.1016/j.immuni.2013.10.017
45. Shibata K, Yamada H, Sato T, Dejima T, Nakamura M, Ikawa T, et al. Notch-Hes1 pathway is required for the development of IL-17-producing gamma delta T cells. *Blood* (2011) 118:586–93. doi: 10.1182/blood-2011-02-334995
46. Petermann F, Rothhammer V, Claussen MC, Haas JD, Blanco LR, Heink S, et al. Gamma delta T cells enhance autoimmunity by restraining regulatory T cell responses via an interleukin-23-dependent mechanism. *Immunity* (2010) 33:351–63. doi: 10.1016/j.immuni.2010.08.013

47. Riol-Blanco L, Ordovas-Montanes J, Perro M, Naval E, Thiriot A, Alvarez D, et al. Nociceptive sensory neurons drive interleukin-23-mediated psoriasiform skin inflammation. *Nature* (2014) 510:157–61. doi: 10.1038/nature13199
48. Chien YH, Zeng X, Prinz I. The natural and the inducible interleukin (IL)-17-producing gamma delta T cells. *Trends Immunol* (2013) 34:151–4. doi: 10.1016/j.it.2012.11.004
49. Haak S, Croxford AL, Kreymborg K, Heppner FL, Pouly S, Becher B, et al. IL-17A and IL-17F do not contribute vitally to autoimmune neuro-inflammation in mice. *J Clin Invest* (2009) 119:61–9. doi: 10.1172/JCI35997
50. Kreymborg K, Etzensperger R, Dumoutier L, Haak S, Rebollo A, Buch T, et al. IL-22 is expressed by Th17 cells in an IL-23-dependent fashion, but not required for the development of autoimmune encephalomyelitis. *J Immunol* (2007) 179:8098–104. doi: 10.4049/jimmunol.179.12.8098
51. Lukens JR, Barr MJ, Chaplin DD, Chi H, Kanneganti TD. Inflammasome-derived IL-1beta regulates the production of GM-CSF by CD4(+) T cells and gammadelta T cells. *J Immunol* (2012) 188:3107–15. doi: 10.4049/jimmunol.1103308
52. McGinley AM, Sutton CE, Edwards SC, Leane CM, DeCourcey J, Teijeiro A, et al. Interleukin-17A serves a priming role in autoimmunity by recruiting IL-1β-producing myeloid cells that promote pathogenic T cells. *Immunity* (2020) 52:342–56. doi: 10.1016/j.immuni.2020.01.002
53. McGeachy MJ, Cua DJ. Th17 cell differentiation: The long and winding road. *Immunity* (2008) 28:445–53. doi: 10.1016/j.immuni.2008.03.001
54. Nurieva R, Yang XXO, Martinez G, Zhang YL, Panopoulos AD, Ma L, et al. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature* (2007) 448:480–U8. doi: 10.1038/nature05969
55. Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, et al. IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol* (2007) 8:967–74. doi: 10.1038/ni1488
56. Bettelli E, Carrier YJ, Gao WD, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector T(H)17 and regulatory T cells. *Nature* (2006) 441:235–8. doi: 10.1038/nature04753
57. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGF beta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* (2006) 24:179–89. doi: 10.1016/j.immuni.2006.01.001
58. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, et al. Transforming growth factor-beta induces development of the T (H)17 lineage. *Nature* (2006) 441:231–4. doi: 10.1038/nature04754
59. Do JS, Fink PJ, Li L, Spolski R, Robinson J, Leonard WJ, et al. Cutting edge: spontaneous development of IL-17-producing gamma delta T cells in the thymus occurs via a TGF-beta 1-dependent mechanism. *J Immunol* (2010) 184:1675–9. doi: 10.4049/jimmunol.0903539
60. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17-producing CD4(+) effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* (2005) 6:1123–32. doi: 10.1038/ni1254
61. Park H, Li ZX, Yang XO, Chang SH, Nurieva R, Wang YH, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* (2005) 6:1133–41. doi: 10.1038/ni1261
62. Korn T, Bettelli E, Gao W, Awasthi A, Jager A, Strom TB, et al. IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. *Nature* (2007) 448:484–U9. doi: 10.1038/nature05970
63. Korn T, Mitsdoerffer M, Croxford AL, Awasthi A, Dardalhon VA, Galileos G, et al. IL-6 controls Th17 immunity in vivo by inhibiting the conversion of conventional T cells into Foxp3(+) regulatory T cells. *Proc Natl Acad Sci U S A* (2008) 105:18460–5. doi: 10.1073/pnas.0809850105
64. Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* (2003) 421:744–8. doi: 10.1038/nature01355
65. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* (2005) 201:233–40. doi: 10.1084/jem.20041257
66. Sutton C, Brereton C, Keogh B, Mills KHG, Lavelle EC. A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. *J Exp Med* (2006) 203:1685–91. doi: 10.1084/jem.20060285
67. Awasthi A, Riol-Blanco L, Jager A, Korn T, Pot C, Galileos G, et al. Cutting edge: IL-23 receptor gfp reporter mice reveal distinct populations of IL-17-producing cells. *J Immunol* (2009) 182:5904–8. doi: 10.4049/jimmunol.0900732
68. El-Behi M, Ciric B, Dai H, Yan YP, Cullimore M, Safavi F, et al. The encephalitogenicity of T(H)17 cells is dependent on IL-1-and IL-23-induced production of the cytokine GM-CSF. *Nat Immunol* (2011) 12:568–U241. doi: 10.1038/ni.2031
69. Codarri L, Gyulveszi G, Tosevski V, Hesske L, Fontana A, Magnenat L, et al. ROR gamma t drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. *Nat Immunol* (2011) 12:560–U248. doi: 10.1038/ni.2027
70. Ponomarev ED, Shriver LP, Maresz K, Pedras-Vasconcelos J, Verthelyi D, Dittel BN. GM-CSF production by autoreactive T cells is required for the activation of microglial cells and the onset of experimental autoimmune encephalomyelitis. *J Immunol* (2007) 178:39–48. doi: 10.4049/jimmunol.178.1.39
71. Croxford AL, Lanzinger M, Hartmann FJ, Schreiner B, Mair F, Pelczar P, et al. The Cytokine GM-CSF Drives the Inflammatory Signature of CCR2(+) Monocytes and Licenses Autoimmunity. *Immunity* (2015) 43:502–14. doi: 10.1016/j.immuni.2015.08.010
72. Shi YF, Liu CH, Roberts AI, Das JT, Xu GW, Ren GW, et al. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and T-cell responses: what we do and don't know. *Cell Res* (2006) 16:126–33. doi: 10.1038/sj.cr.7310017
73. Mokhtarian F, McFarlin DE, Raine CS. Adoptive transfer of myelin basic protein-sensitized T cells produces chronic relapsing demyelinating disease in mice. *Nature* (1984) 309:356–8. doi: 10.1038/309356a0
74. Ferber IA, Brocke S, Taylor-Edwards C, Ridgway W, Dinisco C, Steinman L, et al. Mice with a disrupted IFN-gamma gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). *J Immunol* (1996) 156:5–7.
75. Panitch HS, Hirsch RL, Haley AS, Johnson KP. Exacerbations of multiple sclerosis in patients treated with gamma interferon. *Lancet* (1987) 1:893–5. doi: 10.1016/s0140-6736(87)92863-7
76. Bettelli E, Sullivan B, Szabo SJ, Sobel RA, Glimcher LH, Kuchroo VK. Loss of T-bet, but not STAT1, prevents the development of experimental autoimmune encephalomyelitis. *J Exp Med* (2004) 200:79–87. doi: 10.1084/jem.20031819
77. Gao Y, Yang W, Pan M, Scully E, Girardi M, Augenlicht LH, et al. Gamma delta T cells provide an early source of interferon gamma in tumor immunity. *J Exp Med* (2003) 198:433–42. doi: 10.1084/jem.20030584
78. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Annu Rev Immunol* (2009) 27:485–517. doi: 10.1146/annurev.immunol.021908.132710
79. Roark CL, Simonian PL, Fontenot AP, Born WK, O'Brien RL. gamma delta T cell: an important source of IL-17. *Curr Opin Immunol* (2008) 20:353–7. doi: 10.1016/j.coi.2008.03.006
80. Gao YL, Brosnan CF, Raine CS. Experimental autoimmune encephalomyelitis. Qualitative and semiquantitative differences in heat shock protein 60 expression in the central nervous system. *J Immunol* (1995) 154:3548–56.
81. Shimonkevitz R, Colburn C, Burnham JA, Murray RS, Kotzin BL. Clonal expansions of activated gamma/delta T cells in recent-onset multiple sclerosis. *Proc Natl Acad Sci U S A* (1993) 90:923–7. doi: 10.1073/pnas.90.3.923
82. Schirmer L, Rothhammer V, Hemmer B, Korn T. Enriched CD161high CCR6+ γδ T cells in the cerebrospinal fluid of patients with multiple sclerosis. *JAMA Neurol* (2013) 70:345–51. doi: 10.1001/2013.jamaneurol.409
83. Odyniec A, Szczepanik M, Mycko MP, Stasiolek M, Raine CS, Selmaj KW. gamma delta T cells enhance the expression of experimental autoimmune encephalomyelitis by promoting antigen presentation and IL-12 production. *J Immunol* (2004) 173:682–94. doi: 10.4049/jimmunol.173.1.682
84. Rajan J, Gao YL, Raine CS, Brosnan CF. A pathogenic role for gamma delta T cells in relapsing-remitting experimental allergic encephalomyelitis in the SJL mouse. *J Immunol* (1996) 157:941–9.

85. Rezende RM, Nakagaki BN, Moreira TG, Lopes JR, Kuhn C, Tatematsu BK, et al. γδ T Cell-Secreted XCL1 Mediates Anti-CD3-Induced Oral Tolerance. *J Immunol* (2019) 203:2621–9. doi: 10.4049/jimmunol.1900784
86. Rajan AJ, Asensio VC, Campbell IL, Brosnan CF. Experimental autoimmune encephalomyelitis on the SJL mouse: Effect of gamma delta T cell depletion on chemokine and chemokine receptor expression in the central nervous system. *J Immunol* (2000) 164:2120–30. doi: 10.4049/jimmunol.164.4.2120
87. Freedman MS, Ruijs TC, Selin LK, Antel JP. Peripheral blood gamma-delta T cells lyse fresh human brain-derived oligodendrocytes. *Ann Neurol* (1991) 30:794–800. doi: 10.1002/ana.410300608
88. Duhen R, Glatigny S, Arbelaez CA, Blair TC, Oukka M, Bettelli E. Cutting edge: The pathogenicity of IFN-gamma-producing Th17 cells is independent of T-bet. *J Immunol* (2013) 190:4478–82. doi: 10.4049/jimmunol.1203172
89. Hirota K, Duarte JH, Veldhoen M, Hornsby E, Li Y, Cua DJ, et al. Fate mapping of IL-17-producing T cells in inflammatory responses. *Nat Immunol* (2011) 12:255–U95. doi: 10.1038/ni.1993
90. Singh AK, Novakova L, Axelsson M, Malmeström C, Zetterberg H, Lycke J, et al. High interferon-γ uniquely in Vδ1 T cells correlates with markers of inflammation and axonal damage in early multiple sclerosis. *Front Immunol* (2017) 8:260. doi: 10.3389/fimmu.2017.00260
91. Bloomgren G, Richman S, Hotermans C, Subramanyam M, Goelz S, Natarajan A, et al. Risk of natalizumab-associated progressive multifocal leukoencephalopathy. *N Engl J Med* (2012) 366:1870–80. doi: 10.1056/nejmoa1107829
92. Maeda Y, Seki N, Kataoka H, Takemoto K, Utsumi H, Fukunari A, et al. IL-17-producing Vγ4+ γδ T cells require sphingosine 1-phosphate receptor 1 for their egress from the lymph nodes under homeostatic and inflammatory conditions. *J Immunol* (2015) 195:1408–16. doi: 10.4049/jimmunol.1500599
93. Selmaj K, Brosnan CF, Raine CS. Colocalization of lymphocytes bearing gamma delta T-cell receptor and heat shock protein hsp65+ oligodendrocytes in multiple sclerosis. *Proc Natl Acad Sci U S A* (1991) 88:6452–6. doi: 10.1073/pnas.88.15.6452
94. Wang XQ, Wei YX, Liu XL, Xing C, Han GC, Chen GJ, et al. IL-15-secreting gamma delta T cells induce memory T cells in experimental allergic encephalomyelitis (EAE) mice. *Mol Immunol* (2015) 66:402–8. doi: 10.1016/j.molimm.2015.04.021
95. Ponomarev ED, Dittel BN. gamma delta T cells regulate the extent and duration of inflammation in the central nervous system by a Fas ligand-dependent mechanism. *J Immunol* (2005) 174:4678–87. doi: 10.4049/jimmunol.174.8.4678
96. Gelderblom M, Leypoldt F, Steinbach K, Behrens D, Choe CU, Siler DA, et al. Temporal and spatial dynamics of cerebral immune cell accumulation in stroke. *Stroke* (2009) 40:1849–57. doi: 10.1161/Strokeaha.108.534503
97. Yilmaz G, Arumugam TV, Stokes KY, Granger DN. Role of T lymphocytes and interferon-gamma in ischemic stroke. *Circulation* (2006) 113:2105–12. doi: 10.1161/Circulationaha.105.593046
98. Gelderblom M, Gallizioli M, Ludewig P, Thom V, Arunachalam P, Rissiek B, et al. IL-23-producing conventional dendritic cells control the detrimental IL-17 response in stroke. *Stroke* (2018) 49:155–64. doi: 10.1161/strokeaha.117.019101
99. Shichita T, Sugiyama Y, Ooboshi H, Sugimori H, Nakagawa R, Takada I, et al. Pivotal role of cerebral interleukin-17-producing gammadelta T cells in the delayed phase of ischemic brain injury. *Nat Med* (2009) 15:946–50. doi: 10.1038/nm.1999
100. Kostulas N, Pelidou SH, Kivisakk P, Kostulas V, Link H. Increased IL-1beta, IL-8, and IL-17 mRNA expression in blood mononuclear cells observed in a prospective ischemic stroke study. *Stroke* (1999) 30:2174–9. doi: 10.1161/01.str.30.10.2174
101. Gelderblom M, Weymar A, Bernreuther C, Velden J, Arunachalam P, Steinbach K, et al. Neutralization of the IL-17 axis diminishes neutrophil invasion and protects from ischemic stroke. *Blood* (2012) 120:3793–802. doi: 10.1182/blood-2012-02-412726
102. Kleinschnitz C, Schwab N, Kraft P, Hagedorn I, Dreykluft A, Schwarz T, et al. Early detrimental T-cell effects in experimental cerebral ischemia are neither related to adaptive immunity nor thrombus formation. *Blood* (2010) 115:3835–42. doi: 10.1182/blood-2009-10-249078
103. Arunachalam P, Ludewig P, Melich P, Arumugam TV, Gerloff C, Prinz I, et al. CCR6 is essential for the migration of detrimental natural interleukin-17-producing gammadelta T cells in stroke. *Stroke* (2017) 48:1957–65. doi: 10.1161/strokeaha.117.016753
104. Albertsson AM, Zhang X, Vontell R, Bi D, Bronson RT, Supramaniam V, et al. gammadelta T cells contribute to injury in the developing brain. *Am J Pathol* (2018) 188:757–67. doi: 10.1016/j.ajpath.2017.11.012
105. Kuo PC, Scofield BA, Yu IC, Chang FL, Ganea D, Yen JH. Interferon-beta Modulates Inflammatory Response in Cerebral Ischemia. *J Am Heart Assoc* (2016) 5:2–15. doi: 10.1161/jaha.115.002610
106. Xie L, Sun F, Wang J, Mao X, Xie L, Yang SH, et al. mTOR signaling inhibition modulates macrophage/microglia-mediated neuroinflammation and secondary injury via regulatory T cells after focal ischemia. *J Immunol* (2014) 192:6009–19. doi: 10.4049/jimmunol.1303492
107. Xie G, Luo H, Pang L, Peng BH, Winkelmann E, McGruder B, et al. Dysregulation of toll-like receptor 7 compromises innate and adaptive T cell responses and host resistance to an attenuated west nile virus infection in old mice. *J Virol* (2016) 90:1333–44. doi: 10.1128/jvi.02488-15
108. Sciammas R, Kodukula P, Tang Q, Hendricks RL, Bluestone JA. T cell receptor-gamma/delta cells protect mice from herpes simplex virus type 1-induced lethal encephalitis. *J Exp Med* (1997) 185:1969–75. doi: 10.1084/jem.185.11.1969
109. Ransohoff RM. Natalizumab and PML. *Nat Neurosci* (2005) 8:1275–5. doi: 10.1038/nn1005-1275
110. Deknuydt F, Roquilly A, Cinotti R, Altare F, Asehnoune K. An in vitro model of mycobacterial granuloma to investigate the immune response in brain-injured patients. *Crit Care Med* (2013) 41:245–54. doi: 10.1097/ccm.0b013e3182676052
111. Caccamo N, La Mendola C, Orlando V, Meraviglia S, Todaro M, Stassi G, et al. Differentiation, phenotype, and function of interleukin-17-producing human V gamma 9V delta 2 T cells. *Blood* (2011) 118:129–38. doi: 10.1182/blood-2011-01-331298
112. Nichols JR, Aldrich AL, Mariani MM, Vidlak D, Esen N, Kielian T. TLR2 deficiency leads to increased Th17 infiltrates in experimental brain abscesses. *J Immunol* (2009) 182:7119–30. doi: 10.4049/jimmunol.0802656
113. Takeuchi O, Hoshino K, Kawai T, Sanjo H, Takada H, Ogawa T, et al. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity* (1999) 11:443–51. doi: 10.1016/s1074-7613(00)80119-3
114. Takeuchi O, Hoshino K, Akira S. Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to Staphylococcus aureus infection. *J Immunol* (2000) 165:5392–6. doi: 10.4049/jimmunol.165.10.5392
115. Vidlak D, Kielian T. Differential effects of interleukin-17 receptor signaling on innate and adaptive immunity during central nervous system bacterial infection. *J Neuroinflamm* (2012) 9:128. doi: 10.1186/1742-2094-9-128
116. Stumhofer JS, Laurence A, Wilson EH, Huang E, Tato CM, Johnson LM, et al. Interleukin 27 negatively regulates the development of interleukin 17-producing T helper cells during chronic inflammation of the central nervous system. *Nat Immunol* (2006) 7:937–45. doi: 10.1038/ni1376
117. Bajpai A, Prasad KN, Mishra P, Singh AK, Gupta RK, Ojha BK. Distinct cytokine pattern in response to different bacterial pathogens in human brain abscess. *J Neuroimmunol* (2014) 273:96–102. doi: 10.1016/j.jneuroim.2014.05.009
118. Rubiano AM, Carney N, Chesnut R, Puyana JC. Global neurotrauma research challenges and opportunities. *Nature* (2015) 527:S193–7. doi: 10.1038/nature16035
119. Tobin RP, Mukherjee S, Kain JM, Rogers SK, Henderson SK, Motal HL, et al. Traumatic brain injury causes selective, CD74-dependent peripheral lymphocyte activation that exacerbates neurodegeneration. *Acta Neuropathol Com* (2014) 2:197. doi: 10.1186/s40478-014-0143-5
120. Berer K, Mues M, Koutrolos M, Al Rasbi Z, Boziki M, Johner C, et al. Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. *Nature* (2011) 479:538–U266. doi: 10.1038/nature10554
121. Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A* (2011) 108:4615–22. doi: 10.1073/pnas.1000082107
122. Kadowaki A, Saga R, Lin Y, Sato W, Yamamura T. Gut microbiota-dependent CCR9+CD4+ T cells are altered in secondary progressive multiple sclerosis. *Brain* (2019) 142:916–31. doi: 10.1093/brain/awz012

123. Kadowaki A, Miyake S, Saga R, Chiba A, Mochizuki H, Yamamura T. Gut environment-induced intraepithelial autoreactive CD4(+) T cells suppress central nervous system autoimmunity via LAG-3. *Nat Commun* (2016) 7:116–39. doi: 10.1038/ncomms11639
124. Benakis C, Brea D, Caballero S, Faraco G, Moore J, Murphy M, et al. Commensal microbiota affects ischemic stroke outcome by regulating intestinal gamma delta T cells. *Nat Med* (2016) 22:516–23. doi: 10.1038/nm.4068
125. Xu D, Robinson AP, Ishii T, Duncan DS, Alden TD, Goings GE, et al. Peripherally derived T regulatory and γδ T cells have opposing roles in the pathogenesis of intractable pediatric epilepsy. *J Exp Med* (2018) 215:1169–86. doi: 10.1084/jem.20171285
126. Owens GC, Erickson KL, Malone CC, Pan C, Huynh MN, Chang JW, et al. Evidence for the involvement of gamma delta T cells in the immune response in Rasmussen encephalitis. *J Neuroinflamm* (2015) 12:134. doi: 10.1186/s12974-015-0352-2
127. Al Nimer F, Jelcic I, Kempf C, Pieper T, Budka H, Sospedra M, et al. Phenotypic and functional complexity of brain-infiltrating T cells in Rasmussen encephalitis. *Neurol Neuroimmunol Neuroinflamm* (2018) 5:e419. doi: 10.1212/nxi.0000000000000419
128. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* (2020) 382:1708–20. doi: 10.1056/NEJMoa2002032
129. Troyer EA, Kohn JN, Hong SZ. Are we facing a crashing wave of neuropsychiatric sequelae of COVID-19? Neuropsychiatric symptoms and potential immunologic mechanisms. *Brain Behav Immun* (2020) 87:34–9. doi: 10.1016/j.bbi.2020.04.027

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Diverse Functions of γδ T Cells in the Progression of Hepatitis B Virus and Hepatitis C Virus Infection

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Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are primary risk factors for a wide spectrum of liver diseases that severely affect human health. The liver is an immunological organ that has an abundance of immune cells. Thus, various innate or adaptive immune cells are involved in the progression of HBV or HCV infection. Among those cells, a unique kind of immune cell, the γδ T cell, contributes to promoting or inhibiting the progression of liver diseases. To reveal the diverse roles of γδ T cells in HBV or HCV infection, the properties and functions of these cells in human and mouse models are analyzed. Here, we briefly describe the characteristics and functions of γδ T cells subsets in liver diseases. Then, we fully discuss the diverse roles of γδ T cells in the progression of HBV or HCV infection, including stages of acute infection, chronic infection, liver cirrhosis, and hepatocellular carcinoma. Finally, the functions and existing problems of γδ T cells in HBV or HCV infection are summarized. A better understanding of the function of γδ T cells during the progression of HBV and HCV infection will be helpful for the treatment of virus infection.

Keywords: γδ T cells, hepatitis B virus, hepatitis C virus, progression, cytokines

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INTRODUCTION

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are major risk factors for a wide spectrum of liver diseases. Although most adults recover from HBV infection, about 5% of patients are unable to clear HBV and thus develop chronic HBV infection (1) and experience virus flares and long-term morbidity. Similarly, acute HCV infection can easily convert into chronic HCV infection (2). The persistent inflammatory environment in chronic HBV (CHB) or chronic HCV (CHC) infection patients is associated with the elevated expression of α-smooth muscle actin and collagen fibers in hepatic stellate cells (HSCs), which then develop into liver cirrhosis (2–4). Hepatocellular carcinoma (HCC) is a common cancer and is mainly caused by HBV or HCV infection. HCV patients show a higher probability of developing HCC than HBV patients (5).

The liver is known as an immune tolerance organ. Aside from hepatocytes and stellate cells, there are various hepatic residential immune cells, including Küpffer cells (hepatic macrophages), T cells, natural killer (NK) cells, and dendritic cells (6). These cells play crucial roles in the pathogenesis of HBV or HCV infection. During acute HBV or HCV infection, innate immune cells such as NK cells

are activated and further induce antiviral function of adaptive immune cells (7). In chronic HBV and HCV infections, the liver is infiltrated with impaired antiviral T cells and activated inflammatory cells such as IL-17-producing $CD4^+$ T cells that further exacerbate liver inflammation (8, 9). Moreover, other hepatic immune cells, including regulatory T cells and myeloid-derived suppressor cells (MDSC), prompt the pathogenesis of chronic HBV or HCV infection, liver cirrhosis, or even liver cancer (10). The proportion of hepatic γδ T cells in hepatic T cells in humans and mice is found to be 15%–25% and 4.5%, respectively (6, 11), indicating the crucial role of these cells in liver diseases. However, the current understanding of the function of γδ T cells compared with other immune cells in HBV or HCV infection is limited.

γδ T cells, as the bridge of innate and adaptive immunity, play critical roles in various diseases, including liver diseases, infections, and cancer. γδ T cells can be divided into different subsets through γ and δ TCR chains. Based on δ TCR chains, human γδ T cells can mainly be separated into Vδ1 (in peripheral blood or organs), Vδ2 (peripheral blood dominant γδ T cells, usually combined with Vγ9), and Vδ3 (in intestine and lamina propria) T cell subsets. Based on γ TCR chains, mouse γδ T cells can be divided into Vγ1, Vγ4, Vγ5, Vγ6, and Vγ7 T cell subsets (12). In liver diseases, hepatic γδ T cells usually include Vγ1, Vγ4, and Vγ6 in mice and Vδ1, Vδ2, and Vδ3 in humans (13–15). These cells can produce cytokines such as IFN-γ, TNF-α, IL-17, and IL-22, as well as express cytotoxic and regulatory molecules such as Granzyme B (GrB), perforin, NK receptor, and Toll-like receptors (16). γδ T cells play different roles in the pathogenesis of HBV and HCV infections. In acute HBV infection, human γδ T cells are activated and exhibit antiviral functions by secreting IFN-γ and TNF-α. During other stages of HBV and HCV infections (chronic infection, liver cirrhosis, and HCC), these cells can inhibit or promote progression of the diseases. Surprisingly, different subsets of γδ T cells play contradictory roles in the same stage of liver infection. For example, in chronic HBV infection, human Vδ2 T cell subsets inhibit HBV infection progression by inhibiting Th17-induced liver damage (17). However, human $CD4^-CD8^-$ γδ T cell (18) and mouse IL-17-producing Vγ4 T cell (19) subsets are found to inhibit the function of T cells and promote HBV infection in CHB patients and an HBV mouse model. Similar contradictory functions are also observed in other stages. In HCC, human Vδ2 T cells, which can be activated and proliferate *in vitro* (20), are used in the clinic to prolong the survival time of HCC patients (21).

To determine the precise role of these cells, we summarize the functions of different human and mouse γδ T cells subsets in the different stages of HBV and HCV infections. Moreover, we indicate the opportunities and challenges in clinical application of γδ T cells.

ROLE OF γδ T CELLS IN ACUTE AND CHRONIC HBV INFECTION

During human acute HBV infection, about 5% of adult patients progress to chronic hepatitis B infection, whereas the rest go through a self-limited process that results in recovery (1). Accumulating data have demonstrated that different outcomes of HBV infection are associated with the intensity of antiviral immune responses (22). As shown in our previous study, the numbers of γδ T cells increase in liver tissue, but decrease in the peripheral blood of acute hepatitis B (AHB) patients (3). These peripheral γδ T cells are highly activated and terminally differentiated into memory phenotype, which has increased cytotoxic capacity and enhanced antiviral activity. Interestingly, in asymptomatic HBV infection patients, the frequencies of peripheral Vδ1 and Vδ2 T cells are higher, and the level of peripheral IFN-γ^+Vδ2 T cells is also significantly elevated compared to healthy controls (23). Furthermore, in an AHB infection mouse model, the number of hepatic γδ T cells significantly increases with the upregulation of HBV markers and exhibits elevated expression of the activation marker CD69, IFN-γ production, and IFN-β mRNA abundance in liver tissues (24). The above studies indicate that the antiviral function of γδ T cells in AHB patients can inhibit the progression of AHB infection.

γδ T cells display contradictory roles in CHB infection. Several studies have shown that these cells are impaired and exhibit liver protective functions to inhibit the progression of CHB infection (17). Our study and others show that the frequency of human peripheral and hepatic Vδ2 T cells is significantly lower in severe CHB patients with impaired chemotaxis (17) or degranulation (25). Although they display an active effector-memory phenotype (17), the IFN-γ or TNF-α-induced cytotoxicity of Vδ2 T cells is impaired (26) and can be reversed by IFN-α treatment *in vitro* and *in vivo* (27). In addition, *in vitro* proliferated human Vδ2 T cells can inhibit inflammatory cytokines production in pathogenic Th17 cells (17), which contributes to significant liver damage and pathology. However, a recent study indicates that the frequency of human γδ T cells and their subsets barely change and antiviral function of Vδ2 T cells is enhanced in CHB patients (28). This opposite result maybe because of the different applied standard for patient enrollment, including age, gender, and race, which would interfere the characteristics of γδ T cells (29).

However, other studies report that γδ T cells promote the progression of chronic HBV infection. By suppressing the secretion of HBV core peptide-stimulated IFN-γ and TNF-α by $CD8^+$ T cells, human $CD4^-CD8^-$ γδ T cells limit T cell responses to HBV partially through NKG2A and may impede HBeAg seroconversion during antiviral therapy of CHB patients (18). Moreover, in HBV-associated acute-on-chronic liver failure (CHB-ACLF) patients, more human peripheral γδ T cells exhibit upregulation of TNF-α or IL-17 and GrB or CD107, demonstrating the participation of γδ T cells in liver injury which in turn promote the progression of liver diseases (30). Meanwhile, in an immune tolerance chronic HBV infection mouse model, IL-17-producing Vγ4 T cells recruit MDSCs into the liver and induce $CD8^+$ T cell exhaustion (19).

In conclusion, IFN-γ- or TNF-α-producing γδ T cells can inhibit AHB and CHB infection, while human $CD4^-CD8^-$ γδ T cells and mouse IL-17-producing Vγ4 T cell subsets promote the

progression of chronic HBV infection. The opposite roles of these cells can be attributed to the different subsets of γδ T cells and their variable cytokine production (IFN-γ, TNF-α, or IL-17).

ROLE OF γδ T CELLS IN CHRONIC HCV INFECTION

Numerous researchers have focused on the function of γδ T cells in chronic HCV (CHC) infection. The number of hepatic γδ T cells is higher in CHC patients, and Vδ1 T cells are the predominant subset of hepatic γδ T cells (31, 32). However, the number of peripheral Vγ9Vδ2 and Vδ1 T cells decrease in CHC patients compared with healthy control and asymptomatic HCV carriers (33). Moreover, in mice, the level of hepatic γδ T cells is significantly higher in HCV transgenic mice compared with wild-type mice (34). It is assumed that peripheral γδ T cells are recruited into the liver and contribute to the pathogenesis of HCV infection.

γδ T cells play different roles in the pathogenesis of CHC infection. In some studies, γδ T cells manifest their antiviral role and inhibit the progression of CHC infection. In CHC patients, the cytotoxicity of hepatic γδ T cells is higher than that of hepatic αβ T cells. This is attributable to their elevated secretion of IFN-γ, TNF-α, and IL-8 (31) and their expression of activation marker (human leukocyte antigen-DR) and memory/effector ($CD62L^-CD45RO^+$ $CD95^+$) marker (32). In particular, the frequency of human hepatic IFN-γ^+Vδ1 T cells is positively correlated with the degree of liver necroinflammation, indicating their involvement in liver pathogenesis and liver damage (32). Furthermore, the expression of CD56 and CD16 (markers of natural killer cells) increase in peripheral Vγ9Vδ2 T cells and is further enhanced in hepatic Vγ9Vδ2 T cells of CHC patients (35). In humans, after stimulation by non-peptide antigen-isopentenyl diphosphate (IPP), activated peripheral Vγ9Vδ2 T cells are associated with a dramatic reduction in HCV RNA levels. Neutralizing experiments have further revealed the function of IFN-γ in HCV clearance (36). Moreover, in a mouse model, the number of hepatic γδ T cells increases and activated $CD69^+$ γδ T cells produce more IFN-γ and TNF-α during MHV (mouse hepatitis virus) infection than controls. Interestingly, those activated hepatic γδ T cells can kill MHV-infected hepatocytes *in vitro* by secreting IFN-γ and TNF-α (37).

However, several studies have indicated that human peripheral γδ T cells exhibit impaired function in CHC patients even after antiviral treatment. Human peripheral Vγ9Vδ2 T cells are activated and differentiate into effector cells with upregulated GrB and perforin expression, but have a markedly impaired capacity to produce IFN-γ in CHC patients (38). Furthermore, IFN-α treatments result in the upregulation of cytotoxic markers such as GrB, perforin, and CD107a, but not the IFN-γ production capacity of peripheral Vγ9Vδ2 T cells in CHC patients (35, 38). The above results suggest a functional dichotomy of Vγ9Vδ2 T cells in chronic HCV infections that contribute to both liver inflammation and HCV persistence. Moreover, dysfunction of γδ T cells in CHC patients has also been observed in antiviral therapy. Direct-active antiviral agents (DAAs) are widely used in the treatment of chronic HCV infection. In clinical trials, DAAs have induced minor changes in γδ T cells both in terms of numbers and in alterations of TRG and TRD repertoires 1 year after treatment (39). Although human peripheral Vγ9Vδ2 T cells display an elevated effector phenotype in sustained virologic-response HCV patients, recent DAA treatment research demonstrates that these cells show poor cytokine response and proliferative responses to antigens (40).

In summary, human and mouse hepatic γδ T cells as well as *in vitro* stimulated human peripheral Vγ9Vδ2 T cells can inhibit HCV pathogenesis. However, impaired cytokine response of peripheral Vγ9Vδ2 T cells in CHC patients contributes to HCV infection progression, even after DAA treatment. Further studies on recovery from the cytokine response impairment of Vγ9Vδ2 T cells is very important for CHC treatment.

ROLE OF γδ T CELLS IN LIVER CIRRHOSIS AND HCC

Persistent inflammation of HBV or HCV can lead to liver fibrosis and liver cirrhosis. HSCs are critical cells in the pathogenesis of liver cirrhosis. Activation of these cells promote the progression of liver cirrhosis (41). A liver cirrhosis mouse model shows different relationships between HSCs and hepatic γδ T cells. IL-17-producing $CCR6^+$ γδ T cells induce apoptosis of HSCs in a FasL-dependent manner to inhibit the progression of liver cirrhosis (42). Moreover, IFN-γ-producing γδ T cells can directly kill activated HSCs and increase NK cell-mediated cytotoxicity against activated HSCs partially through a 4-1BB dependent manner (43). However, hepatocyte-secreted exosomes can activate HSCs *via* Toll-like receptor 3. These HSCs further enhance the activity of IL-17-producing γδ T cells, which exacerbates liver fibrosis and promotes the progression of liver cirrhosis (4). In view of the contradictory roles of IL-17-producing γδ T cells in the same mouse model, further studies involving patients and a virus-induced liver cirrhosis mouse model should be performed to elucidate the exact role of γδ T cells.

A recent study has shown that the increased peritumor ratio in human γδ T cells contributes to the progression and recurrence of HCC, indicating the important role of γδ T cells in HCC (44). Interestingly, γδ T cells play different roles in the pathogenesis of HCC. In several studies, γδ T cells display cytotoxicity and inhibit proliferation of tumor cells *in vivo* and *in vitro*. In HCC patients, the number of human peritumoral γδ T cells is positively related to better prognosis of HCC curative resection (45). A recent biostatistics study has shown that the increase of human tumor-infiltrated γδ T cells, which is driven by the accumulation of chemokines such as CCL4 and CCL5, is significantly positively correlated with the survival rate and negatively correlated with HCC recurrence. γδ T cells play protective roles by regulating the infiltration and differentiation of $CD8^+$ T cells in HCC procession (46). Furthermore, human γδ

T cells can induce the death of HCC cell lines and reverse the immune escape of HCC *in vitro* (47). Moreover, the anti-HCC function of peripheral γδ T cells, especially Vγ9Vδ2 T cells, can be further enhanced by activating agents, including histone deacetylase inhibitors (48), pyrophosphate (49), zoledronate (20), CD226 (50), and even the Chinese herb artesunate (51).

However, other studies reveal that impaired human γδ T cells or mouse γδ T cells can also contribute to the progression of HCC. In an immunosuppressed tumor microenvironment, γδ T cells show impaired IFN-γ production and degranulation (perforin and CD107a) capacity, which is attributed to the secretion of TGF-β and IL-10 by tumor-infiltrating Tregs (52). In addition, a decrease in the number and cytotoxicity of peripheral Vδ2 T cells is observed in HCC patients and possibly associated with the lack of IL-2 and IL-21 (53). The total number of γδ T cells and effector γδ T cells is significantly lower in tumors than in peritumoral tissues and non-tumor livers (52, 54). In addition, in an HCC mouse model, IL-17-producing Vγ4 T cells recruit MDSCs in a CXCL5/CXCR2-dependent manner and further suppress the anti-tumor function of $CD8^+$ T cells (55).

Human peripheral Vδ2 T cells can proliferate *in vitro* and kill HCC and thus have been used in clinical immunotherapy of HCC patients. Zoledronate induces the proliferation of γδ T cells in HCC patients who exhibit upregulated expression of IFN-γ, TNF-α, GrB, perforin, and lysosome-associated membrane protein 1 (47). A clinical trial has shown that the combined use of γδ T cells, NK cells, and cytokine-induced killer (CIK) therapy significantly inhibits virus replication and prolongs the survival rate of HCV-positive HCC patients (21).

In conclusion, γδ T cells and their subsets play opposite roles in liver cancer, and their underlying mechanisms require further investigation.

CONCLUSIONS AND PERSPECTIVES

Different subsets of γδ T cells play various roles in pathogenesis of HBV or HCV infection. Most of the mouse and human studies are summarized in **Figure 1**.

In mouse model, IL-17-producing Vγ4 T cells subsets promote the progression of CHB, LC and HCC. However, in

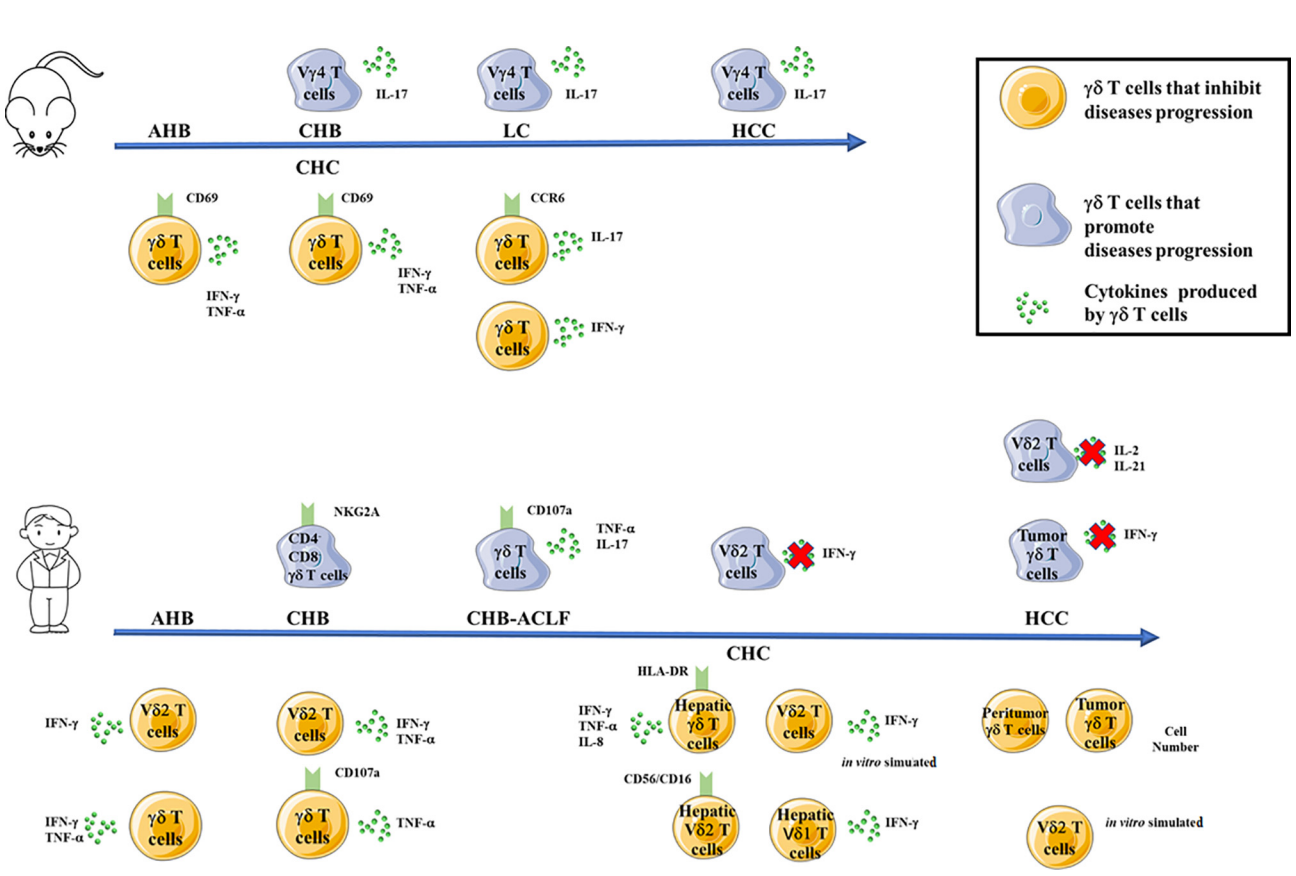

FIGURE 1 | Functions of mouse (top) or human (bottom) γδ T cells and their subsets in inhibiting (yellow cells) or promoting (blue cells) the pathogenesis of HBV or HCV infection. The arrows indicate the progression of HBV or HCV infections, the red fox indicate impaired secretion of cytokines by γδ T cells and their subsets. AHB, acute hepatitis B infection; CHB, chronic hepatitis B infection; CHB-ACLF, HBV-associated acute-on-chronic liver failure; CHC, chronic hepatitis C infection; LC, liver cirrhosis; HCC, hepatocellular carcinoma.

other studies, IFN-γ and TNF-α-producing $CD69^+$ mouse γδ T cells can inhibit the progression of AHB and CHC. Furthermore, IL-17-producing $CCR6^+$ mouse γδ T cells or IFN-γ producing mouse γδ T cells inhibit the progression of LC. (**Figure 1**, top).

In human studies (**Figure 1**, bottom), $CD4^-$ $CD8^-$ γδ T cells subsets and IL-17/TNF-a^+ γδ T cells promote the progression of CHB and CHB-ACLF patients, respectively. Impairment secretion of IFN-γ by peripheral Vδ2 T cells contributes to the progression of CHC. Moreover, impairment secretions of IL-2 and IL-21 by peripheral Vδ2 T cells and IFN-γ by tumor-infiltrating γδ T cells contribute to the progression of HCC. Contradictorily, in AHB patients, IFN-γ-producing peripheral Vδ2 T cells and IFN-γ and TNF-α-producing peripheral γδ T cells can inhibit AHB infection. In addition, IFN-γ and TNF-α-producing peripheral Vδ2 T cells and TNF-α-producing $CD107a^+$ peripheral γδ T cells inhibit the progression of CHB infection. Furthermore, hepatic γδ T cells as well as *in vitro* activated peripheral Vδ2 T cells inhibit the progression of CHC infection. Furthermore, increased number of peritumor and tumor γδ T cells as well as *in vitro* activated peripheral Vδ2 T cells inhibit the progression of HCC (**Figure 1**, bottom).

Although functions of γδ T cells are summarized above, some of their roles in virus infection remain obscure. For instance, IL-17-producing Vγ4 T cells display diverse roles to influence the development of liver cirrhosis in the same mouse model. Furthermore, the role of human peripheral γδ T cells but not hepatic γδ T cells has been extensively studied. Thus, the impact of cytokine production and the functions of hepatic γδ T cell subsets in the pathogenesis of HBV and HCV infections require further investigation. The frequency and function of γδ T cells can be distinguished based on human race, age, and gender, thus these factors have to be considered in related researches (28, 29). Asian Americans display two- to three-fold higher number of peripheral Vδ2 T cells compared to non-Asian Americans (28), which in turn may partially contribute to the immune responses and outcome of virus infection. Moreover, the fate of transferred γδ T cells in the human body as well as the indication and race of liver cancer patients should be assessed to achieve better therapeutic outcomes during treatment. Last but not least, in view of their antiviral function, IFN-γ-producing γδ T cell-based therapies should be developed for patients in stages of virus infection other than HCC. Understanding the roles of γδ T cells in relation to the pathogenesis of HBV and HCV infections may facilitate in the development of γδ T cell-based therapy or γδ T cell-based targets for the treatment of virus infections.

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REFERENCES

1. Rajoriya N, Fergusson JR, Leithead JA, Klenerman P. Gamma Delta T-lymphocytes in Hepatitis C and Chronic Liver Disease. *Front Immunol* (2014) 5:400. doi: 10.3389/fimmu.2014.00400
2. Lee HM, Banini BA. Updates on Chronic HBV: Current Challenges and Future Goals. *Curr Treat Options Gastroenterol* (2019) 17:271–91. doi: 10.1007/s11938-019-00236-3
3. Jia ZH, Li YY, Wang JY, Zhang JY, Huang A, Guo XD, et al. Activated gammadelta T cells exhibit cytotoxicity and the capacity for viral clearance in patients with acute hepatitis B. *Clin Immunol* (2019) 202:40–8. doi: 10.1016/j.clim.2019.03.005
4. Seo W, Eun HS, Kim SY, Yi HS, Lee YS, Park SH, et al. Exosome-mediated activation of toll-like receptor 3 in stellate cells stimulates interleukin-17 production by gammadelta T cells in liver fibrosis. *Hepatology* (2016) 64:616–31. doi: 10.1002/hep.28644
5. Lafaro KJ, Demirjian AN, Pawlik TM. Epidemiology of hepatocellular carcinoma. *Surg Oncol Clinics North America* (2015) 24(1):1–17. doi: 10.1016/j.soc.2014.09.001
6. Racanelli V, Rehermann B. The liver as an immunological organ. *Hepatology* (2006) 43:S54–62. doi: 10.1002/hep.21060
7. Shin EC, Sung PS, Park SH. Immune responses and immunopathology in acute and chronic viral hepatitis. *Nat Rev Immunol* (2016) 16:509–23. doi: 10.1038/nri.2016.69
8. Saeidi A, Zandi K, Cheok YY, Saeidi H, Wong WF, Lee CYQ, et al. T-Cell Exhaustion in Chronic Infections: Reversing the State of Exhaustion and Reinvigorating Optimal Protective Immune Responses. *Front Immunol* (2018) 9:2569. doi: 10.3389/fimmu.2018.02569
9. Zhang JY, Zhang Z, Lin F, Zou ZS, Xu RN, Jin L, et al. Interleukin-17-producing CD4(+) T cells increase with severity of liver damage in patients with chronic hepatitis B. *Hepatology* (2010) 51:81–91. doi: 10.1002/hep.23273
10. Li TY, Yang Y, Zhou G, Tu ZK. Immune suppression in chronic hepatitis B infection associated liver disease: A review. *World J Gastroenterol* (2019) 25:3527–37. doi: 10.3748/wjg.v25.i27.3527
11. Kohlgruber AC, Gal-Oz ST, LaMarche NM, Shimazaki M, Duquette D, Koay HF, et al. γδ T cells producing interleukin-17A regulate adipose regulatory T cell homeostasis and thermogenesis. *Nat Immunol* (2018) 19:464–74. doi: 10.1038/s41590-018-0094-2
12. Pellicci DG, Koay HF, Berzins SP. Thymic development of unconventional T cells: how NKT cells, MAIT cells and gammadelta T cells emerge. *Nat Rev Immunol* (2020) 20:756–70. doi: 10.1038/s41577-020-0345-y
13. Wang X, Tian Z. gammadelta T cells in liver diseases. *Front Med* (2018) 12:262–8. doi: 10.1007/s11684-017-0584-x
14. Papadopoulou M, Sanchez SG, Vermijlen D. Innate and adaptive γδ T cells: How, when, and why. *Immunol Rev* (2020) 00:1–18. doi: 10.1111/imr.12926
15. Hunter S, Willcox CR, Davey MS, Kasatskaya SA, Jeffery HC, Chudakov DM, et al. Human liver infiltrating γδ T cells are composed of clonally expanded circulating and tissue-resident populations. *J Hepatol* (2018) 69(3):654–65. doi: 10.1016/j.jhep.2018.05.007
16. Benova K, Hanckova M, Koci K, Kudelova M, Betakova T. T cells and their function in the immune response to viruses. *Acta Virol* (2020) 64:131–43. doi: 10.4149/av_2020_203
17. Wu X, Zhang JY, Huang A, Li YY, Zhang S, Wei J, et al. Decreased Vdelta2 gammadelta T cells associated with liver damage by regulation of Th17 response in patients with chronic hepatitis B. *J Infect Dis* (2013) 208:1294–304. doi: 10.1093/infdis/jit312
18. Lai Q, Ma S, Ge J, Huang Z, Huang X, Jiang X, et al. TCR gammadelta(+)CD4(-)CD8(-) T cells suppress the CD8(+) T-cell response to hepatitis B virus peptides, and are associated with viral control in chronic hepatitis B. *PloS One* (2014) 9:e88475. doi: 10.1371/journal.pone.0088475

19. Kong X, Sun R, Chen Y, Wei H, Tian Z. gammadeltaT cells drive myeloid-derived suppressor cell-mediated CD8+ T cell exhaustion in hepatitis B virus-induced immunotolerance. *J Immunol* (2014) 193:1645–53. doi: 10.4049/jimmunol.1303432
20. Sugai S, Yoshikawa T, Iwama T, Tsuchiya N, Ueda N, Fujinami N, et al. Hepatocellular carcinoma cell sensitivity to Vgamma9Vdelta2 T lymphocyte-mediated killing is increased by zoledronate. *Int J Oncol* (2016) 48:1794–804. doi: 10.3892/ijo.2016.3403
21. Qian L, Wang N, Tian H, Jin H, Zhao H, Niu C, et al. Dual Effects of Cellular Immunotherapy in Inhibition of Virus Replication and Prolongation of Survival in HCV-Positive Hepatocellular Carcinoma Patients. *J Immunol Res* (2016) 2016:6837241. doi: 10.1155/2016/6837241
22. Ferrari C. HBV and the immune response. *Liver Int* (2015) 35(Suppl 1):121–8. doi: 10.1111/liv.12749
23. Conroy MJ, Mac Nicholas R, Taylor M, O'dea S, Mulcahy F, Norris S, et al. Increased Frequencies of Circulating IFN-gamma-Producing Vdelta1(+) and Vdelta2(+) gammadelta T Cells in Patients with Asymptomatic Persistent Hepatitis B Virus Infection. *Viral Immunol* (2015) 28:201–8. doi: 10.1089/vim.2014.0133
24. Chang L, Wang L, Ling N, Peng H, Chen M. Increase in liver gammadelta T cells with concurrent augmentation of IFN-beta production during the early stages of a mouse model of acute experimental hepatitis B virus infection. *Exp Ther Med* (2020) 19:67–78. doi: 10.3892/etm.2019.8197
25. Cannizzo ES, Tincati C, Binda F, Ronzi P, Cazzaniga FA, Antinori S, et al. Unconventional T cells in chronic hepatitis B patients on long-term suppressive therapy with tenofovir followed by a Peg-IFN add-on strategy: A randomized study. *J Viral Hepat* (2018) 25:381–90. doi: 10.1111/jvh.12820
26. Chen M, Zhang D, Zhen W, Shi Q, Liu Y, Ling N, et al. Characteristics of circulating T cell receptor gamma-delta T cells from individuals chronically infected with hepatitis B virus (HBV): an association between V(delta)2 subtype and chronic HBV infection. *J Infect Dis* (2008) 198:1643–50. doi: 10.1086/593065
27. Chen M, Hu P, Ling N, Peng H, Lei Y, Hu H, et al. Enhanced functions of peripheral gammadelta T cells in chronic hepatitis B infection during interferon alpha treatment in vivo and in vitro. *PLoS One* (2015) 10: e0120086. doi: 10.1371/journal.pone.0120086
28. Chang KM, Traum D, Park JJ, Ho S, Ojiro K, Wong DK, et al. Distinct phenotype and function of circulating Vdelta1+ and Vdelta2+ gammadeltaT-cells in acute and chronic hepatitis B. *PLoS Pathog* (2019) 15:e1007715. doi: 10.1371/journal.ppat.1007715
29. Cairo C, Armstrong CL, Cummings JS, Deetz CO, Tan M, Lu C, et al. Impact of age, gender, and race on circulating γδ T cells. *Hum Immunol* (2010) 71 (10):968–75. doi: 10.1016/j.humimm.2010.06.014
30. Chen M, Hu P, Peng H, Zeng W, Shi X, Lei Y, et al. Enhanced peripheral gammadeltaT cells cytotoxicity potential in patients with HBV-associated acute-on-chronic liver failure might contribute to the disease progression. *J Clin Immunol* (2012) 32:877–85. doi: 10.1007/s10875-012-9678-z
31. Tseng CT, Miskovsky E, Houghton M, Klimpel GR. Characterization of liver T-cell receptor gammadelta T cells obtained from individuals chronically infected with hepatitis C virus (HCV): evidence for these T cells playing a role in the liver pathology associated with HCV infections. *Hepatology* (2001) 33:1312–20. doi: 10.1053/jhep.2001.24269
32. Agrati C, D'offizi G, Narciso P, Abrignani S, Ippolito G, Colizzi V, et al. Vdelta1 T lymphocytes expressing a Th1 phenotype are the major gammadelta T cell subset infiltrating the liver of HCV-infected persons. *Mol Med* (2001) 7:11–9. doi: 10.1007/BF03401834
33. Par G, Rukavina D, Podack ER, Horanyi M, Szekeres-Bartho J, Hegedus G, et al. Decrease in CD3-negative-CD8dim(+) and Vdelta2/Vgamma9 TcR+ peripheral blood lymphocyte counts, low perforin expression and the impairment of natural killer cell activity is associated with chronic hepatitis C virus infection. *J Hepatol* (2002) 37:514–22. doi: 10.1016/S0168-8278(02)00218-0
34. Alonzi T, Agrati C, Costabile B, Cicchini C, Amicone L, Cavallari C, et al. Steatosis and intrahepatic lymphocyte recruitment in hepatitis C virus transgenic mice. *J Gen Virol* (2004) 85:1509–20. doi: 10.1099/vir.0.19724-0
35. Yin W, Tong S, Zhang Q, Shao J, Liu Q, Peng H, et al. Functional dichotomy of Vdelta2 gammadelta T cells in chronic hepatitis C virus infections: role in cytotoxicity but not for IFN-gamma production. *Sci Rep* (2016) 6:26296. doi: 10.1038/srep26296
36. Agrati C, Alonzi T, De Santis R, Castilletti C, Abbate I, Capobianchi MR, et al. Activation of Vgamma9Vdelta2 T cells by non-peptidic antigens induces the inhibition of subgenomic HCV replication. *Int Immunol* (2006) 18:11–8. doi: 10.1093/intimm/dxh337
37. Wu D, Yan WM, Wang HW, Huang D, Luo XP, Ning Q. gammadelta T Cells Contribute to the Outcome of Murine Fulminant Viral Hepatitis via Effector Cytokines TNF-alpha and IFN-gamma. *Curr Med Sci* (2018) 38:648–55. doi: 10.1007/s11596-018-1926-x
38. Lee JW, Kim W, Kwon EK, Kim Y, Shin HM, Kim DH, et al. Immunological dynamics associated with rapid virological response during the early phase of type I interferon therapy in patients with chronic hepatitis C. *PLoS One* (2017) 12:e0179094. doi: 10.1371/journal.pone.0179094
39. Ravens S, Hengst J, Schlapphoff V, Deterding K, Dhingra A, Schultze-Florey C, et al. Human gammadelta T Cell Receptor Repertoires in Peripheral Blood Remain Stable Despite Clearance of Persistent Hepatitis C Virus Infection by Direct-Acting Antiviral Drug Therapy. *Front Immunol* (2018) 9:510. doi: 10.3389/fimmu.2018.00510
40. Ghosh A, Mondal RK, Romani S, Bagchi S, Cairo C, Pauza CD, et al. Persistent gamma delta T-cell dysfunction in chronic HCV infection despite direct-acting antiviral therapy induced cure. *J Viral Hepat* (2019) 26:1105–16. doi: 10.1111/jvh.13121
41. Dewidar B, Meyer C, Dooley S, Meindl-Beinker AN. TGF-beta in Hepatic Stellate Cell Activation and Liver Fibrogenesis-Updated 2019. *Cells* (2019) 8 (11):1419. doi: 10.3390/cells8111419
42. Hammerich L, Bangen JM, Govaere O, Zimmermann HW, Gassler N, Huss S, et al. Chemokine receptor CCR6-dependent accumulation of gammadelta T cells in injured liver restricts hepatic inflammation and fibrosis. *Hepatology* (2014) 59:630–42. doi: 10.1002/hep.26697
43. Liu M, Hu Y, Yuan Y, Tian Z, Zhang C. gammadeltaT Cells Suppress Liver Fibrosis via Strong Cytolysis and Enhanced NK Cell-Mediated Cytotoxicity Against Hepatic Stellate Cells. *Front Immunol* (2019) 10:477. doi: 10.3389/fimmu.2019.00477
44. Zhou BY, Gong JH, Cai XY, Wang JX, Luo F, Jiang N, et al. An imbalance between stellate cells and gammadeltaT cells contributes to hepatocellular carcinoma aggressiveness and recurrence. *Hepatol Int* (2019) 13:631–40. doi: 10.1007/s12072-019-09969-w
45. Cai XY, Wang JX, Yi Y, He HW, Ni XC, Zhou J, et al. Low counts of gammadelta T cells in peritumoral liver tissue are related to more frequent recurrence in patients with hepatocellular carcinoma after curative resection. *Asian Pac J Cancer Prev* (2014) 15:775–80. doi: 10.7314/APJCP.2014.15.2.775
46. Zhao N, Dang H, Ma L, Martin SP, Forgues M, Ylaya K, et al. Intratumoral gammadelta T-cell infiltrates, CCL4/5 protein expression and survival in patients with hepatocellular carcinoma. *Hepatology* (2020). doi: 10.1002/hep.31412
47. Tian W, Ma J, Shi R, Ren C, He J, Zhao H. gammadelta T cell-mediated individualized immunotherapy for hepatocellular carcinoma considering clinicopathological characteristics and immunosuppressive factors. *Oncol Lett* (2018) 15:5433–42. doi: 10.3892/ol.2018.8026
48. Hoh A, Dewerth A, Vogt F, Wenz J, Baeuerle PA, Warmann SW, et al. The activity of gammadelta T cells against paediatric liver tumour cells and spheroids in cell culture. *Liver Int* (2013) 33:127–36. doi: 10.1111/liv.12011
49. Tanaka Y, Kobayashi H, Terasaki T, Toma H, Aruga A, Uchiyama T, et al. Synthesis of pyrophosphate-containing compounds that stimulate Vgamma2Vdelta2 T cells: application to cancer immunotherapy. *Med Chem* (2007) 3:85–99. doi: 10.2174/157340607779317544
50. Toutirais O, Cabillic F, Le Friec G, Salot S, Loyer P, Le Gallo M, et al. DNAX accessory molecule-1 (CD226) promotes human hepatocellular carcinoma cell lysis by Vgamma9Vdelta2 T cells. *Eur J Immunol* (2009) 39:1361–8. doi: 10.1002/eji.200838409
51. Qian P, Zhang YW, Zhou ZH, Liu JQ, Yue SY, Guo XL, et al. Artesunate enhances gammadelta T-cell-mediated antitumor activity through augmenting gammadelta T-cell function and reversing immune escape of HepG2 cells. *Immunopharmacol Immunotoxicol* (2018) 40:107–16. doi: 10.1080/08923973.2017.1386212
52. Yi Y, He HW, Wang JX, Cai XY, Li YW, Zhou J, et al. The functional impairment of HCC-infiltrating gammadelta T cells, partially mediated by regulatory T cells in a TGFbeta- and IL-10-dependent manner. *J Hepatol* (2013) 58:977–83. doi: 10.1016/j.jhep.2012.12.015

53. Jiang H, Yang Z, Song Z, Green M, Song H, Shao Q. gammadelta T cells in hepatocellular carcinoma patients present cytotoxic activity but are reduced in potency due to IL-2 and IL-21 pathways. *Int Immunopharmacol* (2019) 70:167–73. doi: 10.1016/j.intimp.2019.02.019
54. Di Blasi D, Boldanova T, Mori L, Terracciano L, Heim MH, De Libero G. Unique T-Cell Populations Define Immune-Inflamed Hepatocellular Carcinoma. *Cell Mol Gastroenterol Hepatol* (2020) 9:195–218. doi: 10.1016/j.jcmgh.2019.08.004
55. Ma S, Cheng Q, Cai Y, Gong H, Wu Y, Yu X, et al. IL-17A produced by gammadelta T cells promotes tumor growth in hepatocellular carcinoma. *Cancer Res* (2014) 74:1969–82. doi: 10.1158/0008-5472.CAN-13-2534

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REVIEW
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The Dual Roles of Human γδ T Cells: Anti-Tumor or Tumor-Promoting

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γδ T cells are the unique T cell subgroup with their T cell receptors composed of γ chain and δ chain. Unlike αβ T cells, γδ T cells are non-MHC-restricted in recognizing tumor antigens, and therefore defined as innate immune cells. Activated γδ T cells can promote the anti-tumor function of adaptive immune cells. They are considered as a bridge between adaptive immunity and innate immunity. However, several other studies have shown that γδ T cells can also promote tumor progression by inhibiting anti-tumor response. Therefore, γδ T cells may have both anti-tumor and tumor-promoting effects. In order to clarify this contradiction, in this review, we summarized the functions of the main subsets of human γδ T cells in how they exhibit their respective anti-tumor or pro-tumor effects in cancer. Then, we reviewed recent γδ T cell-based anti-tumor immunotherapy. Finally, we summarized the existing problems and prospect of this immunotherapy.

Keywords: γδ T cells, tumor, human, immunity, immunotherapy

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INTRODUCTION

γδ T cells are the non-classical cell subgroup characterized by expression of γδ heterodimeric T cell receptor (TCRγδ) on cell surface. They only account for 1% to 5% of T lymphocytes in peripheral blood circulation and lymphatic circulation, and predominantly reside in the mucosal tissues such as skin, intestine, lung, and uterus (1–3). γδ T cells are the intermediate group of cells between innate and adaptive immune cells, serving as a bridge between innate immunity and adaptive immune response (4, 5). They play important roles in tumor immunity. Depending on the microenvironment, different γδ T cell subgroups can have anti-tumor or pro-tumor activities.

Compare with αβ T cells, γδ T cells have different antigen recognition mechanisms and capabilities without the histocompatibility complex (MHC) and the second signal (CD28 and CD80/86) (6). They can use TCRγδ and natural killer cell receptors (NKR) to recognize a variety of tumor-associated antigens (TAA), including non-peptidic prenyl-pyrophosphate antigens (PAg) and stress proteins (7). The PAg are products of isoprenoid biosynthesis pathways, such as isoprene pyrophosphate (IPP) from mammalian cells and (E)-4-Hydroxy-3-Methylbut-2-Enyl Diphosphate (HMBPP, the strongest stimulant of γδ T cells) from pathogenic microorganisms (8–12). Besides,

the stress proteins will up-regulate or ectopically express under the stress conditions, such as apolipoprotein A1-F1-ATPase complex (F1-ATPase Apo A1) (13), MHC-like molecules MICA/B, UL16 Binding protein (ULBP) (14–18), endothelial cell protein C receptor (EPCR) (19, 20), heat shock protein (21–23) and human MutS homolog 2 (24–26). These antigens can activate γδ T cells to secrete interferon γ (IFN-γ) and tumor necrosis factor α (TNF-α) (6), or kill tumor cells through Fas/FasL and antibody-dependent cell-mediated cytotoxicity (ADCC) (27–30). Moreover, γδ T cells can also enhance the anti-tumor ability of other immune cells by secreting cytokines or expressing costimulatory molecules. For example, human γδ T cells can stimulate the cytotoxicity of NK cells through expressed the costimulatory molecule CD137L (31). γδ T cells have been used in clinic for the treatment of non-small cell lung cancer and breast cancer. Such γδ T-based immunotherapy appeared to be safe and well-tolerated in patients (32–35).

However, it was reported that γδ T cells could also promote cancer development (36). For example, as one of the main sources of interleukin-17 (IL-17), tumor-infiltrating γδ T cells were shown to promote tumor development and metastasis by enhancing angiogenesis and recruiting inhibitory cells (37–40). Tumor-infiltrating γδ T cells could also directly induce the apoptosis of anti-tumor immune cells (41).

In this review, we introduced the classification of human γδ T cells and summarized how γδ T cell subsets play different roles in tumorigenesis. We further discussed the γδ T cell-based anti-tumor immunotherapy which has been widely used in clinic. Finally, we briefly summarized the current limitation and caveats associated with such therapy, and proposed new approach for optimization. We believe that the summary of biological functions of different γδ T cells can help us improve our understanding of tumor microenvironment, and provide novel insights for anti-tumor immunity.

CLASSIFICATION OF γδ T CELLS

Human γδ T cells can be classified into different groups based on the expression of TCRγ chains or TCRδ chains, and they can be further classified by the expression of different CD molecules (42, 43).

Classification Based on the Expression of TCRγ Chain or TCRδ Chain

Different TCRγ chains (Vγ2/3/4/5/8/9) and TCRδ chains (Vδ1/2/3/5) can be combined to form different types of γδ T cells. Interestingly, each TCRδ chain usually forms with one or several dominant TCRγ chains a fixed combination pattern, rather than with random combinations (44–47).

Different γδ T cells have diversified distribution and functions. Vδ1 chain can interact with different γ chains to form various γδ T cells. They are mainly distributed in the skin, intestine, liver, spleen and mucosal tissues. The role of Vδ1 T cells is controversial. In certain situations, they have been shown to have strong anti-tumor effects in colorectal cancer, multiple myeloma, chronic lymphocytic leukemia (48–50). On the other hand, tumor-infiltrating Vδ1 T cells often demonstrate strong immunosuppressive effects. They secreted IL-17 and transforming growth factor-β (TGF-β) (51), expressed programmed cell death 1 ligand 1 (PD-L1), and inhibited the activation of other immune cells (41).

The Vδ2 chain only combines with the Vγ9 chain to form the Vγ9Vδ2 T cells, which mainly exist in peripheral blood. Given that the Vγ9Vδ2 T cells have strong anti-tumor effects in various types of tumors, they were widely used in clinics (52–55). In addition, they have also been shown to kill the cancer stem cells (CSC) in various tumors including colon cancer, ovarian cancer, and neuroblastoma (56, 57).

The Vδ3 chain mainly interacts with the Vγ2 and Vγ3 chains. Vδ3 T cells mainly exist in the liver, and also in a small amount in the peripheral blood of patients with chronic lymphocytic leukemia. The functions of Vδ3 T cells in tumors have not been elucidated in depth (58–61).

The Vδ5 chain usually combines with the Vγ4 chain to form the Vγ4Vδ5 T cells. They mainly exist in peripheral blood. The TCR of Vγ4Vδ5 T cells could directly bind to the endothelial protein C receptor (EPCR) to recognize epithelial tumor cells. Like Vδ3 T cells, they were rarely studied for their tumor-related functions (19, 62) (**Table 1**).

Classifications Based on the Phenotype of CD Molecules

Human γδ T cells can be classified based on the expression of CD27 and CD45RA. The naive type (T_{naive}, $CD27^+CD45RA^+$) and the central-memory phenotype (T_{CM}, $CD27^+CD45RA^-$), mainly exist in the secondary lymphoid organs. T_{CM} can maintain immune memory for a long time and quickly mediate immune response after receiving antigen stimulation. The effector-memory type (T_{EM}, $CD27^-CD45RA^-$) and terminally-differentiated type (T_{EMRA}, $CD27^-CD45RA^+$) mainly exist at the site of inflammation and exert instant effects, namely secreting cytokines and exerting cytotoxicity (63, 64).

Classification of γδ T Cells According to Their Cellular Function

Based on their varied functions, γδ T cells can be divided into several subtypes. Similar to αβ T cells, effector γδ T cells can exert an anti-tumor effect through various pathways. Regulatory γδ T cells ($CD4^+CD25^+Foxp3^+$) or inhibitory γδ T cells can regulate the immune balance and maintain immune tolerance (17, 51). In addition, γδ T17 cells can produce IL-17 to promote tumor development (6, 36, 41).

TABLE 1 | Subsets of human γδ T cells.

Subset	Paired TCRγ chains	Cellular localization
Vδ1	Vγ2, Vγ3, Vγ4, Vγ5, Vγ8 and Vγ9	Skin, intestine, liver, spleen and mucosal tissues
Vδ2	Vγ9	Peripheral blood
Vδ3	Vγ2, Vγ3	Liver and peripheral blood
Vδ5	Vγ4	Peripheral blood

γδ T CELLS PLAY A DIRECT ANTI-TUMOR ROLE

The Tumor-Associated Antigens Recognition by γδ T Cells

Vγ9Vδ2 T cells recognizes TAA through TCRγδ and NKR and Vγ9Vδ2TCR can recognize PAg. This type of antigen was a product of isoprenoid biosynthesis pathway in eukaryotic cells, such as IPP and the adenylated, thymidylated, and uridylated triphosphate derivatives. In tumor cells, the isoprenoid biosynthetic pathway is enhanced to ensure energy supply and PAg accumulation, prompting recognition by the Vγ9Vδ2 T cells (65–68). Vγ9Vδ2TCR requires the help of Butyrophilin (BTN) 3A1 to recognize tumor cells. BTN3A1 is an immunoglobulin-like molecule with immunomodulatory function, which could mediate the interaction between γδ T cells and PAg, and could also be directly recognized by Vγ9Vδ2TCR (69–71). There were two theories on how BTN3A1 helps Vγ9Vδ2TCR recognize PAg. The first proposed mechanism was that BTN3A1 is a sensor that senses the level of PAg inside the cell. The intracellular B30.2 domain of the BTN3A1 molecule is a positively charged pocket that could directly bind to PAg, lead to changes in the structure of the extracellular dimer of BTN3A1 that can be recognized by Vγ9Vδ2TCR, and then activate the γδ T cells (72–77). The second proposed mechanism was that BTN3A1 formed a BTN3A1-PAg complex with PAg, presented PAg to the outside of the cell, and directly bound to Vγ9Vδ2TCR to activate γδ T cells (78). The latest study found that BTN2A1, which was in the same family as BTN3A1, was also a ligand for Vγ9Vδ2TCR and necessary for Vγ9Vδ2 T cells to recognize PAg. BTN2A1 and BTN3A1 can be found on the surface of tumor cells and recognized by two sites of Vγ9Vδ2TCR. BTN2A1 is recognized by the Vγ9 area, and BTN3A1 is recognized by the Vδ2 area (79, 80). In addition, Vγ9Vδ2TCR could recognize the F1-ATPaseApoA1 complex. This complex are normally expressed in the inner membrane of mitochondria, but some tumor cells, such as human leukemia (K562) cells and lymphoma (Raji) cells, could ectopic express it on the cell membrane. ApoA1 in the complex could not directly activate Vγ9Vδ2 T cells, instead it plays a function in stabilizing the interaction between Vγ9Vδ2TCR and F1-ATPase (13, 81).

Vγ9Vδ2 T cells could also recognize TAA through NKR, such as the natural killer 2D receptor (NKG2D) and DNAX accessory molecule 1 (DNAM-1). NKG2D is a lectin-type activation receptor, expressed on most natural killer cells (NK) and natural killer T (NKT) cells and partly expressed on γδ T cells and antigen-activated $CD8^+$ T cells (82). When γδ T cells contacted by the tumor cells, $Vγ9^+$ subpopulations rapidly proliferated, and γδ T cells up-regulated their NKG2D expression (83). NKG2D ligands on tumor cells include MICA, MICB and ULBP1~4 (84, 85). They could be recognized by NKG2D and enable γδ T cells to exert anti-tumor function (82). DNAM-1 is expressed on the γδ T cells and believed to promote the secretion of cytokines and enhance the cytotoxicity of immune cells. Vγ9Vδ2 T cells used DNAM-1 to recognize Nectin-2 and PVR, which were widely expressed on the tumor cells (86–88). Shielding DNAM-1 from the surface of γδ T cells could significantly inhibit its ability to kill tumor cells (89). It was shown that DNAM-1 is one of the important factors mediating γδ T cells to recognize tumor cells.

Vδ1 T cells also recognize tumor cells through TCRγδ and NKR. Vδ1TCR could recognize MHC-like molecule CD1d and the lipid antigen presented by it (90, 91). CD1d is expressed on a variety of cancers, such as myeloma, breast cancer and prostate cancer (92–94). The decrease of CD1d molecules on the primitive neuroectodermal tumor cells would cause these cells to evade immune recognition (95). In addition, Vδ1TCR could recognize tumor cells through MICA, but the MICA bindings by Vδ1TCR and NKG2D were mutually exclusive (96). Vδ1 T cells also express NKR. These cells recognize ULBP3 which is expressed on chronic lymphocytic leukemia of B-cell type (B-CLL) through NKR (97). They recognize human breast cancer cells through NKG2D, significantly preventing the disease progression (35). In addition to NKG2D and DNAM-1, Vδ1 T cells stimulated by IL-2 or IL-15 also express NKp30, NKp44 and NKp46 (48, 98), and have strong IFN-γ secretion ability (99, 100). Moreover, it has been confirmed that in acute myeloid leukemia, the ligand of NKp30 is B7-H6, a member of the B7 family (101).

Other studies have also confirmed that Vγ4Vδ5TCR can recognize EPCR, which is expressed on the epithelial tumor cells (19, 20) (**Figure 1**).

Anti-Tumor Mechanism of γδ T Cells

First, γδ T cells could kill tumor cells directly through secreting perforin and granzyme B (82). γδ T cells recognize tumor cells and release perforin and granzyme B into the synaptic space. They could further activate caspases to break DNA of tumor cells and lead to tumor cell death (102–105). γδ T cells could kill the human squamous cell carcinoma through perforin and granzyme B (106). Perforin and granzyme B inhibitor significantly reduce the ability of Vγ9Vδ2 T cells to lyse breast cancer cells *in vitro* (107). Moreover, in patients with renal carcinoma, activated Vγ9Vδ2 T cells showed a strong cytotoxicity to autologous tumor cells through perforin and granzyme B (108).

Second, γδ T cells kill tumor cells through ADCC. The Fab and Fc segment of antibody could bind to the TAA and γδ T cells, respectively. Then γδ T cells are activated to kill the tumor cells. Upon interaction with tumor cells, the expression of CD16 (FcγRIIIA) could be up-regulated on γδ T cells to induce tumor death through ADCC (82, 109, 110). In chronic lymphocytic leukemia and breast cancer patients, the cytotoxicity of Vγ9Vδ2 T cells is significantly enhanced after treatment with monoclonal antibodies including rituximab, trastuzumab and alemtuzumab (111–113).

Third, γδ T cells kill tumors through the Fas/FasL pathway and TRAIL (106). FasL expressed on γδ T cells could bind to Fas, and formed Fas trimer, which lead to the binding of the death effector domain (DED) to Fas-associated death domain–containing protein (FADD), and then activate caspases to induced the apoptosis of target cells (114–116). Similar to Fas/FasL, TRAIL also activates caspases through FADD, and then leads to apoptosis of tumor cells (117–124). In addition, IFN-γ could enhance the cytotoxicity of γδ T cells by up-regulating the expression of Fas on osteosarcoma cells (125, 126).

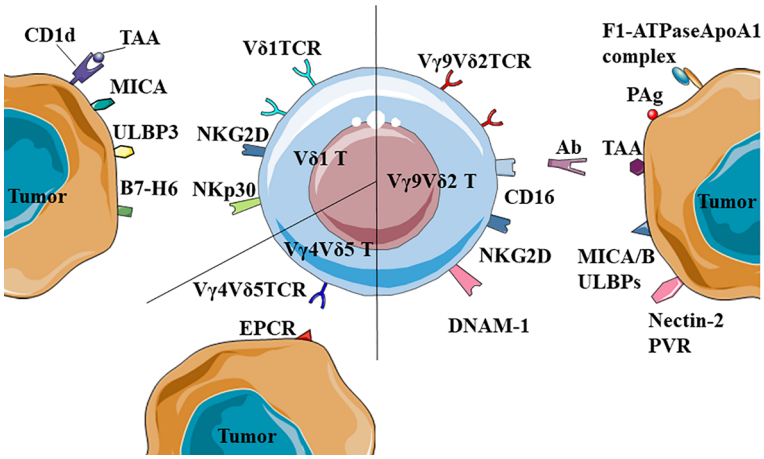

FIGURE 1 | Recognition of tumor-associated antigens (TAA) by different γδ T cells. Vδ1TCR could recognize MICA or the complexes of CD1d and TAA; NKR+ (NKG2D and NKp30) Vδ1 T cells could recognize ULBP3 and B7-H6. Besides, Vγ9Vδ2 T cells could also recognize and kill tumors by CD16-mediated antibody-dependent cell-mediated cytotoxicity (ADCC). Notably, Vγ4Vδ5TCR could recognize the antigen of epithelial tumor cells, EPCR.

Similar to Vγ9Vδ2 T cells, Vδ1 T cells could kill tumor cells through the perforin-granzyme B, Fas/FasL and TRAIL pathway (49, 50, 98, 101). For example, human skin Vδ1 T cells could secrete perforin to kill melanoma cells (127). Granzyme B^+ Vδ1 T cells and $TRAIL^+$ Vδ1 T cells showed strong cytotoxicity to lymphoma cells and chronic lymphocytic leukemia (128–130). Beyond that, *ex vivo* expanded Vδ1 T cells highly express FasL, and have strong cytotoxicity on colon cancer cells (131).

γδ T CELLS ENHANCE THE ANTI-TUMOR ABILITY OF OTHER IMMUNE CELLS

γδ T cells share similar functions as antigen presenting cells (APC), which could activate $CD8^+$T cells (132, 133). When co-cultured with chronic myeloid leukemia (CML) cell lysates, the expression of co-stimulatory molecules (CD40, CD80 and CD86) and antigen-presenting molecule HLA-DR on Vγ9Vδ2 T cells could be strongly up-regulated. When these γδ T cells were co-cultured with $CD8^+$ T cells, the proliferation rate of $CD8^+$ T cells became 3 times faster than that of the control group (134, 135). Tumor cell fragments activate MAPK signaling pathways through Vγ9Vδ2TCR, up-regulate the expression of scavenger receptor CD36, enhance antigen uptake and processing of Vγ9Vδ2 T cells, and then induced tumor antigen-specific $CD8^+$T cell response (136). Furthermore, γδ T cells toned to interact with cell surface-bound antibodies to acquire the ability of APC (137).

In addition, activated γδ T cells could secrete IFN-γ, which stimulates CSC to up-regulate the expression of MHC I molecules and CD54, and enhance the killing effect of $CD8^+$T cells on tumor cells (138). Activated γδ T cells could also express CD137L to stimulate NK cells that upon proliferation exhibit strong anti-tumor activity through cell-to-cell contact (31).

The interaction between γδ T cells and dendritic cells (DC) is mutual. γδ T cells promote the maturation of DC, and mature DC induces the activation and proliferation of γδ T cells, which yield enhanced anti-tumor effect (139, 140). For example, activated Vγ9Vδ2 T cells could secrete IFN-γ and TNF-α to promote DC maturation and increase the expression of CD86 and MHC-I molecules on DC (141, 142). Mature DC could activate γδ T cells through presenting IPP, which synergizes with ATP-binding cassette transporter A1 (ABCA1), ApoA1 and BTN3A1 (143) (**Figure 2**).

TUMOR-INFILTRATING γδ T CELLS PROMOTE TUMOR DEVELOPMENT BY SECRETING IL-17

Interestingly, patients with increased number of tumor-infiltrating γδ T cells have higher recurrence rates and likelihood of metastasis (144–146). Among the tumor-infiltrating γδ T cells, Vδ1 T cells are present as the main population and secrete IL-17 to promote tumor development. IL-17 can promote the proliferation of tumor cells by activating IL-6/STAT3 and NF-κB pathways. In addition, it can also stimulate tumor cells to secrete vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMP) to further help tumor metastasis. High levels of IL-17 have been found in patients with advanced tumor or metastasized tumors (64, 147, 148). For example, in patients with solid tumors, Vδ1 T cells account for a large proportion of tumor-infiltrating γδ T cells; unlike Vδ1 T cells in adjacent non-tumor tissues, tumor-infiltrating γδ T cells do not express granzyme B, perforin, IFN-γ, FasL, TRAIL and NKR, but secrete IL-17 (149–154). Majority of the tumor-infiltrating Vδ1 T cells were T_{EM} phenotype, while most of the Vδ1 T cells in healthy subjects were T_{CM} phenotype

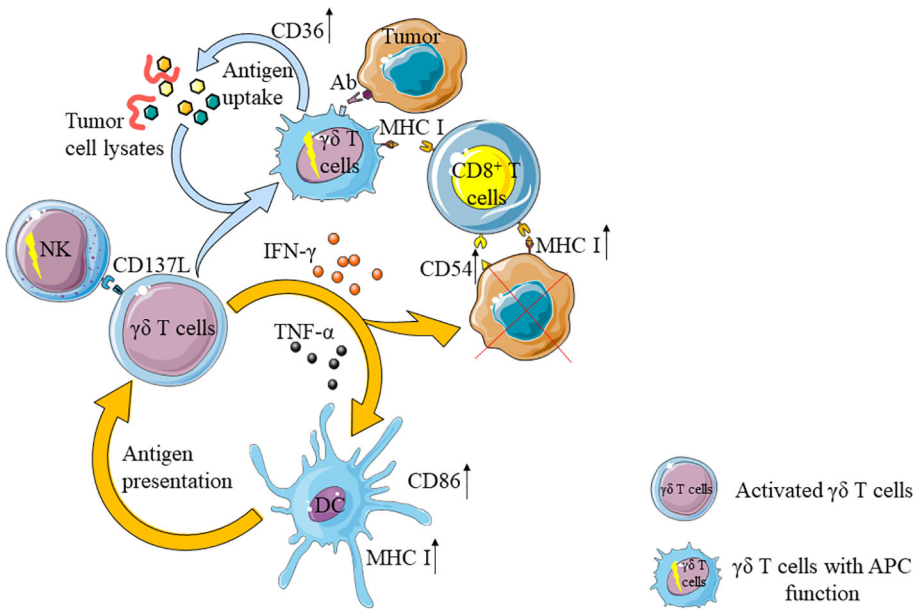

FIGURE 2 | γδ T cells enhance the anti-tumor ability of other immune cells. On the one hand, activated γδ T cells can activate natural killer cells (NK) and dendritic cells (DC), activated DC can further activate γδ T cells. On the other hand, activated γδ T cells can up-regulate the expression of CD36 and enhance their antigen uptake ability after uptake of tumor cell lysates. At the same time, through contact with tumor cells with antibodies, the ability of antigen-presenting cells (APC) is obtained and $CD8^+$T cells are activated. In addition, IFN- γ secreted by γδ T cells can up-regulate the expression of CD54 and MHC I molecules in tumor cells, and further enhance the anti-tumor effect of $CD8^+$T cells.

(64). Similarly and compared with healthy people, cancer patients have a larger proportion of Vδ1 T cells and higher IL-17 levels in their peripheral blood (155, 156).

TUMOR-INFILTRATING γδ T CELLS INHIBIT THE ANTI-TUMOR FUNCTION OF OTHER IMMUNE CELLS

IL-17, secreted by tumor-infiltrating Vδ1 T cells not only acts on tumor cells directly, but can also recruit myeloid-derived suppressor cells (MDSC) to tumor (147, 148, 150). MDSC inhibits the activation of $CD8^+$ T cells by expressing high levels of ARG1, which decomposes arginine in the tumor microenvironment (157–160).

In addition, tumor-infiltrating γδ T cells could significantly inhibit the maturation of $CD4^+$ T cells (155). Studies in breast cancer settings showed that tumor-infiltrating γδ T cells could inhibit the maturation of $CD4^+$ or $CD8^+$ T cells and change their functions by arresting cell cycle in G0/G1 phase and increasing the expression of p53, P21, and P16. Through secreting IL-10 and TGF- β1, these suppressed T cells further amplified the inhibitory effect (161, 162). Beyond that, these cells express high levels of PD-L1 to promote the apoptosis of $CD8^+$ T cells in cancer patients (41).

Vδ1 T cells could also inhibit the maturation of DC, reduce the expression of CD80/86 and HLA-DR on DC, attenuate the secretion of pro-inflammatory cytokines TNF- α, and up-regulate the expression of PD-L1 on the surface of DC (161, 163) (**Figure 3**).

ANTI-TUMOR IMMUNOTHERAPY WITH γδ T CELLS

The unique antigen recognition mechanism of γδ T cells renders them the ability to kill various types of tumors. Therefore, γδ T cell-based therapies have been widely used in clinical as anti-tumor immunotherapies and achieved good results (**Table 2**). At present, the most routine method in these therapies is to activate the anti-tumor activity of natural γδ T cells and the Vγ9Vδ2 T cells, which as the most abundant subtype in peripheral blood are often selected and utilized through transferring back to cancer patient after stimulation *in vitro* or direct activation *in vivo*.

The most widely used stimulants for expanding Vγ9Vδ2 T cells *in vitro* are zoledronic acid (ZOL) and IL-2. As a kind of bisphosphate, ZOL could specifically inhibit farnesyl pyrophosphate synthase (FPPS) in isoprene biosynthesis pathway, thus causing the accumulation of endogenous PAg in cells and promoting the activation of Vγ9Vδ2 T cells (65). This method could effectively expand and activate Vγ9Vδ2 T cells from patients or healthy people *in vitro* (52). In addition, another kind of PAg, 2-methyl-3-butenyl-1-pyrophosphate(2M3B1-PP) could also effectively stimulate and expand Vγ9Vδ2 T cells (164, 165). The activated immune cells are transferred back into the patients to produce anti-tumor effects. In order to track the activated Vγ9Vδ2

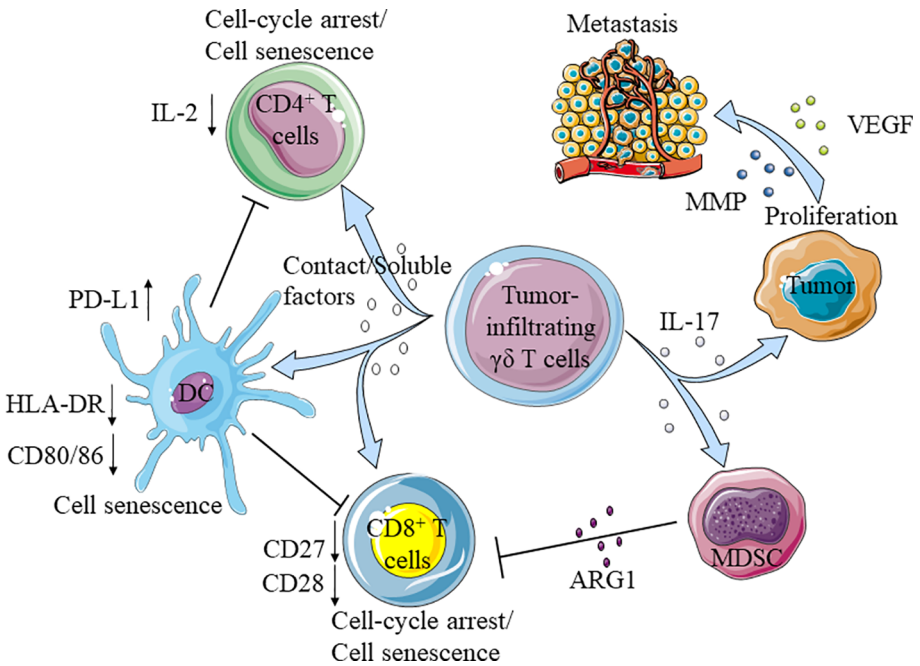

FIGURE 3 | Tumor-infiltrating γδ T cells promote the development of tumor. Tumor-infiltrating γδ T cells secrete IL-17 promote the proliferation and metastasis of tumor cells, and recruit myeloid-derived suppressor cells (MDSC) to inhibit the function of $CD8^+$T cells. Moreover, tumor-infiltrating γδ T cells directly impaired the function of $CD4^+$/$CD8^+$T cells and dendritic cells (DC), the aging DC further inhibited $CD4^+$/$CD8^+$T cells.

TABLE 2 | Clinical trials of γδ T cell-based immunotherapy.

Cell types	Cell source	Stimulation method	Disease	Number of patients	Phase	Ref.
Vγ9Vδ2 T	Peripheral blood of healthy people	ZOL+ a variety of interleukins (Patent pending)	Cholangio-carcinoma	1	IV	(52)
Vγ9Vδ2 T	Peripheral blood	ZM3B1PP+IL-2	Advanced renal cell carcinoma	7	Pilot study	(164)
Vγ9Vδ2 T	Peripheral blood	2M3B1PP+ZOL+IL-2	Advanced renal cell carcinoma	11	I/II	(165)
Vγ9Vδ2 T	Peripheral blood	ZOL+IL-2	Several solid tumors	18	I	(166)
Vγ9Vδ2 T	Peripheral blood	ZOL+IL-2	Multiple myeloma	6	I	(167)
Vγ9Vδ2 T	Peripheral blood	IPH1101+IL-2	Metastatic renal cell carcinoma	10	I	(168)
Vγ9Vδ2 T	Peripheral blood	ZOL+IL-2	Recurrent non-small-cell lung cancer	10	I	(169)
Vγ9Vδ2 T	Peripheral blood	ZOL+IL-2	Advanced non-small lung cancer	15	I	(170)
Vγ9Vδ2 T	Peripheral blood	ZOL+IL-2	Malignant ascites (gastic cancer)	7	Pilot study	(171)
Vγ9Vδ2 T	Peripheral blood	ZOL+IL-2	Refractory renal cell carcinoma	12	Pilot study	(172)
		Injection ZOL+IL-2	Neuroblastoma	4	I	(173)
		Injection ZOL+IL-2	Several advanced tumors	21	I/II	(174)
		Injection ZOL+IL-2	Lymphoid malignacies	19	Pilot study	(175)
		Injection IPH 1101+IL-2	Several solid tumors	28	I	(176)

T cells, they are typically labeled with indium[111]. Studies have confirmed that these cells mainly metastasize to the lung, liver and spleen, as well as to the tumor sites (166). In the treatment of patients with multiple myeloma, the stimulated Vγ9Vδ2 T cells expressed high levels of NKG2D and IFN-γ, but not IL-17. After treatment, the number of Vγ9Vδ2 T cells in the tumor microenvironment increased, lasting as long as 4 weeks (167). In patients with renal cell carcinoma and non-small cell lung cancer, repeated injections of IL-2 has demonstrated good safety (168), enhanced the cytotoxicity of Vγ9Vδ2 T cells. As results, the deterioration of tumor was alleviated with patients' condition stabilized, and the survival time was pro-longed (164, 165, 169,

170). In the clinical study of malignant ascites, after transferring back the activated Vγ9Vδ2 T cells, the number of tumor cells in ascites decreased significantly and the level of IFN-γ in ascites increased. During the course of treatment, there were no significant adverse effects and the amounts of ascites decreased significantly (171). In addition to the direct anti-tumor effect of Vγ9Vδ2 T cells, the numbers of $CD4^+$ T and $CD8^+$ T cells could also get increased after the allogeneic Vγ9Vδ2 T cells were transferred into the patients, as shown in another study of cholangio-carcinoma (52).

Vγ9Vδ2 T cells could also be activated *in vivo*. Injection of ZOL and IL-2 could directly activate these cells in cancer patients. Serval clinical trials have demonstrated that, upon injection and activation of Vγ9Vδ2 T cells *in vivo*, IFN-γ was strongly induced and the deterioration of the tumor was controlled (53, 172–175). Besides ZOL, Vγ9Vδ2 T cells could also be activated by synthetic PAg or bromohydrin pyrophosphate (BrHPP, IPH1101) with good safety tolerability in patients (176).

OPTIMIZATION OF γδ T CELL-BASED IMMUNOTHERAPY

In clinic, repeated use of ZOL and IL-2 carry the liability in inhibiting the proliferation of Vγ9Vδ2 T cells (172), which could be alleviated by vitamin C (VC) and L-ascorbic acid 2-phosphate (pVC). VC has the ability to reduce the apoptosis of γδ T cells during stimulation, and pVC may enhance the expansion of γδ T cells. Therefore, VC and pVC have been utilized to improve the efficacy of the γδ T cells in anti-tumor immunotherapy (177). In addition, cytotoxicity of Vγ9Vδ2 T cells could be improved by adding IL-21, IL-15, or IL-33 *in vitro* (55, 178–182). Anti-cancer drug Gemcitabine or anti-epileptic drug Valproic acid (VPA) in combination with ZOL could also enhance the anti-tumor ability of γδ T cells (183, 184).

In recent years, chitosan nanoparticles (CSNPs) and antibodies have been developed as potential anti-tumor therapies. CSNPs have been shown to regulate γδ T cells by up-regulating the expression of NKG2D, CD56 and FasL, and enhancing their anti-tumor functions (185). TIM-3 could also inhibit the killing effect of Vγ9Vδ2 T cells on tumor by reducing the expression of perforin and granzyme B. PD-1 and TIM-3 antibodies could protect anti-tumor activity of Vγ9Vδ2 T cells (186–188). Beyond these, the application of bispecific antibodies can also promote γδ T cells to inhibit tumor development. For example, in the study of hepatoblastoma and pediatric hepatocellular carcinoma, the application of EpCAM/CD3-bispecific BiTE antibody (MT110) enhanced the anti-tumor ability of γδ T cells; similarly, in epithelial ovarian cancer and pancreatic ductal adenocarcinoma, bispecific antibody [HER2xCD3] and [(HER2)2xVγ9] (tribody format) could also effectively enhance the cytotoxicity of γδ T cells (189–193).

Finally, chimeric antigen receptor engineered γδ T (CAR-γδ T) technology is another new direction in immunotherapy. CAR- γδ T cells could target GD2, a TAA on the surface of neuroblastoma cells, and effectively kill tumors. This kind of CAR-γδ T cells need to recognize GD2 to become activated. Such mechanism ensures the specificity of these cells in killing tumor cells and offering lower toxicities and side effects (194, 195). On the hand, Vγ9Vδ2TCR could also be transduced into αβ T cells. These CAR-T cells are called TEGs, which carry not only the extensive recognition ability of γδ T cells but also and the memory ability of αβ T cells (196–199).

SUMMARY

Taken together, we described in this review that Vδ1 T cells and Vγ9Vδ2 T cells are the two most important subgroups of human γδ T cells. Peripheral Vδ1 T cells and Vγ9Vδ2 T cells could recognize tumor cells through TCRγδ and NKR, and kill them through perforin-granzyme B, Fas/FasL and TRAIL. Activated Vγ9Vδ2 T cells could perform the function of APC, and furthermore, they could activate NK cells and DC directly. On the contrary, tumor-infiltrating Vδ1 T cells promoted tumor development by secreting IL-17 and inhibiting the maturation of $CD4^+$/$CD8^+$ T cells and DC. In immunotherapy, ZOL, 2M3B1-PP or IPH1101 has been commonly used to activate Vγ9Vδ2 T cells to achieve anti-tumor effect. The failure caused by repeated application of this method can be solved by adding VC or replacing cytokines. In addition, new classes of drugs such as CSNPs, were also applied to γδ T cell-based anti-tumor immunotherapy.

It is noteworthy to mention that although Vδ1 T cells account for the majority of tumor-infiltrating γδ T cells, the definition of γδ T cell subsets still rely on their profile in cytokine production (32, 64, 149). Secondly, the interaction mechanism between γδ T cells and the environment or other immune cells remains to be further elucidated. For example, Vγ9Vδ2 T cells could ingest LDL-cholesterol upon activation and lead to reduced function, suggesting that obesity may inhibit the anti-tumor activity of γδ T cells (200). Another myth exists in how exactly soluble molecules mediate the inhibition of γδ T cells in tumor microenvironment (161, 163). In addition, γδ T cell-based immunotherapy needs to be further optimized, with emphasis on how to carry out personalized therapy according to the actual situation of individual patient.

In summary, a more comprehensive understanding of the biological characteristics of γδ T cells, an important group of lymphocytes, will guide the improvement of their clinical application methods and provide new strategies to fight against human cancers.

AUTHOR CONTRIBUTIONS

JZ completed the writing of the classification of γδ T cells. XC and XW completed the writing of introduction and summary. YL and GL completed the writing of the rest of this review. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Carding SR, Egan PJ. Gammadelta T cells: functional plasticity and heterogeneity. *Nat Rev Immunol* (2002) 2(5):336–45. doi: 10.1038/nri797
2. Chien YH, Meyer C, Bonneville M. γδ T cells: first line of defense and beyond. *Annu Rev Immunol* (2014) 32:121–55. doi: 10.1146/annurev-immunol-032713-120216
3. Poggi A, Zocchi MR. γδ T Lymphocytes as a First Line of Immune Defense: Old and New Ways of Antigen Recognition and Implications for Cancer Immunotherapy. *Front Immunol* (2014) 5:575. doi: 10.3389/fimmu.2014.00575
4. Holtmeier W, Kabelitz D. gammadelta T cells link innate and adaptive immune responses. *Chem Immunol Allergy* (2005) 86:151–83. doi: 10.1159/000086659
5. Bonneville M, O'Brien RL, Born WK. Gammadelta T cell effector functions: a blend of innate programming and acquired plasticity. *Nat Rev Immunol* (2010) 10(7):467–78. doi: 10.1038/nri2781
6. Chitadze G, Oberg HH, Wesch D, Kabelitz D. The Ambiguous Role of γδ T Lymphocytes in Antitumor Immunity. *Trends Immunol* (2017) 38(9):668–78. doi: 10.1016/j.it.2017.06.004
7. Correia DV, Lopes A, Silva-Santos B. Tumor cell recognition by γδ T lymphocytes: T-cell receptor vs. NK-cell receptors. *Oncoimmunology* (2013) 2(1):e22892. doi: 10.4161/onci.22892
8. Sanders JM, Ghosh S, Chan JM, Meints G, Wang H, Raker AM, et al. Quantitative structure-activity relationships for gammadelta T cell activation by bisphosphonates. *J Med Chem* (2004) 47(2):375–84. doi: 10.1021/jm0303709
9. Wei H, Huang D, Lai X, Chen M, Zhong W, Wang R, et al. Definition of APC presentation of phosphoantigen (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate to Vgamma2Vdelta 2 TCR. *J Immunol* (2008) 181(7):4798–806. doi: 10.4049/jimmunol.181.7.4798
10. Wang H, Sarikonda G, Puan KJ, Tanaka Y, Feng J, Giner JL, et al. Indirect stimulation of human Vγ2Vδ2 T cells through alterations in isoprenoid metabolism. *J Immunol* (2011) 187(10):5099–113. doi: 10.4049/jimmunol.1002697
11. Puan KJ, Jin C, Wang H, Sarikonda G, Raker AM, Lee HK, et al. Preferential recognition of a microbial metabolite by human Vgamma2Vdelta2 T cells. *Int Immunol* (2007) 19(5):657–73. doi: 10.1093/intimm/dxm031
12. Kondo M, Izumi T, Fujieda N, Kondo A, Morishita T, Matsushita H, et al. Expansion of human peripheral blood γδ T cells using zoledronate. *J Vis Exp* (2011) 55):3182. doi: 10.3791/3182
13. Scotet E, Martinez LO, Grant E, Barbaras R, Jenö P, Guiraud M, et al. Tumor recognition following Vgamma9Vdelta2 T cell receptor interactions with a surface F1-ATPase-related structure and apolipoprotein A-I. *Immunity* (2005) 22(1):71–80. doi: 10.1016/j.immuni.2004.11.012
14. Kong Y, Cao W, Xi X, Ma C, Cui L, He W. The NKG2D ligand ULBP4 binds to TCRgamma9/delta2 and induces cytotoxicity to tumor cells through both TCRgammadelta and NKG2D. *Blood* (2009) 114(2):310–7. doi: 10.1182/blood-2008-12-196287
15. Peipp M, Wesch D, Oberg HH, Lutz S, Muskulus A, van de Winkel JGJ, et al. CD20-Specific Immunoligands Engaging NKG2D Enhance γδ T Cell-Mediated Lysis of Lymphoma Cells. *Scand J Immunol* (2017) 86(4):196–206. doi: 10.1111/sji.12581
16. Gundermann S, Klinker E, Kimmel B, Flierl U, Wilhelm M, Einsele H, et al. A comprehensive analysis of primary acute myeloid leukemia identifies biomarkers predicting susceptibility to human allogeneic Vγ9Vδ2 T cells. *J Immunother* (2014) 37(6):321–30. doi: 10.1097/CJI.0000000000000043
17. Lu J, Aggarwal R, Kanji S, Das M, Joseph M, Pompili V, et al. Human ovarian tumor cells escape γδ T cell recognition partly by down regulating surface expression of MICA and limiting cell cycle related molecules. *PloS One* (2011) 6(9):e23348. doi: 10.1371/journal.pone.0023348
18. Knight A, Arnouk H, Britt W, Gillespie GY, Cloud GA, Harkins L, et al. CMV-independent lysis of glioblastoma by ex vivo expanded/activated Vδ1+ γδ T cells. *PloS One* (2013) 8(8):e68729. doi: 10.1371/journal.pone.0068729
19. Willcox CR, Pitard V, Netzer S, Couzi L, Salim M, Silberzahn T, et al. Cytomegalovirus and tumor stress surveillance by binding of a human γδ T cell antigen receptor to endothelial protein C receptor. *Nat Immunol* (2012) 13(9):872–9. doi: 10.1038/ni.2394
20. Witherden DA, Havran WL. EPCR: a stress trigger for γδ T cells. *Nat Immunol* (2012) 13(9):812–4. doi: 10.1038/ni.2398
21. Zhang H, Hu H, Jiang X, He H, Cui L, He W. Membrane HSP70: the molecule triggering gammadelta T cells in the early stage of tumorigenesis. *Immunol Invest* (2005) 34(4):453–68. doi: 10.1080/08820130500265349
22. Thomas ML, Samant UC, Deshpande RK, Chiplunkar SV. gammadelta T cells lyse autologous and allogenic oesophageal tumours: involvement of heat-shock proteins in the tumour cell lysis. *Cancer Immunol Immunother* (2000) 48(11):653–9. doi: 10.1007/s002620050014
23. Laad AD, Thomas ML, Fakih AR, Chiplunkar SV. Human gamma delta T cells recognize heat shock protein-60 on oral tumor cells. *Int J Cancer* (1999) 80(5):709–14. doi: 10.1002/(sici)1097-0215(19990301)80:5<709::aid-ijc14>3.0.co;2-r
24. Mo C, Dai Y, Kang N, Cui L, He W. Ectopic expression of human MutS homologue 2 on renal carcinoma cells is induced by oxidative stress with interleukin-18 promotion via p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) signaling pathways. *J Biol Chem* (2012) 287(23):19242–54. doi: 10.1074/jbc.M112.349936\
25. Dai Y, Chen H, Mo C, Cui L, He W. Ectopically expressed human tumor biomarker MutS homologue 2 is a novel endogenous ligand that is recognized by human γδ T cells to induce innate anti-tumor/virus immunity. *J Biol Chem* (2012) 287(20):16812–9. doi: 10.1074/jbc.M111.327650
26. Chen H, He X, Wang Z, Wu D, Zhang H, Xu C, et al. Identification of human T cell receptor gammadelta-recognized epitopes/proteins via CDR3delta peptide-based immunobiochemical strategy. *J Biol Chem* (2008) 283(18):12528–37. doi: 10.1074/jbc.M708067200
27. Meraviglia S, Caccamo N, Guggino G, Tolomeo M, Siragusa S, Stassi G, et al. Optimizing tumor-reactive γδ T cells for antibody-based cancer immunotherapy. *Curr Mol Med* (2010) 10(8):719–26. doi: 10.2174/156652410793384150
28. Hoeres T, Smetak M, Pretscher D, Wilhelm M. Improving the Efficiency of Vγ9Vδ2 T-Cell Immunotherapy in Cancer. *Front Immunol* (2018) 9:800. doi: 10.3389/fimmu.2018.00800
29. Chen J, Fan Y, Cui B, Li X, Yu Y, Du Y, et al. HMGN2: An Antitumor Effector Molecule of γδT Cells. *J Immunother* (2018) 41(3):118–24. doi: 10.1097/CJI.0000000000000211
30. Iovino F, Meraviglia S, Spina M, Orlando V, Saladino V, Dieli F, et al. Immunotherapy targeting colon cancer stem cells. *Immunotherapy* (2011) 3(1):97–106. doi: 10.2217/imt.10.87
31. Maniar A, Zhang X, Lin W, Gastman BR, Pauza CD, Strome SE, et al. Human gammadelta T lymphocytes induce robust NK cell-mediated antitumor cytotoxicity through CD137 engagement. *Blood* (2010) 116(10):1726–33. doi: 10.1182/blood-2009-07-234211
32. Silva-Santos B, Mensurado S, Coffelt SB. γδ T cells: pleiotropic immune effectors with therapeutic potential in cancer. *Nat Rev Cancer* (2019) 19(7):392–404. doi: 10.1038/s41568-019-0153-5
33. Sebestyen Z, Prinz I, Déchanet-Merville J, Silva-Santos B, Kuball J. Translating gammadelta (γδ) T cells and their receptors into cancer cell therapies. *Nat Rev Drug Discovery* (2020) 19(3):169–84. doi: 10.1038/s41573-019-0038-z
34. Kakimi K, Matsushita H, Murakawa T, Nakajima J. γδ T cell therapy for the treatment of non-small cell lung cancer. *Transl Lung Cancer Res* (2014) 3(1):23–33. doi: 10.3978/j.issn.2218-6751.2013.11.01
35. Wu Y, Kyle-Cezar F, Woolf RT, Naceur-Lombardelli C, Owen J, Biswas D, et al. An innate-like Vδ1+ γδ T cell compartment in the human breast is associated with remission in triple-negative breast cancer. *Sci Transl Med* (2019) 11(513):eaax9364. doi: 10.1126/scitranslmed.aax9364
36. Rei M, Pennington DJ, Silva-Santos B. The emerging Protumor role of γδ T lymphocytes: implications for cancer immunotherapy. *Cancer Res* (2015) 75(5):798–802. doi: 10.1158/0008-5472.CAN-14-3228
37. Wu X, Yang T, Liu X, Guo JN, Xie T, Ding Y, et al. IL-17 promotes tumor angiogenesis through Stat3 pathway mediated upregulation of VEGF in gastric cancer. *Tumour Biol* (2016) 37(4):5493–501. doi: 10.1007/s13277-015-4372-4
38. Pan B, Shen J, Cao J, Zhou Y, Shang L, Jin S, et al. Interleukin-17 promotes angiogenesis by stimulating VEGF production of cancer cells via the STAT3/GIV signaling pathway in non-small-cell lung cancer. *Sci Rep* (2015) 5:16053. doi: 10.1038/srep16053

39. Yan J, Huang J. Innate γδT17 cells convert cancer-elicited inflammation into immunosuppression through myeloid-derived suppressor cells. *Oncoimmunology* (2014) 3(8):e953423. doi: 10.4161/21624011.2014.953423
40. Qu P, Wang LZ, Lin PC. Expansion and functions of myeloid-derived suppressor cells in the tumor microenvironment. *Cancer Lett* (2016) 380 (1):253–6. doi: 10.1016/j.canlet.2015.10.022
41. Daley D, Zambirinis CP, Seifert L, Akkad N, Mohan N, Werba G, et al. γδ T Cells Support Pancreatic Oncogenesis by Restraining αβ T Cell Activation. *Cell* (2016) 166(6):1485–99.e15. doi: 10.1016/j.cell.2016.07.046
42. Lafont V, Sanchez F, Laprevotte E, Michaud HA, Gros L, Eliaou JF, et al. Plasticity of γδ T Cells: Impact on the Anti-Tumor Response. *Front Immunol* (2014) 5:622. doi: 10.3389/fimmu.2014.00622
43. Lee HW, Chung YS, Kim TJ. Heterogeneity of Human γδ T Cells and Their Role in Cancer Immunity. *Immune Netw* (2020) 20(1):e5. doi: 10.4110/in.2020.20.e5
44. Pang DJ, Neves JF, Sumaria N, Pennington DJ. Understanding the complexity of γδ T-cell subsets in mouse and human. *Immunology* (2012) 136(3):283–90. doi: 10.1111/j.1365-2567.2012.03582.x
45. Adams EJ, Gu S, Luoma AM. Human gamma delta T cells: Evolution and ligand recognition. *Cell Immunol* (2015) 296(1):31–40. doi: 10.1016/j.cellimm.2015.04.008
46. Wu D, Wu P, Qiu F, Wei Q, Huang J. Human γδT-cell subsets and their involvement in tumor immunity. *Cell Mol Immunol* (2017) 14(3):245–53. doi: 10.1038/cmi.2016.55
47. Fichtner AS, Ravens S, Prinz I. Human γδ TCR Repertoires in Health and Disease. *Cells* (2020) 9(4):800. doi: 10.3390/cells9040800
48. Mikulak J, Oriolo F, Bruni E, Roberto A, Colombo FS, Villa A, et al. NKp46-expressing human gut-resident intraepithelial Vδ1 T cell subpopulation exhibits high antitumor activity against colorectal cancer. *JCI Insight* (2019) 4(24):e125884. doi: 10.1172/jci.insight.125884
49. Knight A, Mackinnon S, Lowdell MW. Human Vdelta1 gamma-delta T cells exert potent specific cytotoxicity against primary multiple myeloma cells. *Cytotherapy* (2012) 14(9):1110–8. doi: 10.3109/14653249.2012.700766
50. Siegers GM, Dhamko H, Wang XH, Mathieson AM, Kosaka Y, Felizardo TC, et al. Human Vδ1 γδ T cells expanded from peripheral blood exhibit specific cytotoxicity against B-cell chronic lymphocytic leukemia-derived cells. *Cytotherapy* (2011) 13(6):753–64. doi: 10.3109/14653249.2011.553595
51. Kühl AA, Pawlowski NN, Grollich K, Blessenohl M, Westermann J, Zeitz M, et al. Human peripheral gammadelta T cells possess regulatory potential. *Immunology* (2009) 128(4):580–8. doi: 10.1111/j.1365-2567.2009.03162.x
52. Alnaggar M, Xu Y, Li J, He J, Chen J, Li M, et al. Allogenic Vγ9Vδ2 T cell as new potential immunotherapy drug for solid tumor: a case study for cholangiocarcinoma. *J Immunother Cancer* (2019) 7(1):36. doi: 10.1186/s40425-019-0501-8
53. Meraviglia S, Eberl M, Vermijlen D, Todaro M, Buccheri S, Cicero G, et al. In vivo manipulation of Vgamma9Vdelta2 T cells with zoledronate and low-dose interleukin-2 for immunotherapy of advanced breast cancer patients. *Clin Exp Immunol* (2010) 161(2):290–7. doi: 10.1111/j.1365-2249.2010.04167.x
54. Di Carlo E, Bocca P, Emionite L, Cilli M, Cipollone G, Morandi F, et al. Mechanisms of the antitumor activity of human Vγ9Vδ2 T cells in combination with zoledronic acid in a preclinical model of neuroblastoma. *Mol Ther* (2013) 21(5):1034–43. doi: 10.1038/mt.2013.38
55. Joalland N, Chauvin C, Oliver L, Vallette FM, Pecqueur C, Jarry U, et al. IL-21 Increases the Reactivity of Allogeneic Human Vγ9Vδ2 T Cells Against Primary Glioblastoma Tumors. *J Immunother* (2018) 41(5):224–31. doi: 10.1097/CJI.0000000000000225
56. Lai D, Wang F, Chen Y, Wang C, Liu S, Lu B, et al. Human ovarian cancer stem-like cells can be efficiently killed by γδ T lymphocytes. *Cancer Immunol Immunother* (2012) 61(7):979–89. doi: 10.1007/s00262-011-1166-4
57. Nishio N, Fujita M, Tanaka Y, Maki H, Zhang R, Hirosawa T, et al. Zoledronate sensitizes neuroblastoma-derived tumor-initiating cells to cytolysis mediated by human γδ T cells. *J Immunother* (2012) 35(8):598–606. doi: 10.1097/CJI.0b013e31826a745a
58. Bartkowiak J, Kulczyck-Wojdala D, Blonski JZ, Robak T. Molecular diversity of gammadelta T cells in peripheral blood from patients with B-cell chronic lymphocytic leukaemia. *Neoplasma* (2002) 49(2):86–90. doi: 10.1038/sj.neo.7900211
59. Kenna T, Golden-Mason L, Norris S, Hegarty JE, O'Farrelly C, Doherty DG. Distinct subpopulations of gamma delta T cells are present in normal and tumor-bearing human liver. *Clin Immunol* (2004) 113(1):56–63. doi: 10.1016/j.clim.2004.05.003
60. Petrasca A, Melo AM, Breen EP, Doherty DG. Human Vδ3+ γδ T cells induce maturation and IgM secretion by B cells. *Immunol Lett* (2018) 196:126–34. doi: 10.1016/j.imlet.2018.02.002
61. Mangan BA, Dunne MR, O'Reilly VP, Dunne PJ, Exley MA, O'Shea D, et al. Cutting edge: CD1d restriction and Th1/Th2/Th17 cytokine secretion by human Vδ3 T cells. *J Immunol* (2013) 191(1):30–4. doi: 10.4049/jimmunol.1300121
62. Zhao Y, Niu C, Cui J. Gamma-delta (γδ) T cells: friend or foe in cancer development? *J Transl Med* (2018) 16(1):3. doi: 10.1186/s12967-017-1378-2
63. Qin G, Liu Y, Zheng J, Xiang Z, Ng IH, Malik Peiris JS, et al. Phenotypic and functional characterization of human γδ T-cell subsets in response to influenza A viruses. *J Infect Dis* (2012) 205(11):1646–53. doi: 10.1093/infdis/jis253
64. Lo Presti E, Toia F, Oieni S, Buccheri S, Turdo A, Mangiapane LR, et al. Squamous Cell Tumors Recruit γδ T Cells Producing either IL17 or IFNγ Depending on the Tumor Stage. *Cancer Immunol Res* (2017) 5(5):397–407. doi: 10.1158/2326-6066.CIR-16-0348
65. Gober HJ, Kistowska M, Angman L, Jenö P, Mori L, De Libero G. Human T cell receptor gammadelta cells recognize endogenous mevalonate metabolites in tumor cells. *J Exp Med* (2003) 197(2):163–8. doi: 10.1084/jem.20021500
66. Silva-Santos B, Serre K, Norell H. γδ T cells in cancer. *Nat Rev Immunol* (2015) 15(11):683–91. doi: 10.1038/nri3904
67. Mullen PJ, Yu R, Longo J, Archer MC, Penn LZ. The interplay between cell signalling and the mevalonate pathway in cancer. *Nat Rev Cancer* (2016) 16 (11):718–31. doi: 10.1038/nrc.2016.76
68. Moulin M, Alguacil J, Gu S, Mehtougui A, Adams EJ, Peyrottes S, et al. Vγ9Vδ2 T cell activation by strongly agonistic nucleotidic phosphoantigens. *Cell Mol Life Sci* (2017) 74(23):4353–67. doi: 10.1007/s00018-017-2583-0
69. Kabelitz D. CD277 takes the lead in human γδ T-cell activation. *Blood* (2012) 120(11):2159–61. doi: 10.1182/blood-2012-07-442731
70. Peigné CM, Léger A, Gesnel MC, Konczak F, Olive D, Bonneville M, et al. The Juxtamembrane Domain of Butyrophilin BTN3A1 Controls Phosphoantigen-Mediated Activation of Human Vγ9Vδ2 T Cells. *J Immunol* (2017) 198(11):4228–34. doi: 10.4049/jimmunol.1601910
71. Gu S, Sachleben JR, Boughter CT, Nawrocka WI, Borowska MT, Tarrasch JT, et al. Phosphoantigen-induced conformational change of butyrophilin 3A1 (BTN3A1) and its implication on Vγ9Vδ2 T cell activation. *Proc Natl Acad Sci U.S.A.* (2017) 114(35):E7311–20. doi: 10.1073/pnas.1707547114
72. Sandstrom A, Peigné CM, Léger A, Crooks JE, Konczak F, Gesnel MC, et al. The intracellular B30.2 domain of butyrophilin 3A1 binds phosphoantigens to mediate activation of human Vγ9Vδ2 T cells. *Immunity* (2014) 40(4):490–500. doi: 10.1016/j.immuni.2014.03.003
73. Wang H, Morita CT. Sensor Function for Butyrophilin 3A1 in Prenyl Pyrophosphate Stimulation of Human Vγ2Vδ2 T Cells. *J Immunol* (2015) 195(10):4583–94. doi: 10.4049/jimmunol.1500314
74. Gu S, Nawrocka W, Adams EJ. Sensing of Pyrophosphate Metabolites by Vγ9Vδ2 T Cells. *Front Immunol* (2015) 5:688. doi: 10.3389/fimmu.2014.00688
75. Nguyen K, Li J, Puthenveetil R, Lin X, Poe MM, Hsiao CC, et al. The butyrophilin 3A1 intracellular domain undergoes a conformational change involving the juxtamembrane region. *FASEB J* (2017) 31(11):4697–706. doi: 10.1096/fj.201601370RR
76. Vantourout P, Laing A, Woodward MJ, Zlatareva I, Apolonia L, Jones AW, et al. Heteromeric interactions regulate butyrophilin (BTN) and BTN-like molecules governing γδ T cell biology. *Proc Natl Acad Sci U S A* (2018) 115 (5):1039–44. doi: 10.1073/pnas.1701237115
77. Yang Y, Li L, Yuan L, Zhou X, Duan J, Xiao H, et al. A Structural Change in Butyrophilin upon Phosphoantigen Binding Underlies Phosphoantigen-Mediated Vγ9Vδ2 T Cell Activation. *Immunity* (2019) 50(4):1043–1053.e5. doi: 10.1016/j.immuni.2019.02.016
78. Vavassori S, Kumar A, Wan GS, Ramanjaneyulu GS, Cavallari M, El Daker S, et al. Butyrophilin 3A1 binds phosphorylated antigens and stimulates human γδ T cells. *Nat Immunol* (2013) 14(9):908–16. doi: 10.1038/ni.2665

79. Karunakaran MM, Willcox CR, Salim M, Paletta D, Fichtner AS, Noll A, et al. Butyrophilin-2A1 Directly Binds Germline-Encoded Regions of the Vγ9Vδ2 TCR and Is Essential for Phosphoantigen Sensing. *Immunity* (2020) 52(3):487–498.e6. doi: 10.1016/j.immuni.2020.02.014
80. Rigau M, Ostrouska S, Fulford TS, Johnson DN, Woods K, Ruan Z, et al. Butyrophilin 2A1 is essential for phosphoantigen reactivity by γδ T cells. *Science* (2020) 367(6478):eaay5516. doi: 10.1126/science.aay5516
81. Mookerjee-Basu J, Vantourout P, Martinez LO, Perret B, Collet X, Périgaud C, et al. F1-adenosine triphosphatase displays properties characteristic of an antigen presentation molecule for Vgamma9Vdelta2 T cells. *J Immunol* (2010) 184(12):6920–8. doi: 10.4049/jimmunol.0904024
82. Dhar P, Wu JD. NKG2D and its ligands in cancer. *Curr Opin Immunol* (2018) 51:55–61. doi: 10.1016/j.coi.2018.02.004
83. Niu C, Jin H, Li M, Xu J, Xu D, Hu J, et al. In vitro analysis of the proliferative capacity and cytotoxic effects of ex vivo induced natural killer cells, cytokine-induced killer cells, and gamma-delta T cells. *BMC Immunol* (2015) 16:61. doi: 10.1186/s12865-015-0124-x
84. Corvaisier M, Moreau-Aubry A, Diez E, Bennouna J, Mosnier JF, Scotet E, et al. V gamma 9V delta 2 T cell response to colon carcinoma cells. *J Immunol* (2005) 175(8):5481–8. doi: 10.4049/jimmunol.175.8.5481
85. Wang Z, Wang Z, Li S, Li B, Sun L, Li H, et al. Decitabine Enhances Vγ9Vδ2 T Cell-Mediated Cytotoxic Effects on Osteosarcoma Cells via the NKG2DL-NKG2D Axis. *Front Immunol* (2018) 9:1239. doi: 10.3389/fimmu.2018.01239
86. Toutirais O, Cabillic F, Le Friec G, Salot S, Loyer P, Le Gallo M, et al. DNAX accessory molecule-1 (CD226) promotes human hepatocellular carcinoma cell lysis by Vgamma9Vdelta2 T cells. *Eur J Immunol* (2009) 39(5):1361–8. doi: 10.1002/eji.200838409
87. Gertner-Dardenne J, Castellano R, Mamessier E, Garbit S, Kochbati E, Etienne A, et al. Human Vγ9Vδ2 T cells specifically recognize and kill acute myeloid leukemic blasts. *J Immunol* (2012) 188(9):4701–8. doi: 10.4049/jimmunol.1103710
88. Deniger DC, Maiti SN, Mi T, Switzer KC, Ramachandran V, Hurton LV, et al. Activating and propagating polyclonal gamma delta T cells with broad specificity for malignancies. *Clin Cancer Res* (2014) 20(22):5708–19. doi: 10.1158/1078-0432.CCR-13-3451
89. Wang X, Mou W, Han W, Xi Y, Chen X, Zhang H, et al. Diminished cytolytic activity of γδ T cells with reduced DNAM-1 expression in neuroblastoma patients. *Clin Immunol* (2019) 203:63–71. doi: 10.1016/j.clim.2019.04.006
90. Luoma AM, Castro CD, Adams EJ. γδ T cell surveillance via CD1 molecules. *Trends Immunol* (2014) 35(12):613–21. doi: 10.1016/j.it.2014.09.003
91. Luoma AM, Castro CD, Mayassi T, Bembinster LA, Bai L, Picard D, et al. Crystal structure of Vδ1 T cell receptor in complex with CD1d-sulfatide shows MHC-like recognition of a self-lipid by human γδ T cells. *Immunity* (2013) 39(6):1032–42. doi: 10.1016/j.immuni.2013.11.001
92. Dhodapkar MV, Geller MD, Chang DH, Shimizu K, Fujii S, Dhodapkar KM, et al. A reversible defect in natural killer T cell function characterizes the progression of premalignant to malignant multiple myeloma. *J Exp Med* (2003) 197(12):1667–76. doi: 10.1084/jem.20021650
93. Shyanti RK, Sehrawat A, Singh SV, Mishra JPN, Singh RP. Zerumbone modulates CD1d expression and lipid antigen presentation pathway in breast cancer cells. *Toxicol In Vitro* (2017) 44:74–84. doi: 10.1016/j.tiv.2017.06.016
94. Nowak M, Arredouani MS, Tun-Kyi A, Schmidt-Wolf I, Sanda MG, Balk SP, et al. Defective NKT cell activation by CD1d+ TRAMP prostate tumor cells is corrected by interleukin-12 with α-galactosylceramide. *PloS One* (2010) 5 (6):e11311. doi: 10.1371/journal.pone.0011311
95. Vermeulen JF, van Hecke W, Spliet WG, Villacorta Hidalgo J, Fisch P, Broekhuizen R, et al. Pediatric Primitive Neuroectodermal Tumors of the Central Nervous System Differentially Express Granzyme Inhibitors. *PloS One* (2016) 11(3):e0151465. doi: 10.1371/journal.pone.0151465
96. Xu B, Pizarro JC, Holmes MA, McBeth C, Groh V, Spies T, et al. Crystal structure of a gammadelta T-cell receptor specific for the human MHC class I homolog MICA. *Proc Natl Acad Sci U S A* (2011) 108(6):2414–9. doi: 10.1073/pnas.1015433108
97. Poggi A, Venturino C, Catellani S, Clavio M, Miglino M, Gobbi M, et al. Vdelta1 T lymphocytes from B-CLL patients recognize ULBP3 expressed on leukemic B cells and up-regulated by trans-retinoic acid. *Cancer Res* (2004) 64(24):9172–9. doi: 10.1158/0008-5472.CAN-04-2417
98. Almeida AR, Correia DV, Fernandes-Platzgummer A, da Silva CL, da Silva MG, Anjos DR, et al. Delta One T Cells for Immunotherapy of Chronic Lymphocytic Leukemia: Clinical-Grade Expansion/Differentiation and Preclinical Proof of Concept. *Clin Cancer Res* (2016) 22(23):5795–804. doi: 10.1158/1078-0432.CCR-16-0597
99. Hudspeth K, Silva-Santos B, Mavilio D. Natural cytotoxicity receptors: broader expression patterns and functions in innate and adaptive immune cells. *Front Immunol* (2013) 4:69. doi: 10.3389/fimmu.2013.00069
100. Silva-Santos B, Strid J. Working in "NK Mode": Natural Killer Group 2 Member D and Natural Cytotoxicity Receptors in Stress-Surveillance by γδ T Cells. *Front Immunol* (2018) 9:851. doi: 10.3389/fimmu.2018.00851
101. Di Lorenzo B, Simões AE, Caiado F, Tieppo P, Correia DV, Carvalho T, et al. Broad Cytotoxic Targeting of Acute Myeloid Leukemia by Polyclonal Delta One T Cells. *Cancer Immunol Res* (2019) 7(4):552–8. doi: 10.1158/2326-6066.CIR-18-0647
102. Voskoboinik I, Whisstock JC, Trapani JA. Perforin and granzymes: function, dysfunction and human pathology. *Nat Rev Immunol* (2015) 15(6):388–400. doi: 10.1038/nri3839
103. Praper T, Sonnen A, Viero G, Kladnik A, Froelich CJ, Anderluh G, et al. Human perforin employs different avenues to damage membranes. *J Biol Chem* (2011) 286(4):2946–55. doi: 10.1074/jbc.M110.169417
104. Prager I, Liesche C, van Ooijen H, Urlaub D, Verron Q, Sandström N, et al. NK cells switch from granzyme B to death receptor-mediated cytotoxicity during serial killing. *J Exp Med* (2019) 216(9):2113–27. doi: 10.1084/jem.20181454
105. van Tetering G, Bovenschen N, Meeldijk J, van Diest PJ, Vooijs M. Cleavage of Notch1 by granzyme B disables its transcriptional activity. *Biochem J* (2011) 437(2):313–22. doi: 10.1042/BJ20110226
106. Alexander AA, Maniar A, Cummings JS, Hebbeler AM, Schulze DH, Gastman BR, et al. Isopentenyl pyrophosphate-activated CD56+ {gamma}{delta} T lymphocytes display potent antitumor activity toward human squamous cell carcinoma. *Clin Cancer Res* (2008) 14(13):4232–40. doi: 10.1158/1078-0432.CCR-07-4912
107. Dhar S, Chiplunkar SV. Lysis of aminobisphosphonate-sensitized MCF-7 breast tumor cells by Vγ9Vδ2 T cells. *Cancer Immun* (2010) 10:10.
108. Viey E, Fromont G, Escudier B, Morel Y, Da Rocha S, Chouaib S, et al. Phosphostim-activated gamma delta T cells kill autologous metastatic renal cell carcinoma. *J Immunol* (2005) 174(3):1338–47. doi: 10.4049/jimmunol.174.3.1338
109. Lafont V, Liautard J, Liautard JP, Favero J. Production of TNF-alpha by human V gamma 9V delta 2 T cells via engagement of Fc gamma RIIIA, the low affinity type 3 receptor for the Fc portion of IgG, expressed upon TCR activation by nonpeptidic antigen. *J Immunol* (2001) 166(12):7190–9. doi: 10.4049/jimmunol.166.12.7190
110. Fisher JP, Yan M, Heuijerjans J, Carter L, Abolhassani A, Frosch J, et al. Neuroblastoma killing properties of Vδ2 and Vδ2-negative γδT cells following expansion by artificial antigen-presenting cells. *Clin Cancer Res* (2014) 20(22):5720–32. doi: 10.1158/1078-0432.CCR-13-3464
111. Tokuyama H, Hagi T, Mattarollo SR, Morley J, Wang Q, So HF, et al. V gamma 9 V delta 2 T cell cytotoxicity against tumor cells is enhanced by monoclonal antibody drugs–rituximab and trastuzumab. *Int J Cancer* (2008) 122(11):2526–34. doi: 10.1002/ijc.23365
112. Gertner-Dardenne J, Bonnafous C, Bezombes C, Capietto AH, Scaglione V, Ingoure S, et al. Bromohydrin pyrophosphate enhances antibody-dependent cell-mediated cytotoxicity induced by therapeutic antibodies. *Blood* (2009) 113(20):4875–84. doi: 10.1182/blood-2008-08-172296
113. Capietto AH, Martinet L, Fournié JJ. Stimulated γδ T cells increase the in vivo efficacy of trastuzumab in HER-2+ breast cancer. *J Immunol* (2011) 187 (2):1031–8. doi: 10.4049/jimmunol.1100681
114. Tatsuta T, Shiraishi A, Mountz JD. The prodomain of caspase-1 enhances Fas-mediated apoptosis through facilitation of caspase-8 activation. *J Biol Chem* (2000) 275(19):14248–54. doi: 10.1074/jbc.275.19.14248
115. Sprick MR, Rieser E, Stahl H, Grosse-Wilde A, Weigand MA, Walczak H. Caspase-10 is recruited to and activated at the native TRAIL and CD95 death-inducing signalling complexes in a FADD-dependent manner but can

not functionally substitute caspase-8. *EMBO J* (2002) 21(17):4520–30. doi: 10.1093/emboj/cdf441
116. Milhas D, Cuvillier O, Therville N, Clavé P, Thomsen M, Levade T, et al. Caspase-10 triggers Bid cleavage and caspase cascade activation in FasL-induced apoptosis. *J Biol Chem* (2005) 280(20):19836–42. doi: 10.1074/jbc.M414358200
117. Todaro M, D'Asaro M, Caccamo N, Iovino F, Francipane MG, Meraviglia S, et al. Efficient killing of human colon cancer stem cells by gammadelta T lymphocytes. *J Immunol* (2009) 182(11):7287–96. doi: 10.4049/jimmunol.0804288
118. Dieli F, Vermijlen D, Fulfaro F, Caccamo N, Meraviglia S, Cicero G, et al. Targeting human {gamma}delta} T cells with zoledronate and interleukin-2 for immunotherapy of hormone-refractory prostate cancer. *Cancer Res* (2007) 67(15):7450–7. doi: 10.1158/0008-5472.CAN-07-0199
119. Li Z, Peng H, Xu Q, Ye Z. Sensitization of human osteosarcoma cells to Vγ9Vδ2 T-cell-mediated cytotoxicity by zoledronate. *J Orthop Res* (2012) 30 (5):824–30. doi: 10.1002/jor.21579
120. Dokouhaki P, Schuh NW, Joe B, Allen CA, Der SD, Tsao MS, et al. NKG2D regulates production of soluble TRAIL by ex vivo expanded human γδ T cells. *Eur J Immunol* (2013) 43(12):3175–82. doi: 10.1002/eji.201243150
121. Fiandalo MV, Schwarze SR, Kyprianou N. Proteasomal regulation of caspase-8 in cancer cell apoptosis. *Apoptosis* (2013) 18(6):766–76. doi: 10.1007/s10495-013-0821-y
122. Li M, Le W, Zhang XM, Zhang YJ, Jiang J, Liu PY. The M476W/Q482H mutation of procaspase-8 restored caspase-8-mediated apoptosis. *Biochem Biophys Res Commun* (2019) 514(3):653–8. doi: 10.1016/j.bbrc.2019.05.023
123. Mao ZG, Jiang CC, Yang F, Thorne RF, Hersey P, Zhang XD. TRAIL-induced apoptosis of human melanoma cells involves activation of caspase-4. *Apoptosis* (2010) 15(10):1211–22. doi: 10.1007/s10495-010-0513-9
124. Seo J, Lee EW, Shin J, Seong D, Nam YW, Jeong M, et al. K6 linked polyubiquitylation of FADD by CHIP prevents death inducing signaling complex formation suppressing cell death. *Oncogene* (2018) 37(36):4994–5006. doi: 10.1038/s41388-018-0323-z
125. Li Z, Xu Q, Peng H, Cheng R, Sun Z, Ye Z. IFN-γ enhances HOS and U2OS cell lines susceptibility to γδ T cell-mediated killing through the Fas/Fas ligand pathway. *Int Immunopharmacol* (2011) 11(4):496–503. doi: 10.1016/j.intimp.2011.01.001
126. Reboursiere E, Gac AC, Garnier A, Salaun V, Reman O, Pham AD, et al. Increased frequencies of circulating and tumor-resident Vδ1+ T cells in patients with diffuse large B-cell lymphoma. *Leuk Lymphoma* (2018) 59 (1):187–95. doi: 10.1080/10428194.2017.1321751
127. Ebert LM, Meuter S, Moser B. Homing and function of human skin gammadelta T cells and NK cells: relevance for tumor surveillance. *J Immunol* (2006) 176(7):4331–6. doi: 10.4049/jimmunol.176.7.4331
128. Zhou J, Kang N, Cui L, Ba D, He W. Anti-γδ TCR antibody-expanded γδ T cells: a better choice for the adoptive immunotherapy of lymphoid malignancies. *Cell Mol Immunol* (2012) 9(1):34–44. doi: 10.1038/cmi.2011.16
129. Simões C, Silva I, Carvalho A, Silva S, Santos S, Marques G, et al. Quantification and phenotypic characterization of peripheral blood Vδ1 + T cells in chronic lymphocytic leukemia and monoclonal B cell lymphocytosis. *Cytometry B Clin Cytom* (2019) 96(2):164–8. doi: 10.1002/cyto.b.21645
130. Correia DV, Fogli M, Hudspeth K, da Silva MG, Mavilio D, Silva-Santos B. Differentiation of human peripheral blood Vδ1+ T cells expressing the natural cytotoxicity receptor NKp30 for recognition of lymphoid leukemia cells. *Blood* (2011) 118(4):992–1001. doi: 10.1182/blood-2011-02-339135
131. Wu D, Wu P, Wu X, Ye J, Wang Z, Zhao S, et al. Ex vivo expanded human circulating Vδ1 γδT cells exhibit favorable therapeutic potential for colon cancer. *Oncoimmunology* (2015) 4(3):e992749. doi: 10.4161/2162402X.2014.992749
132. Brandes M, Willimann K, Moser B. Professional antigen-presentation function by human gammadelta T Cells. *Science* (2005) 309(5732):264–8. doi: 10.1126/science.1110267
133. Khan MW, Curbishley SM, Chen HC, Thomas AD, Pircher H, Mavilio D, et al. Expanded Human Blood-Derived γδT Cells Display Potent Antigen-Presentation Functions. *Front Immunol* (2014) 5:344. doi: 10.3389/fimmu.2014.00344
134. Mao C, Mou X, Zhou Y, Yuan G, Xu C, Liu H, et al. Tumor-activated TCRγδ$^+$ T cells from gastric cancer patients induce the antitumor immune response of TCRαβ$^+$ T cells via their antigen-presenting cell-like effects. *J Immunol Res* (2014) 2014:593562. doi: 10.1155/2014/593562
135. Sawaisorn P, Tangchaikeeree T, Chan-On W, Leepiyasakulchai C, Udomsangpetch R, Hongeng S, et al. Antigen-Presenting Cell Characteristics of Human γδ T Lymphocytes in Chronic Myeloid Leukemia. *Immunol Invest* (2019) 48(1):11–26. doi: 10.1080/08820139.2018.1529039
136. Muto M, Baghdadi M, Maekawa R, Wada H, Seino K. Myeloid molecular characteristics of human γδ T cells support their acquisition of tumor antigen-presenting capacity. *Cancer Immunol Immunother* (2015) 64 (8):941–9. doi: 10.1007/s00262-015-1700-x
137. Himoudi N, Morgenstern DA, Yan M, Vernay B, Saraiva L, Wu Y, et al. Human γδ T lymphocytes are licensed for professional antigen presentation by interaction with opsonized target cells. *J Immunol* (2012) 188(4):1708–16. doi: 10.4049/jimmunol.1102654
138. Chen HC, Joalland N, Bridgeman JS, Alchami FS, Jarry U, Khan MWA, et al. Synergistic targeting of breast cancer stem-like cells by human γδ T cells and CD8+ T cells. *Immunol Cell Biol* (2017) 95(7):620–9. doi: 10.1038/icb.2017.21
139. Münz C, Steinman RM, Fujii S. Dendritic cell maturation by innate lymphocytes: coordinated stimulation of innate and adaptive immunity. *J Exp Med* (2005) 202(2):203–7. doi: 10.1084/jem.20050810
140. Devilder MC, Maillet S, Bouyge-Moreau I, Donnadieu E, Bonneville M, Scotet E. Potentiation of antigen-stimulated V gamma 9V delta 2 T cell cytokine production by immature dendritic cells (DC) and reciprocal effect on DC maturation. *J Immunol* (2006) 176(3):1386–93. doi: 10.4049/jimmunol.176.3.1386
141. Conti L, Casetti R, Cardone M, Varano B, Martino A, Belardelli F, et al. Reciprocal activating interaction between dendritic cells and pamidronate-stimulated gammadelta T cells: role of CD86 and inflammatory cytokines. *J Immunol* (2005) 174(1):252–60. doi: 10.4049/jimmunol.174.1.252
142. Girard P, Ponsard B, Charles J, Chaperot L, Aspord C. Potent Bidirectional Cross-Talk Between Plasmacytoid Dendritic Cells and γδT Cells Through BTN3A, Type I/II IFNs and Immune Checkpoints. *Front Immunol* (2020) 11:861. doi: 10.3389/fimmu.2020.00861
143. Castella B, Kopecka J, Sciancalepore P, Mandili G, Foglietta M, Mitro N, et al. The ATP-binding cassette transporter A1 regulates phosphoantigen release and Vγ9Vδ2 T cell activation by dendritic cells. *Nat Commun* (2017) 8:15663. doi: 10.1038/ncomms15663
144. Nabholtz JM, Abrial C, Mouret-Reynier MA, Dauplat MM, Weber B, Gligorov J, et al. Multicentric neoadjuvant phase II study of panitumumab combined with an anthracycline/taxane-based chemotherapy in operable triple-negative breast cancer: identification of biologically defined signatures predicting treatment impact. *Ann Oncol* (2014) 25(8):1570–7. doi: 10.1093/annonc/mdu183
145. Van Hede D, Polese B, Humblet C, Wilharm A, Renoux V, Dortu E, et al. Human papillomavirus oncoproteins induce a reorganization of epithelial-associated γδ T cells promoting tumor formation. *Proc Natl Acad Sci U S A* (2017) 114(43):E9056–65. doi: 10.1073/pnas.1712883114
146. Zhou BY, Gong JH, Cai XY, Wang JX, Luo F, Jiang N, et al. An imbalance between stellate cells and γδT cells contributes to hepatocellular carcinoma aggressiveness and recurrence. *Hepatol Int* (2019) 13(5):631–40. doi: 10.1007/s12072-019-09969-w
147. Welte T, Zhang XH. Interleukin-17 Could Promote Breast Cancer Progression at Several Stages of the Disease. *Mediators Inflamm* (2015) 2015:804347. doi: 10.1155/2015/804347
148. Qian X, Chen H, Wu X, Hu L, Huang Q, Jin Y. Interleukin-17 acts as double-edged sword in anti-tumor immunity and tumorigenesis. *Cytokine* (2017) 89:34–44. doi: 10.1016/j.cyto.2015.09.011
149. Meraviglia S, Lo Presti E, Tosolini M, La Mendola C, Orlando V, Todaro M, et al. Distinctive features of tumor-infiltrating γδ T lymphocytes in human colorectal cancer. *Oncoimmunology* (2017) 6(10):e1347742. doi: 10.1080/2162402X.2017.1347742
150. Wu P, Wu D, Ni C, Ye J, Chen W, Hu G, et al. γδT17 cells promote the accumulation and expansion of myeloid-derived suppressor cells in human

colorectal cancer. *Immunity* (2014) 40(5):785–800. doi: 10.1016/j.immuni.2014.03.013
151. Aotsuka A, Matsumoto Y, Arimoto T, Kawata A, Ogishima J, Taguchi A, et al. Interleukin-17 is associated with expression of programmed cell death 1 ligand 1 in ovarian carcinoma. *Cancer Sci* (2019) 110(10):3068–78. doi: 10.1111/cas.14174
152. Wistuba-Hamprecht K, Di Benedetto S, Schilling B, Sucker A, Schadendorf D, Garbe C, et al. Phenotypic characterization and prognostic impact of circulating γδ and αβ T-cells in metastatic malignant melanoma. *Int J Cancer* (2016) 138(3):698–704. doi: 10.1002/ijc.29818
153. Wistuba-Hamprecht K, Martens A, Haehnel K, Geukes Foppen M, Yuan J, Postow MA, et al. Proportions of blood-borne Vδ1+ and Vδ2+ T-cells are associated with overall survival of melanoma patients treated with ipilimumab. *Eur J Cancer* (2016) 64:116–26. doi: 10.1016/j.ejca.2016.06.001
154. Rong L, Li K, Li R, Liu HM, Sun R, Liu XY. Analysis of tumor-infiltrating gamma delta T cells in rectal cancer. *World J Gastroenterol* (2016) 22 (13):3573–80. doi: 10.3748/wjg.v22.i13.3573
155. Chen X, Shang W, Xu R, Wu M, Zhang X, Huang P, et al. Distribution and functions of γδ T cells infiltrated in the ovarian cancer microenvironment. *J Transl Med* (2019) 17(1):144. doi: 10.1186/s12967-019-1897-0
156. Patil RS, Shah SU, Shrikhande SV, Goel M, Dikshit RP, Chiplunkar SV. IL17 producing γδT cells induce angiogenesis and are associated with poor survival in gallbladder cancer patients. *Int J Cancer* (2016) 139(4):869–81. doi: 10.1002/ijc.30134
157. Sacchi A, Tumino N, Sabatini A, Cimini E, Casetti R, Bordoni V, et al. Myeloid-Derived Suppressor Cells Specifically Suppress IFN-γ Production and Antitumor Cytotoxic Activity of Vδ2 T Cells. *Front Immunol* (2018) 9:1271. doi: 10.3389/fimmu.2018.01271
158. Yu SJ, Ma C, Heinrich B, Brown ZJ, Sandhu M, Zhang Q, et al. Targeting the crosstalk between cytokine-induced killer cells and myeloid-derived suppressor cells in hepatocellular carcinoma. *J Hepatol* (2019) 70(3):449–57. doi: 10.1016/j.jhep.2018.10.040
159. Romano A, Parrinello NL, La Cava P, Tibullo D, Giallongo C, Camiolo G, et al. PMN-MDSC and arginase are increased in myeloma and may contribute to resistance to therapy. *Expert Rev Mol Diagn* (2018) 18 (7):675–83. doi: 10.1080/14737159.2018.1470929
160. Okła K, Czerwonka A, Wawruszak A, Bobiński M, Bilska M, Tarkowski R, et al. Clinical Relevance and Immunosuppressive Pattern of Circulating and Infiltrating Subsets of Myeloid-Derived Suppressor Cells (MDSCs) in Epithelial Ovarian Cancer. *Front Immunol* (2019) 10:691. doi: 10.3389/fimmu.2019.00691
161. Peng G, Wang HY, Peng W, Kiniwa Y, Seo KH, Wang RF. Tumor-infiltrating gammadelta T cells suppress T and dendritic cell function via mechanisms controlled by a unique toll-like receptor signaling pathway. *Immunity* (2007) 27(2):334–48. doi: 10.1016/j.immuni.2007.05.020
162. Ye J, Ma C, Wang F, Hsueh EC, Toth K, Huang Y, et al. Specific recruitment of γδ regulatory T cells in human breast cancer. *Cancer Res* (2013) 73 (20):6137–48. doi: 10.1158/0008-5472.CAN-13-0348
163. Ye J, Ma C, Hsueh EC, Eickhoff CS, Zhang Y, Varvares MA, et al. Tumor-derived γδ regulatory T cells suppress innate and adaptive immunity through the induction of immunosenescence. *J Immunol* (2013) 190(5):2403–14. doi: 10.4049/jimmunol.1202369
164. Kobayashi H, Tanaka Y, Yagi J, Osaka Y, Nakazawa H, Uchiyama T, et al. Safety profile and anti-tumor effects of adoptive immunotherapy using gamma-delta T cells against advanced renal cell carcinoma: a pilot study. *Cancer Immunol Immunother* (2007) 56(4):469–76. doi: 10.1007/s00262-006-0199-6
165. Kobayashi H, Tanaka Y, Yagi J, Minato N, Tanabe K. Phase I/II study of adoptive transfer of γδ T cells in combination with zoledronic acid and IL-2 to patients with advanced renal cell carcinoma. *Cancer Immunol Immunother* (2011) 60(8):1075–84. doi: 10.1007/s00262-011-1021-7
166. Nicol AJ, Tokuyama H, Mattarollo SR, Hagi T, Suzuki K, Yokokawa K, et al. Clinical evaluation of autologous gamma delta T cell-based immunotherapy for metastatic solid tumours. *Br J Cancer* (2011) 105(6):778–86. doi: 10.1038/bjc.2011.293
167. Abe Y, Muto M, Nieda M, Nakagawa Y, Nicol A, Kaneko T, et al. Clinical and immunological evaluation of zoledronate-activated Vgamma9gammadelta T-cell-based immunotherapy for patients with multiple myeloma. *Exp Hematol* (2009) 37(8):956–68. doi: 10.1016/j.exphem.2009.04.008
168. Bennouna J, Bompas E, Neidhardt EM, Rolland F, Philip I, Galéa C, et al. Phase-I study of Innacell gammadelta, an autologous cell-therapy product highly enriched in gamma9delta2 T lymphocytes, in combination with IL-2, in patients with metastatic renal cell carcinoma. *Cancer Immunol Immunother* (2008) 57(11):1599–609. doi: 10.1007/s00262-008-0491-8
169. Nakajima J, Murakawa T, Fukami T, Goto S, Kaneko T, Yoshida Y, et al. A phase I study of adoptive immunotherapy for recurrent non-small-cell lung cancer patients with autologous gammadelta T cells. *Eur J Cardiothorac Surg* (2010) 37(5):1191–7. doi: 10.1016/j.ejcts.2009.11.051
170. Sakamoto M, Nakajima J, Murakawa T, Fukami T, Yoshida Y, Murayama T, et al. Adoptive immunotherapy for advanced non-small cell lung cancer using zoledronate-expanded γδTcells: a phase I clinical study. *J Immunother* (2011) 34(2):202–11. doi: 10.1097/CJI.0b013e318207ecfb
171. Wada I, Matsushita H, Noji S, Mori K, Yamashita H, Nomura S, et al. Intraperitoneal injection of in vitro expanded Vγ9Vδ2 T cells together with zoledronate for the treatment of malignant ascites due to gastric cancer. *Cancer Med* (2014) 3(2):362–75. doi: 10.1002/cam4.196
172. Lang JM, Kaikobad MR, Wallace M, Staab MJ, Horvath DL, Wilding G, et al. Pilot trial of interleukin-2 and zoledronic acid to augment γδ T cells as treatment for patients with refractory renal cell carcinoma. *Cancer Immunol Immunother* (2011) 60(10):1447–60. doi: 10.1007/s00262-011-1049-8
173. Pressey JG, Adams J, Harkins L, Kelly D, You Z, Lamb LS Jr. In vivo expansion and activation of γδ T cells as immunotherapy for refractory neuroblastoma: A phase 1 study. *Med (Baltimore)* (2016) 95(39):e4909. doi: 10.1097/MD.0000000000004909
174. Kunzmann V, Smetak M, Kimmel B, Weigang-Koehler K, Goebeler M, Birkmann J, et al. Tumor-promoting versus tumor-antagonizing roles of γδ T cells in cancer immunotherapy: results from a prospective phase I/II trial. *J Immunother* (2012) 35(2):205–13. doi: 10.1097/CJI.0b013e318245bb1e
175. Wilhelm M, Kunzmann V, Eckstein S, Reimer P, Weissinger F, Ruediger T, et al. Gammadelta T cells for immune therapy of patients with lymphoid malignancies. *Blood* (2003) 102(1):200–6. doi: 10.1182/blood-2002-12-3665
176. Bennouna J, Levy V, Sicard H, Senellart H, Audrain M, Hiret S, et al. Phase I study of bromohydrin pyrophosphate (BrHPP, IPH 1101), a Vgamma9Vdelta2 T lymphocyte agonist in patients with solid tumors. *Cancer Immunol Immunother* (2010) 59(10):1521–30. doi: 10.1007/s00262-010-0879-0
177. Kouakanou L, Xu Y, Peters C, He J, Wu Y, Yin Z, et al. Vitamin C promotes the proliferation and effector functions of human γδ T cells. *Cell Mol Immunol* (2020) 17(5):462–73. doi: 10.1038/s41423-019-0247-8
178. Jiang H, Yang Z, Song Z, Green M, Song H, Shao Q. γδ T cells in hepatocellular carcinoma patients present cytotoxic activity but are reduced in potency due to IL-2 and IL-21 pathways. *Int Immunopharmacol* (2019) 70:167–73. doi: 10.1016/j.intimp.2019.02.019
179. Duault C, Betous D, Bezombes C, Roga S, Cayrol C, Girard JP, et al. IL-33-expanded human Vγ9Vδ2 T cells have anti-lymphoma effect in a mouse tumor model. *Eur J Immunol* (2017) 47(12):2137–41. doi: 10.1002/eji.201747093
180. Van Acker HH, Anguille S, Willemen Y, Van den Bergh JM, Berneman ZN, Lion E, et al. Interleukin-15 enhances the proliferation, stimulatory phenotype, and antitumor effector functions of human gamma delta T cells. *J Hematol Oncol* (2016) 9(1):101. doi: 10.1186/s13045-016-0329-3
181. Cairo C, Sagnia B, Cappelli G, Colizzi V, Leke RG, Leke RJ, et al. Human cord blood γδ T cells expressing public Vγ2 chains dominate the response to bisphosphonate plus interleukin-15. *Immunology* (2013) 138(4):346–60. doi: 10.1111/imm.12039
182. Duault C, Franchini DM, Familliades J, Cayrol C, Roga S, Girard JP, et al. TCRVγ9 γδ T Cell Response to IL-33: A CD4 T Cell-Dependent Mechanism. *J Immunol* (2016) 196(1):493–502. doi: 10.4049/jimmunol.1500260
183. Aoki T, Matsushita H, Hoshikawa M, Hasegawa K, Kokudo N, Kakimi K. Adjuvant combination therapy with gemcitabine and autologous γδ T-cell transfer in patients with curatively resected pancreatic cancer. *Cytotherapy* (2017) 19(4):473–85. doi: 10.1016/j.jcyt.2017.01.002
184. Wang S, Li H, Ye C, Lin P, Li B, Zhang W, et al. Valproic Acid Combined with Zoledronate Enhance γδ T Cell-Mediated Cytotoxicity against Osteosarcoma Cells via the Accumulation of Mevalonate Pathway

Intermediates. *Front Immunol* (2018) 9:377:377. doi: 10.3389/fimmu.2018.00377
185. Lin L, He J, Li J, Xu Y, Li J, Wu Y. Chitosan Nanoparticles Strengthen Vγ9Vδ2 T-Cell Cytotoxicity Through Upregulation Of Killing Molecules And Cytoskeleton Polarization. *Int J Nanomedicine* (2019) 14:9325–36. doi: 10.2147/IJN.S212898
186. Rossi C, Gravelle P, Decaup E, Bordenave J, Poupot M, Tosolini M, et al. Boosting γδ T cell-mediated antibody-dependent cellular cytotoxicity by PD-1 blockade in follicular lymphoma. *Oncoimmunology* (2018) 8(3):1554175. doi: 10.1080/2162402X.2018.1554175
187. Wu K, Zhao H, Xiu Y, Li Z, Zhao J, Xie S, et al. IL-21-mediated expansion of Vγ9Vδ2 T cells is limited by the Tim-3 pathway. *Int Immunopharmacol* (2019) 69:136–42. doi: 10.1016/j.intimp.2019.01.027
188. Li X, Lu H, Gu Y, Zhang X, Zhang G, Shi T, et al. Tim-3 suppresses the killing effect of $V\gamma9V\delta2^{+}$T cells on colon cancer cells by reducing perforin and granzyme B expression. *Exp Cell Res* (2020) 386(1):111719. doi: 10.1016/j.yexcr.2019.111719
189. Hoh A, Dewerth A, Vogt F, Wenz J, Baeuerle PA, Warmann SW, et al. The activity of γδ T cells against paediatric liver tumour cells and spheroids in cell culture. *Liver Int* (2013) 33(1):127–36. doi: 10.1111/liv.12011
190. Oberg HH, Janitschke L, Sulaj V, Weimer J, Gonnermann D, Hedemann N, et al. Bispecific antibodies enhance tumor-infiltrating T cell cytotoxicity against autologous HER-2-expressing high-grade ovarian tumors. *J Leukoc Biol* (2020) 107(6):1081–95. doi: 10.1002/JLB.5MA1119-265R
191. Oberg HH, Peipp M, Kellner C, Sebens S, Krause S, Petrick D, et al. Novel bispecific antibodies increase γδ T-cell cytotoxicity against pancreatic cancer cells. *Cancer Res* (2014) 74(5):1349–60. doi: 10.1158/0008-5472.CAN-13-0675
192. Oberg HH, Kellner C, Gonnermann D, Peipp M, Peters C, Sebens S, et al. γδ T cell activation by bispecific antibodies. *Cell Immunol* (2015) 296(1):41–9. doi: 10.1016/j.cellimm.2015.04.009
193. Tawfik D, Groth C, Gundlach JP, Peipp M, Kabelitz D, Becker T, et al. TRAIL-Receptor 4 Modulates γδ T Cell-Cytotoxicity Toward Cancer Cells. *Front Immunol* (2019) 10:2044. doi: 10.3389/fimmu.2019.02044
194. Fisher J, Abramowski P, Wisidagamage Don ND, Flutter B, Capsomidis A, Cheung GW, et al. Avoidance of On-Target Off-Tumor Activation Using a Co-stimulation-Only Chimeric Antigen Receptor. *Mol Ther* (2017) 25(5):1234–47. doi: 10.1016/j.ymthe.2017.03.002
195. Capsomidis A, Benthall G, Van Acker HH, Fisher J, Kramer AM, Abeln Z, et al. Chimeric Antigen Receptor-Engineered Human Gamma Delta T Cells: Enhanced Cytotoxicity with Retention of Cross Presentation. *Mol Ther* (2018) 26(2):354–65. doi: 10.1016/j.ymthe.2017.12.001
196. Marcu-Malina V, Heijhuurs S, van Buuren M, Hartkamp L, Strand S, Sebestyen Z, et al. Redirecting αβ T cells against cancer cells by transfer of a broadly tumor-reactive γδT-cell receptor. *Blood* (2011) 118(1):50–9. doi: 10.1182/blood-2010-12-325993
197. Gründer C, van Dorp S, Hol S, Drent E, Straetemans T, Heijhuurs S, et al. γ9 and δ2CDR3 domains regulate functional avidity of T cells harboring γ9δ2TCRs. *Blood* (2012) 120(26):5153–62. doi: 10.1182/blood-2012-05-432427
198. Braham MVJ, Minnema MC, Aarts T, Sebestyen Z, Straetemans T, Vyborova A, et al. Cellular immunotherapy on primary multiple myeloma expanded in a 3D bone marrow niche model. *Oncoimmunology* (2018) 7(6):e1434465. doi: 10.1080/2162402X.2018.1434465
199. Straetemans T, Kierkels GJJ, Doorn R, Jansen K, Heijhuurs S, Dos Santos JM, et al. GMP-Grade Manufacturing of T Cells Engineered to Express a Defined γδTCR. *Front Immunol* (2018) 9:1062. doi: 10.3389/fimmu.2018.01062
200. Rodrigues NV, Correia DV, Mensurado S, Nóbrega-Pereira S, deBarros A, Kyle-Cezar F, et al. Low-Density Lipoprotein Uptake Inhibits the Activation and Antitumor Functions of Human Vγ9Vδ2 T Cells. *Cancer Immunol Res* (2018) 6(4):448–57. doi: 10.1158/2326-6066.CIR-17-0327

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RNA-Seq Identifies Marked Th17 Cell Activation and Altered CFTR Expression in Different Atopic Dermatitis Subtypes in Chinese Han Populations

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Background: Patients with atopic dermatitis (AD) exhibit phenotypic variability in ethnicity and IgE status. In addition, some patients develop other allergic conditions, such as allergic rhinitis (AR), in subsequent life. Understanding the heterogeneity of AD would be beneficial to phenotype-specific therapies.

Methods: Twenty-eight Chinese AD patients and 8 healthy volunteers were enrolled in the study. High-throughput transcriptome sequencing was conducted on lesional and nonlesional skin samples from 10 AD patients and matched normal skin samples from 5 healthy volunteers. Identification of differentially expressed genes (DEGs), KEGG pathway analyses, and sample cluster analyses were conducted in the R software environment using the DEseq2, ClusterProfiler, and pheatmap R packages, respectively. qRT-PCR, Western blotting, and ELISA were used to detect gene expression levels among subtypes. Correlation analysis was performed to further investigate their correlation with disease severity.

Results: A total of 25,798 genes were detected per sample. Subgroup differential expression analysis and functional enrichment analysis revealed significant changes in the IL17 signaling pathway in Chinese EAD patients but not in IAD patients. DEGs enriched in cytokine-cytokine receptor interactions and gland secretion were considered to be associated with atopic march. Further investigations confirmed a marked IL17A upregulation in Chinese EAD with a positive relationship with total IgE level and AD severity. In addition, increased IL17A in AD patients with AR demonstrated a closer association with AR severity than IL4R. Moreover, AQP5 and CFTR were decreased in the lesions of AD patients with AR. The CFTR mRNA expression level was negatively associated with the skin IL17A level and AR severity.

Conclusion: Our research characterized marked Th17 activation in Chinese EAD patients, and altered expression of IL17A, IL4R, AQP5, and CFTR in AD patients with

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AR was associated with AR severity. It partially explained the phenotypic differences of AD subtypes and provided potential references for endotype-targeted therapy.

Keywords: atopic dermatitis, extrinsic AD, intrinsic AD, heterogeneity, atopic march

INTRODUCTION

Atopic dermatitis (AD) is characterized by pruritus with a chronic course of exacerbation and remission (1, 2). Patients with AD have a high propensity to develop other allergic conditions, such as asthma, allergic rhinitis (AR), and food allergies, which is called "atopic march" (AM) (2, 3). The clinical signs of AD often predate the development of atopic comorbidities, suggesting that AD might be an "entry point" for subsequent allergic diseases (3).

Recent studies have provided insights into the heterogeneity of AD among ethnicities and subtypes (1). It was reported that susceptibility loci associated with AD were only partially overlapped among different ethnicities by Genome-wide association study (GWAS) (4, 5), and Asian patients showed characteristic remarkable Th17 axis activation (1, 6). Besides, it is widely accepted that AD can be divided into two subtypes based on lgE levels (1, 2). Patients with high total and environmental serum IgE levels are diagnosed with extrinsic AD (EAD), which is the predominant subtype (accounting for 80%). This subtype has a close relationship with filaggrin mutation, personal and family atopic background, eosinophilia, and tendency to develop AR or asthma (1, 7). Intrinsic AD (IAD) is characterized by normal IgE levels, the absence of specific IgE for environmental and food allergens, less barrier function damage, and more similar to allergic contact dermatitis (2, 7, 8). Although the molecular endotypic difference between EAD and IAD has been reported by several studies (1, 2, 7), ethnic factors were not taken into account. Given AD heterogeneity exists across ethnicities and subtypes, previous studies based on a single aspect may result in inaccurate conclusions.

High-throughput sequencing allows the analysis of the global transcriptome and accurate detection of gene expression on a larger scale (9). To characterize the heterogeneity with precise stratifications, we performed high-throughput RNA-seq on lesional and nonlesional skin samples from 10 AD patients and normal skin samples from 5 healthy volunteers and analyzed the difference between the IAD and EAD and the genes related to the AM in the Chinese Han population. Our results showed that a marked Th17 activation in EAD rather than IAD. In addition, IL17A, IL4R, AQP5, and CFTR were associated with AM in the Chinese Han population.

MATERIALS AND METHODS

Patients Enrollment and Samples Collection

Twenty-eight Chinese Han AD patients and 8 age- and sex-matched healthy volunteers were recruited with signed consent obtained. Among all participants, 19 subjects were from Guangzhou, and 17 were from Shanghai, representing different regions in China. The enrollment criteria for patients were as follows: (1) Met Williams diagnostic criteria clinically and the main characteristics of AD pathologically; (2) SCORAD scores ≥ 25; and (3) did not receive any treatment in the past two weeks. The subjects were divided into two groups (10 IAD patients and 18 EAD patients) based on raised levels of IgE (> 500 kU/L) and/or personal or familial history of atopy (8). In these 28 AD patients, 15 patients had AR, 11 patients did not have AR, and the condition of 2 patients was unknown. In those 8 healthy volunteers, 3 volunteers had AR, and 5 volunteers did not have AR. The total nasal symptom score (TNSS) was used to measure the severity of AR (10). The study was approved by the local ethics committee and conducted according to the principles of the Declaration of Helsinki. A 4 mm-diameter skin biopsy was taken from lesional and nonlesional skin in every AD patient and in 8 healthy volunteers. Samples were stored at −80°C until processing for extraction of RNA and protein. The information for the participants is shown in **Supplementary Table S1**.

RNA-Seq

RNA-seq was conducted on nonlesional and lesional skin samples from 10 patients (P1-P10) and 5 healthy skin samples (H1-H5). Sample details of RNA-seq are shown in **Supplementary Table S2**. Total RNA was extracted using TRIzol (Invitrogen, USA) following the manufacturer's instructions. The total RNA concentration, the RIN value, 28S/18S, and the fragment size were measured using an Agilent 2100 Bioanalyzer (Agilent, USA). The BGISEQ-500 (Shenzhen Huada Gene, China) platform based on sequencing by synthesis (SBS) technology was used for high-throughput sequencing to obtain a 100 bp sequencing read. The raw data were subjected to quality control to obtain effective reads. SOAPnuke (v1.4.0) and Trimmomatic (v0.36) were used to perform statistical analysis and filter out reads of low to moderate quality, polluted connectors, and unknown nucleotides with high N content before data analysis to ensure reliability.

RNA-Seq Analyses

RNA-seq analyses were conducted in the R environment (v3.6.2). The DEseq2 R package was used to identify the differentially expressed genes (DEGs) ($|\log2FC| \geq 1$, adj. $P \geq 0.05$) between each group following the previously described methods (11). After constructing appropriate gene sets, two types of enrichment analyses were conducted: overrepresentation analysis (ORA) and gene set enrichment analysis (GSEA) using the ClusterProfiler R package (12). Sample cluster analysis of the DEGs was performed using the pheatmap R package (13). Annotation of the genes and pathways was provided by the

KEGG (Kyoto Encyclopedia of Genes and Genomes) database (http://www.genome.jp/kegg). Only KEGG pathways enriched at $P < 0.05$ were considered. The workflow of the RNA-seq analyses is shown in **Supplementary Figure S1**.

Microarray Analysis

Three microarray studies from the Gene Expression Omnibus (GEO) database were utilized, including GSE75890, GSE146352, and GSE124700. GSE75890 included 9 IAD, 5 EAD, and 8 normal skin (HS) samples from Denmark to analyze the heterogeneity between IAD and EAD in patients from Europe. In brief, the raw data were downloaded from the repository. DEGs ($|\log2FC| \geq 1$, adj. $P \leq 0.05$) between each group were identified by the limma R package, and KEGG pathway enrichment was conducted by the methods described above in the R environment. GSE146352 and GSE124700 were conducted by the same platforms, including 4 AD, 3 AM, and 3 HS skin samples from Korea in total. The two datasets were merged as a microarray of AM and mined for the expression levels of AM-associated genes involved in the IL17 signaling pathway and gland secretion.

Quantitative Real-Time RT-PCR (qRT-PCR)

Total RNA was extracted using TRIzol (Invitrogen, USA) following the manufacturer's instructions and transcribed into cDNA using PrimeScript™ RT Master Mix (Takara, Japan). The relative expression of various target genes was determined by RT-PCR using TB Green® Premix Ex Taq™ II (Takara, Japan). Gene expression was normalized to that of *GAPDH*. The primer sequences used in this study are listed in **Supplementary Table S3**.

ELISA Assessment

The IL17A and IL4R proteins were measured by ELISA (ab216167 and ab243668, Abcam, USA). Briefly, standards and samples were added into the wells and incubated with antibody cocktails for 1 h at room temperature on a plate shaker set to 400 rpm. Then, aspirate and wash each well with 350 μL of 1X wash buffer three times. After washing, 100 μL of TMB development solution was added to each well and further incubated for 10 min in the dark on a plate shaker set to 400 rpm. Finally, 100 μL of stop solution was added to each well and shaken for 1 min, and then the intensity of the color was measured at 450 nm.

Western Blotting

Frozen skin samples were cut into pieces and lysed with ice-cold radioimmunoprecipitation (RIPA) lysis buffer containing protease inhibitors (Beyotime, China) to obtain tissue homogenates. The protein concentration of each sample was detected using a bicinchoninic acid (BCA) kit (Beyotime, China). After normalization to PBS, the lysates were mixed with loading buffer and incubated for 10 min at 95°C. Approximately 20 μg of protein from each sample was separated by 10% SDS-PAGE and transferred to PVDF membranes (Merck Millipore, USA). After blocking in 5% bovine serum albumin (Yeasen, China) for 1 h, the membranes were incubated with primary antibodies overnight at 4°C and with secondary antibodies for 1 h. GAPDH and β-tubulin were used as loading controls. Bound antibodies were detected using the ECL Western blot detection system (Merck Millipore, USA) and quantified by ImageJ (National Institutes of Health, USA). The information for the primary antibodies used in the experiment is listed in **Supplementary Table S4**.

Statistical Analysis

Statistical analysis of RNA-seq was performed by using R software (www.R-project.org) and packages available through Bioconductor (www.bioconductor.org). The experimental data are presented as the mean ± SD and were analyzed using unpaired Student's *t*-test and one-way ANOVA followed by Tukey's or Dunnett's multiple comparisons posttest. Correlations were determined by Pearson correlation analysis. All analyses of experimental data and clinical features were performed using GraphPad Prism 7 software (San Diego, USA). Differences were regarded as significant at $P < 0.05$.

RESULTS

Significant Alterations in Focal Adhesion and the NOD-Like Receptor Signaling Pathway in AD Skin From the Chinese Han Population

A total of 25 skin samples, including 10 lesional (L) and 10 nonlesional skin samples (NL) from AD patients and 5 HS from healthy volunteers, were subjected to RNA-seq. Through RNA-seq, we generated 107 ± 7 million (mean ± SD) reads per sample, with reads averaging 100 bp in length, producing 11 Gb of cDNA sequence per sample. After mapping the RNA-seq reads to the reference genome, we obtained a high mapping rate of 85% on average. A total of 25,798 genes were detected, including 19,483 annotated genes.

Then, differential expression analyses between groups were performed using the DEseq2 R package. The results showed a total of 4924 DEGs between the L and HS groups, 4924 DEGs between the L and NL groups, and 4281 DEGs between the NL and HS groups (**Figure 1A**). KEGG analyses were employed by ORA and GSEA using the ClusterProfiler R package to explore changes that occurred in lesional and nonlesional skin. All the identified DEGs were subjected to KEGG analysis by ORA, and all annotated genes were input into GSEA. As shown in **Figures 1B, C**, significant changes in cytokine-cytokine receptor interactions were identified among the three groups by both ORA and GSEA. In addition, GSEA detected significant alterations in the NOD-like receptor signaling pathway in both the L vs. NL group and L vs. HS group, as well as changes in focal adhesion in both the L vs. HS group and NL vs. HS group. Sample cluster analyses were further performed to investigate the DEGs and/or core enriched genes involved in these three pathways. DEGs (or core enrichment genes) in the three pathways were upregulated in most samples from AD patients, especially in lesional skin (**Figures 1D, E, G**). As expected, the Western blotting results illustrated that NLRP3

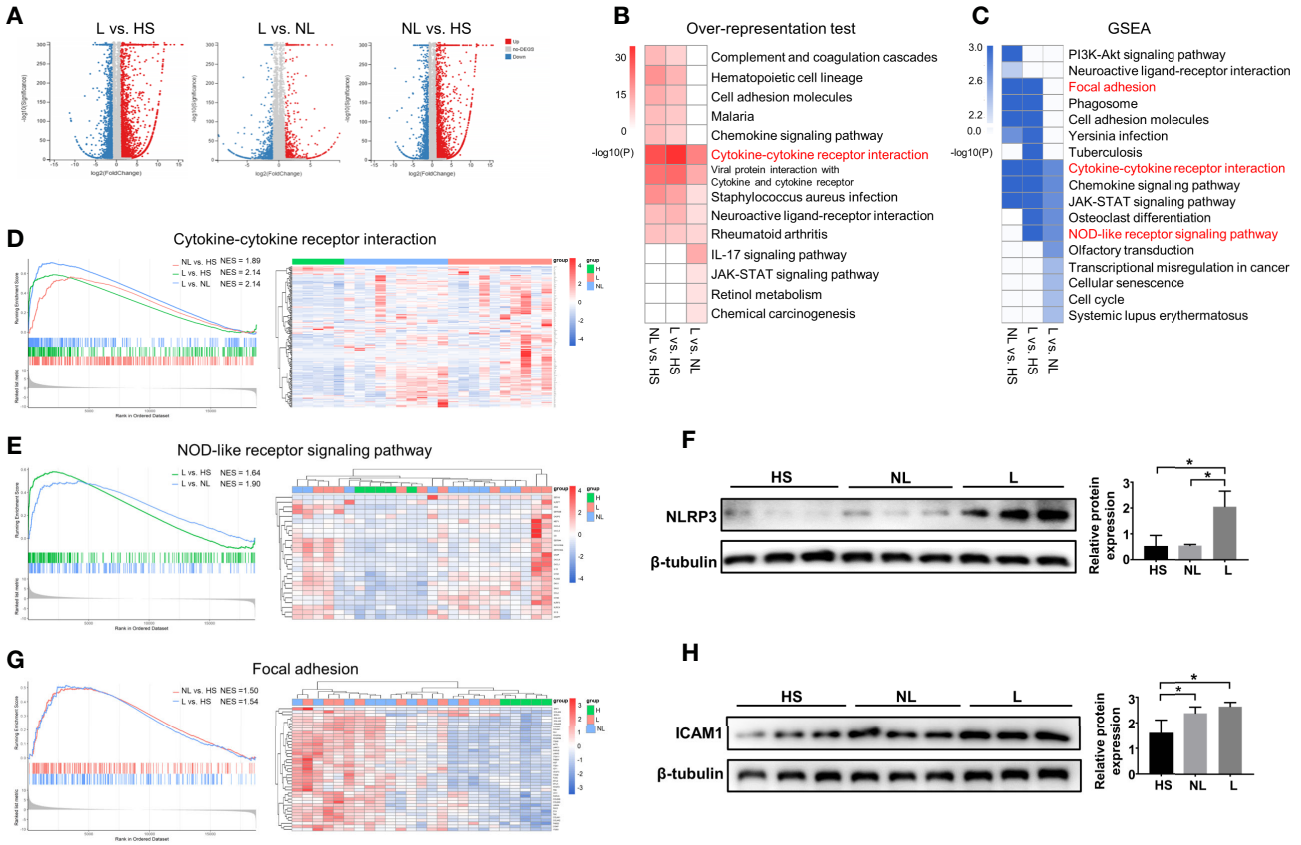

FIGURE 1 | DEG identification and KEGG enrichment pathway analyses between the HS and NL groups, HS and L groups, and NL and L groups. **(A)** Volcano plot of DEGs between the L and HS groups, the L and NL groups, and the NL and HS groups identified by differential expression analyses. HS group (n = 5), NL group (n = 10), and L group (n = 10). **(B)** Top 10 enriched KEGG pathways analysis by overrepresentation test and **(C)** by GSEA among the three groups. ($P < 0.05$) The GSEA enrichment plot and the sample cluster analyses based on the expression pattern of involved DEGs of **(D)** cytokine-cytokine receptor interaction, **(E)** NOD-like receptor signaling pathway, and **(G)** focal adhesion. Western blot and quantitative analysis of **(F)** NLRP3 and **(H)** ICAM1 among the HS (n = 3), NL (n = 3), and L (n = 3) groups. *$P < 0.05$ according to one-way ANOVA followed by Tukey's multiple comparisons.

was upregulated in lesional skin compared to nonlesional and healthy skin and was enriched in the NOD-like receptor signaling pathway (**Figure 1F**). The protein expression of ICAM1 involved in focal adhesion was increased in both nonlesional and lesional skin compared to healthy skin (**Figure 1H**).

Increased IL17A Characterizes Chinese EAD Patients Rather Than IAD Patients

To determine if there were specific pathways involved in different subtypes, differential expression analyses were performed between lesional and nonlesional skin samples from IAD patients (IAD-L vs. IAD-NL) and EAD patients (EAD-L vs. EAD-NL). A total of 2250 DEGs were identified in the IAD-L vs. IAD-NL group, as well as 1267 DEGs in the EAD-L vs. EAD-NL group (**Supplementary Figure S2**). The results of KEGG analyses revealed a remarkable change in the PPAR signaling pathway in IAD, while alterations in the IL17 signaling pathway were significant in EAD (**Figure 2A**). The interaction network of subtype-specific pathways and genes is shown in **Figure 2B**. We further performed differential expression analyses and enrichment analyses between each subtype and healthy skin samples from Denmark. Surprisingly, KEGG analysis results demonstrated a remarkable change in the IL17 signaling pathway in IAD but not EAD (**Figure 2C**). As shown in **Figures 2D, E**, an opposite expression pattern of genes enriched in the IL17 signaling pathway was identified between Chinese and Denmark AD patients.

We detected the mRNA and protein expression levels of IL17A and PPARG by qRT-PCR and Western blotting (or ELISA) to confirm the RNA-seq analysis results in the Chinese Han population. Consistently, the mRNA and protein expression levels of IL17A were significantly upregulated in the lesions and serum of EAD patients compared to the IAD and HS groups (**Figure 2F**). In addition, the correlation analysis revealed that skin IL17A levels were significantly and positively related to the total IgE level and AD severity (SCORAD) (**Figure 2G**). However, although the mRNA expression level of PPARG was increased in the IAD-L group compared to the HS group, its

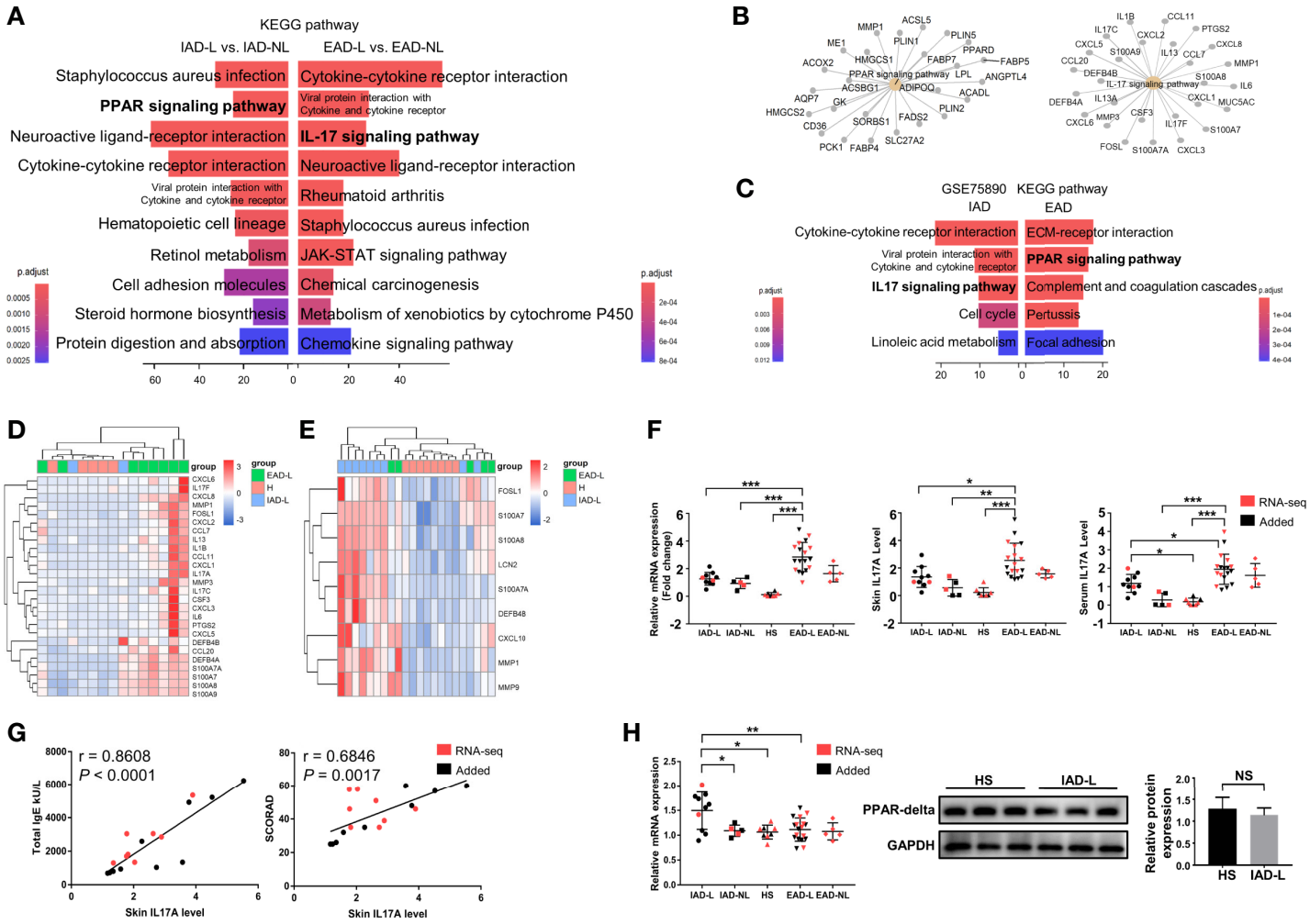

FIGURE 2 | KEGG pathway enrichment analysis of DEGs in intrinsic and extrinsic AD. **(A)** Top 10 enriched KEGG pathways of the DEGs involved in Chinese IAD and EAD patients, $P < 0.05$. **(B)** The interaction networks of IAD- and EAD-specific pathways and the DEGs involved. **(C)** Top 5 enriched KEGG pathways of the DEGs involved in Denmark IAD and EAD patients, $P < 0.05$. **(D)** Sample cluster analyses based on the expression pattern of DEGs involved in the IL-17 signaling pathway in the Chinese Han population (HS group (n = 5), IAD-L group (n = 2), EAD-L group (n = 8)], and **(E)** in the Denmark population [HS group (n = 8), IAD-L group (n = 9), EAD-L group (n = 5)]. **(F)** The IL17A expression level in the skin and serum in the Chinese Han population [HS group (n = 8), IAD-L group (n = 10), IAD-NL group (n = 5), EAD-L group (n = 18), EAD-NL group (n = 5)]. **(G)** The correlation of skin IL17A levels with total IgE and SCORAD (n = 18). **(H)** The mRNA [HS group (n = 8), IAD-L group (n = 10), IAD-NL group (n = 5), EAD-L group (n = 18), EAD-NL group (n = 5)], protein expression level and quantitative analysis [HS group (n = 3), IAD-NL group (n = 3)] of PPARG. NS, no significance, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ according to Student's *t*-test analysis or one-way ANOVA followed by Tukey's multiple comparisons.

protein expression level showed no significant difference between the two groups (**Figure 2H**).

Mild Immune Activation in Chinese IAD Patients

We further performed differential expression analyses and enrichment analyses of lesional (IAD-L vs. EAD-L) and nonlesional (IAD-NL vs. EAD-NL) skin samples. Similar to the above enrichment results, remarkable changes in cytokine-cytokine receptor interaction and *Staphylococcus aureus* infection were also identified in both groups (**Supplementary Figure S3**). As shown in **Figure 3A**, heatmaps and cluster analyses demonstrated that the expression level of DEGs in these two pathways was significantly upregulated in EAD, whereas the DEG expression pattern of IAD was more similar to those of healthy controls, indicating that immune activation in IAD was mild. To confirm these results, qRT-PCR was performed to investigate the mRNA levels of several cytokines. The results revealed that the expression levels of IL2 and IL9 were not obviously changed in IAD but were significantly increased in EAD. Elevated expression of IL4R, IL13, and IL22 was detected in both EAD and IAD (**Figures 3B, C**).

Elevated IL17A and IL4R in Lesions Were Associated With Rhinitis Severity

To understand the specific changes that occurred in AD patients with AR, differential expression analyses were performed among lesional skin samples from AD patients with AR (LR) or without AR (LNR) and from healthy volunteers without rhinitis (HNR). A total of 5980 DEGs were detected in the LR vs. HNR group, 4888 DEGs in the LR vs. LNR group, and 4233 DEGs in the LNR vs. HNR group (**Supplementary Figure S4**). AM-associated genes were set by combining the DEGs from the LR vs. LNR group and LR vs. HNR group and then removing the DEGs from the LNR vs. HNR group using a Venn diagram (**Figure 4A**). A total of 1932 AM-associated genes were obtained. KEGG analysis was employed to explore the potential biological function of these genes. As shown in **Figure 4B**, cytokine-cytokine receptor interaction was identified as the most significant pathway.

As shown in **Figure 4C**, 53 genes were enriched in cytokine-cytokine receptor interactions. The expression levels of those genes were further detected in the microarray of AM. As shown in **Figure 4D**, the expression levels of CCL1, CCL20, CCL22, EDAR, IL1B, IL34, and IL4R in the AM group were significantly different from those in the HS group. Among them, the mRNA and protein levels of IL4R and IL17A were significantly increased in the skin of the LR group compared to those of the HNR and LNR groups (**Figures 4E, F**). In addition, the ELISA results found that serum IL17A and IL4R were upregulated in the LR group compared to the HNR and LNR groups (**Figure 4G**). Subsequently, correlation analysis revealed that the mRNA expression levels of IL17A and IL4R

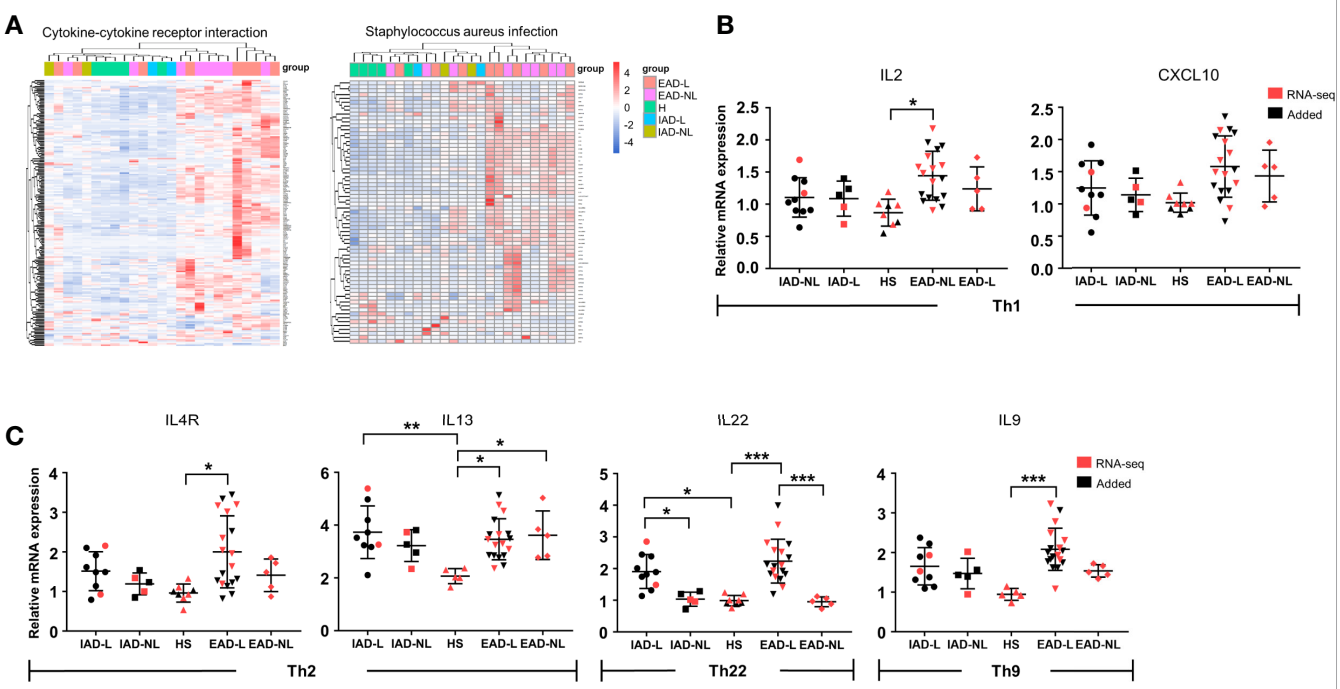

FIGURE 3 | Cytokine levels in Chinese intrinsic and extrinsic AD patients. **(A)** Sample cluster analyses based on the expression pattern of DEGs involved in cytokine-cytokine receptor interactions and *Staphylococcus aureus* infection [HS group (n = 5), IAD-L group (n = 2), IAD-NL group (n = 2), EAD-L group (n = 8), EAD-NL group (n = 8)]. **(B, C)** The mRNA expression levels of cytokines involved in Th1, Th2, Th22, and Th9 cells in the skin [HS group (n = 8), IAD-L group (n = 10), IAD-NL group (n = 5), EAD-L group (n = 18), EAD-NL group (n = 5)]. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ according to one-way ANOVA followed by Tukey's multiple comparisons.

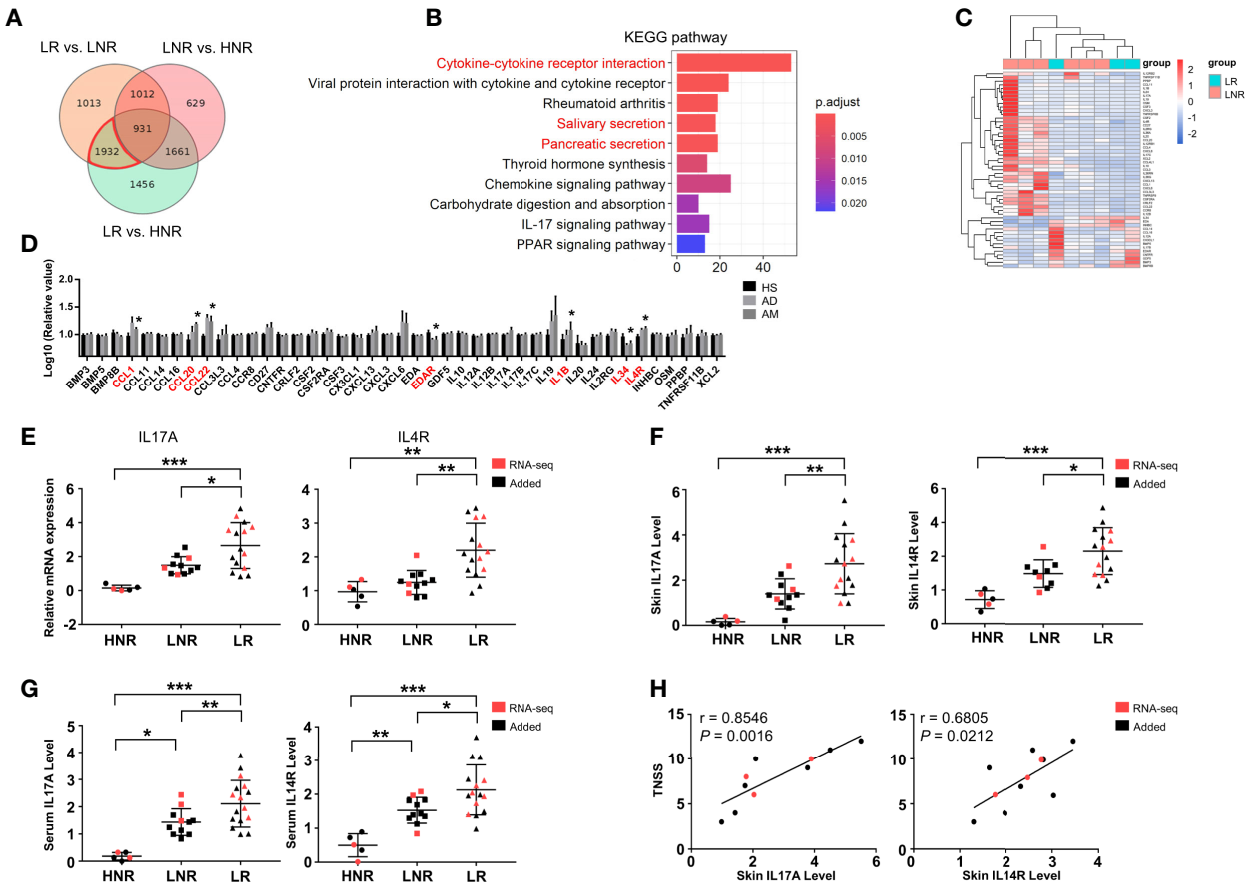

FIGURE 4 | Aberrant cytokine identification in lesions of AD patients with atopic march. **(A)** Vann diagram showing the number of AM-associated genes in the red circle. **(B)** Top 10 enriched KEGG pathways, $P < 0.05$. **(C)** Sample cluster analyses based on the expression pattern of DEGs involved in cytokine-cytokine receptor interactions [LNR group (n = 3), LR group (n = 6)]. **(D)** The expression levels of DEGs enriched in cytokine-cytokine receptor interactions in the microarray of AM [HS group (n = 3), AD group (n = 4), AM group (n = 3)]. **(E)** The mRNA expression levels of IL17A and IL4R in the skin. **(F)** The protein expression levels of IL17A and IL4R in the skin and **(G)** serum [HNR group (n = 5), LNR group (n = 11), LR group (n = 15)]. **(H)** The correlation of skin IL17A and IL4R with TNSS (n = 11). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ according to one-way ANOVA followed by Tukey's or Dunnett's multiple comparisons.

in LR lesions were significantly and positively related to TNSS, and the IL17A expression level showed a closer and more significant association with TNSS than the IL4R expression level did (**Figure 4H**).

Decreased Expression of Genes Involved in Gland Secretion Contributes to Atopic March

Interestingly, significant changes in salivary secretion and pancreatic secretion were also identified by KEGG analysis (**Figure 4B**), in which a total of 24 genes were involved. These genes were also considered AM-associated genes (**Figure 5A**). Interestingly, we supposed that the expression pattern of those genes could distinguish LR from LNR (**Figure 5B**) via cluster analysis. Among the 24 genes, five genes displayed remarkably aberrant expression in the AM group compared to the HS group (**Figure 5C**).

The qRT-PCR and Western blotting results confirmed that AQP5 and CFTR were significantly downregulated in the LR group compared to those in the HNR and LNR groups (**Figures 5D, E**). Correlation analysis illustrated that the CFTR mRNA expression level was significantly and negatively associated with skin IL17A level and TNSS, while no remarkable relationship was found between AQP5 and skin IL17A level and TNSS (**Figures 5F, G**).

DISCUSSION

AD affects up to 20% of children and 2-3% of adults worldwide, in which the Chinese AD patient population may be the largest ethnic AD population (~9% in adults) (14, 15). The heterogeneity of Chinese AD patients remains poorly understood. Compared with previous studies on Chinese patients (6, 16), our study conducted more precise

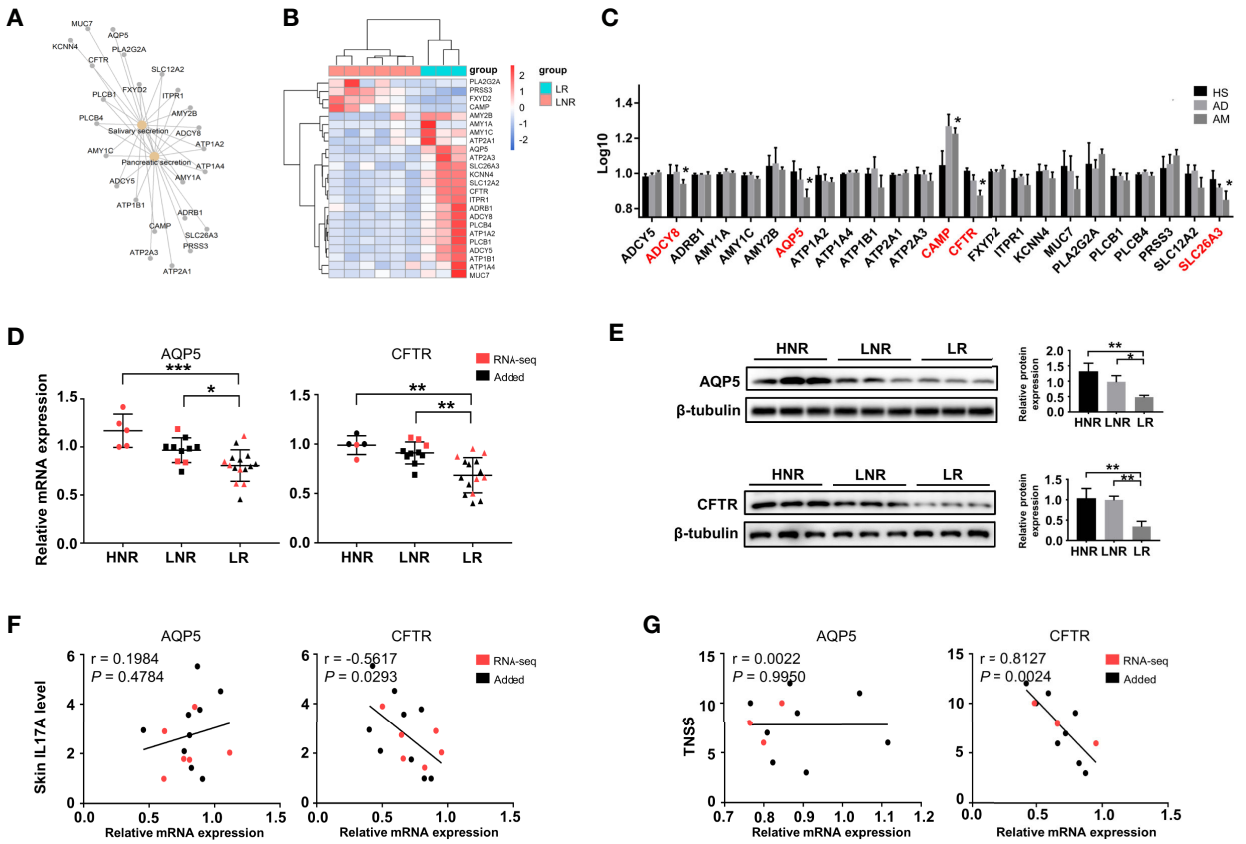

FIGURE 5 | Altered expression of genes involved in gland secretion identification in lesions of AD patients with atopic march. **(A)** The interaction network of salivary and pancreatic secretion and the DEGs involved. **(B)** Sample cluster analysis based on the expression pattern of the genes enriched in salivary and pancreatic secretion [LNR group (n = 3), LR group (n = 6)]. **(C)** The expression level of DEGs enriched in gland secretion in the microarray of AM [HS group (n = 3), AD group (n = 4), AM group (n = 3]. **(D)** The mRNA expression levels of AQP5 and CFTR in the skin [HNR group (n = 5), LNR group (n = 11), LR group (n = 15)]. **(E)** The protein expression levels and quantitative analysis of AQP5 and CFTR in the skin [HNR group (n = 3), LNR group (n = 3), LR group (n = 3)]. **(F)** The correlation of AQP5 and CFTR with skin IL17A levels (n = 15). **(G)** The correlation of AQP5 and CFTR with TNSS (n = 11). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ according to one-way ANOVA followed by Tukey's or Dunnett's multiple comparisons.

stratifications based on phenotypes and showed marked Th17 activation in EAD rather than IAD. Moreover, the expression of *IL17A*, *IL4R*, *AQP5*, and *CFTR* was associated with AM in the Chinese Han population by functional analyses of the transcriptome and further experiments.

It is well established that AD is characterized by Th2 bias, and elevated Th17 level distinguish Asian AD patients from European AD patients (1, 6). However, increased IL17 signaling pathway also was found in European IAD patients (17). Our results further demonstrated that the enhanced IL17 signaling pathway was significant in Chinese EAD patients rather than in IAD patients, indicating that not all Chinese AD patient had high IL17 level. Furthermore, correlation analysis revealed that IL17A was positively associated with IgE level and AD severity in EAD. IgE is produced by B cells in the Th2-biased cytokine microenvironment and represents a good cumulative readout of systemic Th2 bias (18). However, recent studies reported that B cells could also express IL17RA, and IL17 plays an important role in Th2 differentiation and IgE response (19). In line with these notions, IL17A might function as an enhancer of Th2 cytokine and serum IgE production in Chinese EAD patients (20).

Besides, we analyzed the genes related to the AM in the Chinese Han population. The results showed that patients with AM exhibited higher IL17A and IL4R levels both in the skin and serum with a significantly positive correlation with AR severity (TNSS), indicating that the Th2/Th17 axis might contribute to AM. Interestingly, *IL17A* showed a more significant association with TNSS than *IL4R* in Han Chinese patients and has been considered a risk allele gene related to AM by GWAS (21). A recent study revealed that IL17A could induce nasal fibroblasts to produce thymic stromal lymphopoietin (TSLP), which promotes Th2 inflammation in AR (22). In addition, IL17A could regulate DC migration to the peribronchial lymph nodes and allergen presentation in experimental allergic asthma (23). These findings implied a close relationship between IL17A and AR in the Th2-biased cytokine microenvironment. Given that Asian patients show higher IL17 than other races, whether IL17A displays a

closer correlation with AR than IL4R in other races needs further investigation.

In addition to abnormal cytokine-cytokine receptor interactions, our results revealed a remarkable transcriptomic alteration in gland secretion in lesional skin from AD patients with AR compared to those without AR. Indeed, dysfunction of the sweat gland was reported in some AD patients (24). This leads the skin with impaired barrier function to be more sensitive to antigens, and sweat containing inflammatory cytokines could initiate inflammation at points of allergen entry (24). In addition, *AQP5* and *CFTR* were identified as AM-associated genes in our study. AQP5 is an essential factor that can modulate the fluid content of the upper airway and nasopharyngeal secretions (Skowron-zwarg et al., 2007). In the skin, AQP5 is mainly located in the luminal and basolateral surfaces of eccrine secretory cells in sweat glands (25). Although it was significantly decreased in the lesions of AD patients with AR, its expression level was barely related to serum IL17A or rhinitis severity. However, the downregulated CFTR in the lesions of AD patients with AR was negatively correlated with serum IL17A and TNSS. As an apical membrane anion channel, CFTR regulates fluid homeostasis in both the airway and sweat glands (26). It was reported that chemokines induced by IL17 were enhanced in the absence of CFTR activity in human airway epithelial cells (27). Thus, we speculated that a low level of CFTR in the lesion might amplify the IL17A-induced response contributing to AM.

Recently, it has been proposed that AD and psoriasis could be considered diseases occurring across a spectrum, with certain subtypes having overlapping characteristics (28). Anti-IL17 treatment seems to be promising for atopic patients (1, 29). However, some psoriasis patients present eczematous reactions with atopic dermatitis-like features after receiving IL17 antibody treatment (30). This implied that inhibition of Th17 alone might tilt the balance toward the Th2 axis. Therefore, although our study highlighted the role of IL17A in EAD and AM, anti-Th17 therapy for AD should be made cautiously.

There were some limitations in our study. The sample size was small and obtained from only two regions of China. Given that the source of human samples is influenced by many factors, such as region, environment, climate, diet, and allergen, a larger-scale study with an extended sample size needs to be conducted to represent the average characteristics of AD among the Chinese Han population. In addition, the sample from other races needs to be studied to describe national heterogeneity.

In conclusion, our research characterized the contribution of IL17A in EAD and AM in the Chinese Han population. Furthermore, abnormal expression of genes involved in gland secretion in lesions might contribute to the development of AM. This finding partially explained the phenotypic differences of AD subtypes and provided potential references for phenotype-specific therapies.

DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the NCBI Bioproject repository, accession number Bioproject ID: PRJNA691738.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by ethics committee of Guangzhou Institute of Dermatology (201803). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

YL, ZW, and XZ: study design. XT and YL: data collection. BL, LC, YX, JW, and JL: analysis of data. YY and LS: interpretation of data. ZW, BL, and JZ: drafting the article. All authors contributed to the article and approved the submitted version.

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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.2021.628512/full#supplementary-material

REFERENCES

1. Czarnowicki T, He H, Krueger JG, Guttman-Yassky E. Atopic dermatitis endotypes and implications for targeted therapeutics. *J Allergy Clin Immunol* (2019) 143(1):1–11. doi: 10.1016/j.jaci.2018.10.032
2. Nomura T, Honda T, Kabashima K. Multipolarity of cytokine axes in the pathogenesis of atopic dermatitis in terms of age, race, species, disease stage and biomarkers. *Int Immunol* (2018) 30(9):419–28. doi: 10.1093/intimm/dxy015
3. Han H, Roan F, Ziegler SF. The atopic march: current insights into skin barrier dysfunction and epithelial cell-derived cytokines. *Immunol Rev* (2017) 278(1):116–30. doi: 10.1111/imr.12546
4. Hirota T, Takahashi A, Kubo M, Tsunoda T, Tomita K, Sakashita M, et al. Genome-wide association study identifies eight new susceptibility loci for

atopic dermatitis in the Japanese population. *Nat Genet* (2012) 44(11):1222–6. doi: 10.1038/ng.2438
5. Paternoster L, Standl M, Waage J, Baurecht H, Hotze M, Strachan DP, et al. Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis. *Nat Genet* (2015) 47 (12):1449–56. doi: 10.1038/ng.3424
6. Chan TC, Sanyal RD, Pavel AB, Glickman J, Zheng X, Xu H, et al. Atopic dermatitis in Chinese patients shows TH2/TH17 skewing with psoriasiform features. *J Allergy Clin Immunol* (2018) 142(3):1013–7. doi: 10.1016/j.jaci.2018.06.016
7. Tokura Y. Extrinsic and intrinsic types of atopic dermatitis. *J Dermatol Sci* (2010) 58(1):1–7. doi: 10.1016/j.jdermsci.2010.02.008
8. Ingordo V, D'Andria G, D'Andria C, Tortora A. Results of atopy patch tests with house dust mites in adults with 'intrinsic' and'extrinsic' atopic dermatitis. *J Eur Acad Dermatol Venereol* (2002) 16(5):450–4. doi: 10.1046/j.1468-3083.2002.00525.x
9. Suárez-Fariñas M, Ungar B, Correa da Rosa J, Ewald DA, Rozenblit M, Gonzalez J, et al. RNA sequencing atopic dermatitis transcriptome profiling provides insights into novel disease mechanisms with potential therapeutic implications. *J Allergy Clin Immunol* (2015) 135(5):1218–27. doi: 10.1016/j.jaci.2015.03.003
10. Qian X, Shi S, Zhang G. Long non-coding RNA antisense non-coding RNA in the INK4 locus expression correlates with increased disease risk, severity, and inflammation of allergic rhinitis. *Med (Baltimore)* (2019) 98(20):e15247. doi: 10.1097/MD.0000000000015247
11. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* (2014) 15(12):550. doi: 10.1186/s13059-014-0550-8
12. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *Omics* (2012) 16(5):284–7. doi: 10.1089/omi.2011.0118
13. Zou C, Wang J, Huang X, Jian C, Zou D, Li X. Analysis of transcription factor- and ncRNA-mediated potential pathogenic gene modules in Alzheimer's disease. *Aging (Albany NY)* (2019) 11(16):6109–19. doi: 10.18632/aging.102169
14. Wang X, Shi XD, Li LF, Zhou P, Shen YW, Song QK. Prevalence and clinical features of adult atopic dermatitis in tertiary hospitals of China. *Med (Baltimore)* (2017) 96(11):e6317. doi: 10.1097/md.0000000000006317
15. Yamazaki Y, Nakamura Y, Nunez G. Role of the microbiota in skin immunity and atopic dermatitis. *Allergol Int* (2017) 66(4):539–44. doi: 10.1016/j.alit.2017.08.004
16. Sun LD, Xiao FL, Li Y, Zhou WM, Tang HY, Tang XF, et al. Genome-wide association study identifies two new susceptibility loci for atopic dermatitis in the Chinese Han population. *Nat Genet* (2011) 43(7):690–4. doi: 10.1038/ng.851
17. Martel BC, Litman T, Hald A, Norsgaard H, Lovato P, Dyring-Andersen B, et al. Distinct molecular signatures of mild extrinsic and intrinsic atopic dermatitis. *Exp Dermatol* (2016) 25(6):453–9. doi: 10.1111/exd.12967
18. An HJ, Kim JY, Kim WH, Gwon MG, Gu HM, Jeon MJ, et al. Therapeutic effects of bee venom and its major component, melittin, on atopic dermatitis in vivo and in vitro. *Br J Pharmacol* (2018) 175(23):4310–24. doi: 10.1111/bph.14487
19. Kamijo S, Hara M, Suzuki M, Nakae S, Ogawa H, Okumura K, et al. Innate IL-17A Enhances IL-33-Independent Skin Eosinophilia and IgE Response on Subcutaneous Papain Sensitization. *J Invest Dermatol* (2021) 141(1):105–113 e14. doi: 10.1016/j.jid.2020.05.088
20. Nakajima S, Kitoh A, Egawa G, Natsuaki Y, Nakamizo S, Moniaga CS, et al. IL-17A as an inducer for Th2 immune responses in murine atopic dermatitis models. *J Invest Dermatol* (2014) 134(8):2122–30. doi: 10.1038/jid.2014.51
21. Ferreira MA, Vonk JM, Baurecht H, Marenholz I, Tian C, Hoffman JD, et al. Shared genetic origin of asthma, hay fever and eczema elucidates allergic disease biology. *Nat Genet* (2017) 49(12):1752–7. doi: 10.1038/ng.3985
22. Wang WW, Yu HW, Zhang B, Pan YL, Shao SW. Interleukin-17A up-regulates thymic stromal lymphopoietin production by nasal fibroblasts from patients with allergic rhinitis. *Eur Arch Otorhinolaryngol* (2020) 278(1):127–33. doi: 10.1007/s00405-020-06274-3
23. Jirmo AC, Busse M, Happle C, Skuljec J, Daluge K, Habener A, et al. IL-17 regulates DC migration to the peribronchial LNs and allergen presentation in experimental allergic asthma. *Eur J Immunol* (2020) 50(7):1019–33. doi: 10.1002/eji.201948409
24. Murota H, Yamaga K, Ono E, Katayama I. Sweat in the pathogenesis of atopic dermatitis. *Allergol Int* (2018) 67(4):455–9. doi: 10.1016/j.alit.2018.06.003
25. Iizuka T, Suzuki T, Nakano K, Sueki H. Immunolocalization of aquaporin-5 in normal human skin and hypohidrotic skin diseases. *J Dermatol* (2012) 39 (4):344–9. doi: 10.1111/j.1346-8138.2011.01327.x
26. Watson MJ, Lee SL, Marklew AJ, Gilmore RC, Gentzsch M, Sassano MF, et al. The Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Uses its C-Terminus to Regulate the A2B Adenosine Receptor. *Sci Rep* (2016) 6:27390. doi: 10.1038/srep27390
27. Mizunoe S, Shuto T, Suzuki S, Matsumoto C, Watanabe K, Ueno-Shuto K, et al. Synergism between interleukin (IL)-17 and Toll-like receptor 2 and 4 signals to induce IL-8 expression in cystic fibrosis airway epithelial cells. *J Pharmacol Sci* (2012) 118(4):512–20. doi: 10.1254/jphs.11240fp
28. Guttman-Yassky E, Krueger JG. Atopic dermatitis and psoriasis: two different immune diseases or one spectrum? *Curr Opin Immunol* (2017) 48:68–73. doi: 10.1016/j.coi.2017.08.008
29. Galili E, Barzilai A, Twig G, Caspi T, Daniely D, Shreberk-Hassidim R, et al. Allergic Rhinitis and Asthma Among Adolescents with Psoriasis: A Population-based Cross-sectional Study. *Acta Derm Venereol* (2020) 100 (10):adv00133. doi: 10.2340/00015555-3485
30. Caldarola G, Pirro F, Di Stefani A, Talamonti M, Galluzzo M, D'Adamio S, et al. Clinical and histopathological characterization of eczematous eruptions occurring in course of anti IL-17 treatment: a case series and review of the literature. *Expert Opin Biol Ther* (2020) 20(6):665–72. doi: 10.1080/14712598.2020.1727439

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Hyperforin Ameliorates Imiquimod-Induced Psoriasis-Like Murine Skin Inflammation by Modulating IL-17A–Producing γδ T Cells

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Hyperforin is a major active constituent of *Hypericum perforatum* L. extract, which is widely used for the treatment of depressive disorders. Recent studies have reported that hyperforin reduced inflammation in stroke and suppressed proliferation and differentiation in keratinocytes. Psoriasis is a chronic immune-mediated inflammatory skin disease in which the IL-23/IL-17 axis plays an important role. To investigate the underlying inflammatory mechanisms and response of hyperforin in psoriasis, we use imiquimod (IMQ)-induced mice model, *in vitro* cultured murine splenic γδ T cells, and HaCaT cells in this study. Data showed that hyperforin reduced epidermal thickness and decreased IMQ-induced pathological scores of cutaneous skin lesions in mice. Meanwhile we proved that hyperforin suppressed infiltration of $CD3^+$ T cells and downregulated expression of *Il1*, *Il6*, *Il23*, *Il17a*, *Il22*, antimicrobial peptides (AMPs) in the skin lesion. Hyperforin significantly inhibited imiquimod-induced splenomegaly, reduced serum levels of TNF-α and IL-6, and IL-17A in splenocytes and draining lymph nodes. Our study also suggested that hyperforin lessened the infiltration of γδ T cell and $CCR6^+$ γδ T cells in spleen and lymph nodes. Hyperforin also suppressed the typical psoriasis-like inflammatory responses and the infiltration of IL-$17A^+$ cells in dermal γδ T cells of IMQ treated *Tcrd*$^{-/-}$ mice transferred with γδ T cells. *In vitro* studies, hyperforin reduced the expression and secretion of IL-17A in γδ T cells, and suppressed the activation of MAPK/STAT3 pathways in human keratinocyte HaCaT cells and γδ T cells. In conclusion, hyperforin alleviates IMQ-induced inflammation in psoriasis through suppressing the immune responses exerted by IL-17 A-producing γδ T cells and related cytokines by modulating MAPK/STAT3 pathways. Our study provided a novel therapeutic tragedy for psoriasis by which hyperforin attenuates psoriasis-related inflammatory responses.

Keywords: IL-17A, psoriasis, hyperforin, γδT cells, Stat3

INTRODUCTION

Psoriasis is a chronic immune-mediated inflammatory skin disease, which is associated with high prevalence, disfigurement, and comorbid diseases (1). IL-23/IL-17 cytokine axis has been repeatedly confirmed to play the key role in the pathogenesis of human psoriasis (1). Novel biologics, such as Ustekinumab and Secukinumab, have showed good curative effects in moderate-to-severe plaque psoriasis (2). T helper 17 cells (Th17), a subtype of $CD4^+$ T cells producing IL-17, were reported to play an important role in psoriasis (3, 4). However, recent studies revealed that other innate immune cells, such as IL-17–producing γδ T cells, also involved in the pathogenesis of psoriasis (5, 6). As a commonly studied transcription factor, STAT3 has recently performed to be crucial in psoriatic-like inflammatory conditions (7). This factor also emerges great influence on the pathogenesis of psoriasis through regulating cytokines including the main IL-23/IL-17 axis (8).

Hyperforin is a major active constituent of *Hypericum perforatum* L. extract, which has antidepressant, bactericidal, anti-inflammatory, antioxidant, and other effects (9–13). Clinical studies have supported the topical use of St. John's wort as an effective treatment for psoriasis (14, 15), but the specific mechanisms are still not completely explored. Previous study has indicated the effects of hyperforin on keratinocytes. Margarethe Muller had reported that hyperforin modulates the differentiation and proliferation of HaCaT cells and primary cultures of human keratinocytes *via* TRPC6 channels by inducing Ca^{2+} influx (16), which may be partially involved in the pathogenesis of psoriasis. Studies had reported the anti-inflammatory effects of hyperforin in pancreatic β cells, microglia, vascular endothelial cells, and neuronal cells (17–19). However, the specific mechanism of hyperforin on inflammatory response mediated by active immune cells and cytokines in psoriasis has not been perfectly proved.

In this study, we used the imiquimod (IMQ)-induced murine psoriatic models, *in vitro* cultured γδ T cells and HaCaT cells to find out the effects of hyperforin on (1): the change of inflammatory cytokines expression and inflammatory cells infiltration in the skin lesion (2); systemic inflammation of spleen and lymph nodes (3); the quantity of γδ T cells in the spleen and lymph nodes (4); the activation of MAPK and STAT3 pathway in *in vitro* cultured γδ T cells and HaCaT cells. Our study will support the novel therapeutic potential of hyperforin in alleviating psoriasis.

MATERIALS AND METHODS

Reagents and Antibodies

Hyperforin (dicyclohexylammonium salt, Product Code: 19572, Cayman Chemical Company, Ann Arbor, Michigan) were dissolved in dimethyl sulfoxide (DMSO, D8414, Sigma Chemical Co., St. Louis, MO, USA) to make the stock solutions (c = 1.4 mmol/L). Before addition to the cell culture or injection to the mice, working solutions were freshly prepared through diluted. The final concentration of DMSO was less than 0.1% and the cytokinetic parameters were not affected. Chemicals including MTX (CAS: 133073-73-1), LPS (L2880), TNF-α (CAS: 94948-59-1) were obtained from Sigma Chemical Co., St. Louis, MO, USA. Soluble γδTCR antibody (107502), anti-CD28 (102101), and IFN-γ (517904) were obtained from BioLegend. IL-2 (212–12), IL-1β (211-11B), and IL-23 (200–23) were purchased from PeproTech. CD4+ sorting magnetic beads were obtained from Miltenyi Biotec. PCR primers were purchased from Takara Biotechnology, Dalian, China. Anti-human antibodies including STAT3 (ab119352), p-STAT3 (705) (ab76315), ERK (ab32537), p-ERK (ab79483), JNK (ab213521), p-JNK (ab131499), p38 (ab31828), p-p38 (ab178867) were purchased from Abcam Company. Anti-mouse antibodies including STAT3 (AF6294), p-STAT3 (705) (AF3293), ERK (AF0115), p-ERK (AF1015), JNK (AF6318), p-JNK (AF3318), p38 (AF6456), p-p38 (AF4001) were purchased from Affinity Company.

Animal Use

BALB/c and C57BL/6J mice used in this experiment were purchased from Beijing HFK Bioscience Co., Ltd. *Tcrd*$^{-/-}$ mice on a C57BL/6J background were kindly donated by Jing Luo. They were then bred in the animal facility under specific pathogen-free conditions for more than a week before the experiment. All mice were age- and weight-matched when used in experiments. Animal experiments were performed in the USUHS laboratory animal facility. The protocol used in these experiments was approved by the USUHS Institutional Animal Care and Use Committee.

IMQ-Induced Psoriatic-Like Mouse Model

Remove the dorsal hair of mice (8–10 weeks of age) at a surface area of about 4 to 5 cm^2 as described in our previous publication (13), 5% imiquimod (IMQ) cream (Mingxin Lidi Laboratory, China) was applied daily at a topical dose of 62.5 mg for 7 days to establish IMQ-induced psoriasis mouse model (20).

Groups of Mice Model

BALB/c mice were randomly divided into the following groups: control group; IMQ treated groups (topical dose with 62.5 mg of 5% IMQ cream alone); MTX treated positive control group (1.0 mg/kg/week, intraperitoneal injection); Hyperforin group (5 mg/kg/day, intraperitoneal injection), as used by others (10). MTX was dissolved in the saline while Hyperforin was dissolved in DMSO. Each group have five mice, and seven consecutive days of administration would be necessary for all mice. γδ T cells (2×10^6) pretreated or not with hyperforin (suspended in 200 μl sterile PBS) were administered into *Tcrd*$^{-/-}$ mice *via* tail vein injection. Control *Tcrd*$^{-/-}$ mice were injected with 200 μl PBS. Five days later, for the mouse model, the reconstituted mice were subjected to induction of psoriasis-like disease. All mice were sacrificed, and samples were collected for analysis on day 8.

Measurement of Skin Inflammation Severity

The Psoriasis Area and Severity Index (PASI) consists of measurements of skin erythema, scale, and thickness. In our previous study (20), we have used this measurement to assess the

severity of skin lesions. The specific criteria of PASI scores have been described in detail in our previous studies (20). Mice were evaluated since the first day that IMQ was administrated for seven consecutive days. The thickness of the mouse skin was measured using a micrometer, and the average value was measured three times a day. To measure cellular accumulation and epidermal thickness, surgical specimens of dorsal skin tissues were paraffin-embedded for H&E staining. Paraffin-embedded tissues sections (4 μm) were stained with hematoxylin (Beyotime, China) for 40 s and with eosin (Beyotime, China) for 30 s. The tissue sections were examined under an OLYMPUS light microscope. The thickness of mouse epidermis was measured using Photoshop software in three separated fields of view.

Immunohistochemistry

Skin tissues were fixed with 4% paraformaldehyde for 48 h and embedded with paraffin. Then samples were cut into 4-μm-thick slides. For immunohistochemistry, sections were sequentially incubated with the primary antibody anti-CD3 (1:100, Abcam) and the secondary antibody HRP anti-rabbit IgG (Maxim), and then the color was developed with diaminobenzidine. Using Image J software to measure the region where $CD3^+$ cell accumulate for quantitative analyses and expressed as the percentage of positive cells.

Cytokine Detection by Enzyme-Linked Immunosorbent Assay (ELISA)

Collect the dorsal skin of the control or experimental mouse, remove the attached connective tissue, then put a $0.1cm^3$ tissue into 1ml of saline. Carefully cut the tissue, and grind it thoroughly in a homogenizer to obtain the tissue suspension. The prepared 10% homogenate was centrifuged at 4 degrees at 3,000 rpm for 15 min, and the supernatant was taken for ELISA detection. The level of IL-17A in the skin of control or experimental groups and in the supernatant of *in vitro* cultured γδ T cells were measured by commercial ELISA kits following the manufacturer's instructions (MultiSciences, China).

Reverse Transcription and Real-time PCR Analysis

Collect the dorsal skin of the control or experimental mouse, remove the attached connective tissue, then put a $0.1\text{-}cm^3$ tissue into 1 ml of saline. Carefully cut the tissue, and grind it thoroughly in a homogenizer to obtain the tissue suspension. The prepared 10% homogenate was centrifuged at 4 degrees at 3,000 rpm for 15 min, and the precipitate was taken for RT-PCR detection. RT-PCR was performed according to the manufacturer's instructions. Briefly, total RNA was isolated using TRIzol (Invitrogen, Australia Pty. Ltd.), and cDNA was synthesized using RevertAid First Strand cDNA Synthesis Kit (K1622, Thermo Scientific). Quantitative real-time PCR was performed using the SYBR Green kit (Takara Biotechnology) on a real-time PCR system (StepOnePlus™ Real-Time PCR System, Thermo Scientific). The reaction was performed with a denaturation step at 95°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s for 45 cycles. The relative quantity of the target mRNA was normalized to the level of GAPDH mRNA (the internal control).

Primers used in this experiment showed below: IL-1 mice F: GCAACTGTTCCTG AACTCAACT R: ATCTTTTGGGGTCC GTCCAACT IL-6 mice F: GACAAAGCC AGAGTCCTTCAGA GAGA R: GGTCTTGGTCCTTAGCCACTCCTT IL-10 mice F: CACAAAGCAGCCTTGCAGAA R: AGA GCAGGCAG CATAGCAGTG IL-17A mice F: CCTCAGACTACCTCAACC GTTCC R: AGGCTCC CTCTTCAGGACCAG IL-22 mice F: GGTGACGACCAGAACATCCA R: CAGCAGGTCCAGTT CCCCAAT IL-23p40 mice F: AATGTGCCCCGTATCCAGTG R: GAAGATGTCAGAGTCAAGC A GGTG GAPDH mice F: AACTTTGGCATTGTGGAAGG R: ACACATTGGG GGTA GGAACA CRAMP mice F: AGG AGATCTTGGGAACCA TGCAGTT R: GCAGATCTACTGCTCCGGCTG AGGTA S100A7 F: GCCTCGCTTCATGGA CAC R:CGGAACA GCTCTGTGATGTAGT S100A8 F: TGCGATGGTGATAAAA GTGG R: GGCCAGAAGCTCTGCTACTC S100A9 F: CACAGTTG GCAACCTTT ATG R:CAGCTGATTGTCCT GGTTTG LL37 human F: GCAGTCACCAGAGGAT TGTGAC R: CACCGCTTCACCAGCCC.

Flow Cytometric Assays

The mice were sacrificed by cervical dislocation, spleens, axilliary, inguinal lymph node and skin were separated and placed in pre-cooled PBS. Grind the spleen and lymph node, use a 1 mL syringe core to grind the tissue cell mixture through a 70 μM cell screen, place the prepared spleen cell suspension in 5 ml of pre-cooled PBS, centrifuge at 400*g* for 5 min, and discard the supernatant. Resuspend the spleen cells in 10 ml RBC Lysis Buffer (prepo tech 10× RBC Lysis Buffer diluted 10-fold) and resuspend the lymph node cells in PBS, incubate for 10 min, centrifuge the cells at 500*g* for 5 min, and remove the supernatant. Resuspend the spleen cells and the lymph node cells in 100 μl PBS again, add 0.5 μg (1 μl) gamma delta TCR antibody (Catalog #11-5711-81, Invitrogen) and 1 μg (5 μl) CCR6 antibody (Catalog# 50-7196-80, Invitrogen). For the mice skin, cut off the skin sample and gently eliminate the subcutaneous fat. Chop skin samples into small pieces with 10 ml of Collagenase from clostridium histolyticum Type IV (1 mg/ml, Catalog #9001-12-1, Sigma) and DNase I (100 μg/ml, Catalog #10104159001, Roche). Incubate at 37°C for 90 min. Use a 1-ml syringe core to grind the skin cell mixture through a 70-μM cell screen, place the prepared skin cell suspension in 10 ml of pre-cooled PBS, centrifuge, and resuspend the skin cells in 100 μl PBS. Add 0.5 μg (1 μl) γδ TCR antibody (Catalog #11-5711-81, Invitrogen) and 1 μg (5 μl) CD3 antibody (Catalog #70-AM003E07-100, Multisciences) and incubate for 30 min. For intracellular staining, after incubating FIX&PERM MEDIUM (Catalog #70-GAS005/2, Multisciences), add 0.5 μg (1 μl) IL-17A antibody (Catalog# 70-AM0I1704-100, Multisciences) and 1 μg (5 μl) IFN-γ antibody (Catalog# 70-AM0IF05-100, Multisciences). Cell samples were finally administrated utilizing the flow cytometer (FACSCalibur, BD Biosciences).

Isolation of γδ T Cells and in Experimental Design

The mice were sacrificed by cervical dislocation, spleens were ground into a single cell suspension. Place the prepared spleen cell suspension in Tris-NH4Cl for 5 min, centrifuge at 2,000*g* for

7 min, washed twice with RPMI-1640 medium, and then resuspended in 4 ml of pre-warmed RPMI-1640 medium containing 10% serum. Incubate the single cell suspension to the treated nylon wool column at 37°C for 50 min, the eluted cells are spleen-derived T lymphocytes. The collected T lymphocytes were resuspended in PBS, centrifuged, and then supernatant was discarded. Add 40 μl of pre-chilled PBS sorting buffer containing 0.5% BSA and 2 mM EDTA and 20 μl of $CD4^+$ sorting magnetic beads to 1×10^7 cells. After mixing, incubate at 4°C for 10 min, and separate them with a separator and LS column. The isolated $CD4^+$ T cells were cultured in anti-mouse γδTCR (10 μg/ml) coated Corning plates at 37°C and 5% CO_2 for 6 days, and soluble anti-mouse CD28 antibody (1 μg/ml) and IL-2 (2 ng/ml) were added. The model group was supplemented with IL-1β (5 ng/ml), IL-23 (5 ng/ml) and anti-mouse IFN-γ antibody (5 μg/ml). The hyperforin groups were supplemented with IL-1β (5 ng/ml), IL-23 (5 ng/ml), anti-mouse IFN-γ antibody (5 μg/ml), and hyperforin (0.1, 1, 10 μM).

HaCaT Cells Culture and Experimental Design

Human keratinocyte HaCaT cells were purchased from the China Center for Type Culture Collection and cultured in 1,640 containing 10% heat-inactivated FBS, 100-U/ml penicillin and 100-μg/ml streptomycin. The cells were kept in a cell incubator at 37°C under 5% CO_2 and 95% humidified atmosphere. Cells were incubated with TNF-α (10, 20 ng/ml) for 4 h to induce psoriatic inflammation. The cells were then incubated with hyperforin (0.1, 1, 10 μM) for another 2 h.

Western Blotting

Total cell and nuclear lysates were prepared as protocol using the following antibodies: STAT3, p-STAT3 (705), ERK, p-ERK, JNK, p-JNK, p-38, p-p38 followed by incubation with a horseradish peroxidase-conjugated secondary antibody and visualized using a Bio-Rad ChemiDoc XRS Imaging System with an XRS camera (Bio-Rad, Hercules, CA, U.S.A.).

Statistical Analyses

Statistical comparisons between two groups were performed using a Student's t test. GraphPad Software Prism 6.0 was used for statistical analysis. P values < 0.05 were considered significant.

RESULTS

Hyperforin Ameliorated IMQ-Induced Psoriatic Skin Lesion in Mice

To confirm whether hyperforin ameliorated skin lesion in psoriasis, we utilized the IMQ-induced psoriasis-like mice models with or without hyperforin treatment. **Figure 1A** showed the results of this test. IMQ cream was smeared to the shaved back skin of Balb/c mice for eight consecutive days with or without intraperitoneal injection of hyperforin (5 mg/kg/d) and MTX (1 mg/kg/w). As shown in **Figure 1B**, the IMQ treated mice emerged typical psoriasis-like inflammatory responses on back, such as erythema, scaling and thickening, compared to control mice group. Intraperitoneal injection of hyperforin significantly ameliorated skin lesion throughout the treatment period, demonstrated by the reduced severity score of skin inflammation. Therapeutic effect of hyperforin was comparable with MTX, which is effectively used in psoriasis treatment (**Figure 1B**). Administration of hyperforin notably alleviated the severity of IMQ induced psoriasis compared to the IMQ model group according to the scores of erythema, thickness and cumulative score on day 7 (**Figure 1C**). Furthermore, H&E staining suggested that IMQ induced psoriatic lesions, indicated by the presence of epidermal parakeratosis, thickening of acanthosis cell layer, and downward epidermal extension of in-depth dermis. Meanwhile, administration of hyperforin alleviated the severity of the skin lesion (**Figure 1D**). In general, hyperforin exerted similar therapeutic effect in alleviating psoriatic skin lesion compared with MTX (**Figures 1C, D**).

Hyperforin Inhibited Inflammatory Cell Infiltration and Inflammatory Cytokines Released in Skin of IMQ-Induced Mouse Model

To assess the effect of hyperforin on inflammatory cell accumulation in skin of IMQ-induced mouse model, immunohistochemistry staining of $CD3^+$ T cell were performed. Results showed that IMQ induced the accumulation of T cells in dermis and epidermis compared to the control group. Reduced abundance of $CD3^+$ T cells in the dermis of hyperforin-treated groups was observed even compared to the MTX group (**Figures 2A, B**).

Furthermore, we tested the mRNA expression of a set of psoriasis related inflammatory cytokines in the skin lesions, and suggested that the mRNA levels of *Il1*, *Il6*, *Il23p40*, *Il17a* and *Il22* in lesions of the model group were highly enhanced, while *Ifng* level showed no remarked change compared with the control group. Also, mRNA level of *Il4* in Th2 cells which exert anti-inflammatory effect did not change markedly. Meanwhile, compared to the model group, the mRNA levels of *Il1, Il6, Il23, Il17a* and *Il22* were decreased in the hyperforin group, of which *Il17a* mRNA declined notably (**Figures 2C–I**).

Hyperforin Suppressed Imiquimod-Induced Systemic Inflammation

As shown in **Figure 3A**, imiquimod increased relative spleen weight, and hyperforin significantly inhibited imiquimod-induced splenomegaly (**Figure 3B**). Hyperforin also significantly inhibited serum levels of TNF-α and IL-6 in IMQ model (**Figures 3C, D**). To evaluate the IL-17A mRNA levels in mice spleen and axillary lymph nodes, **Figure 3E** showed that hyperforin inhibited the IL-17A levels in spleen compared to the model group. Meanwhile, the IL-17A mRNA levels in the axillary lymph nodes exhibited no notable change compared with normal mice (**Figure 3F**).

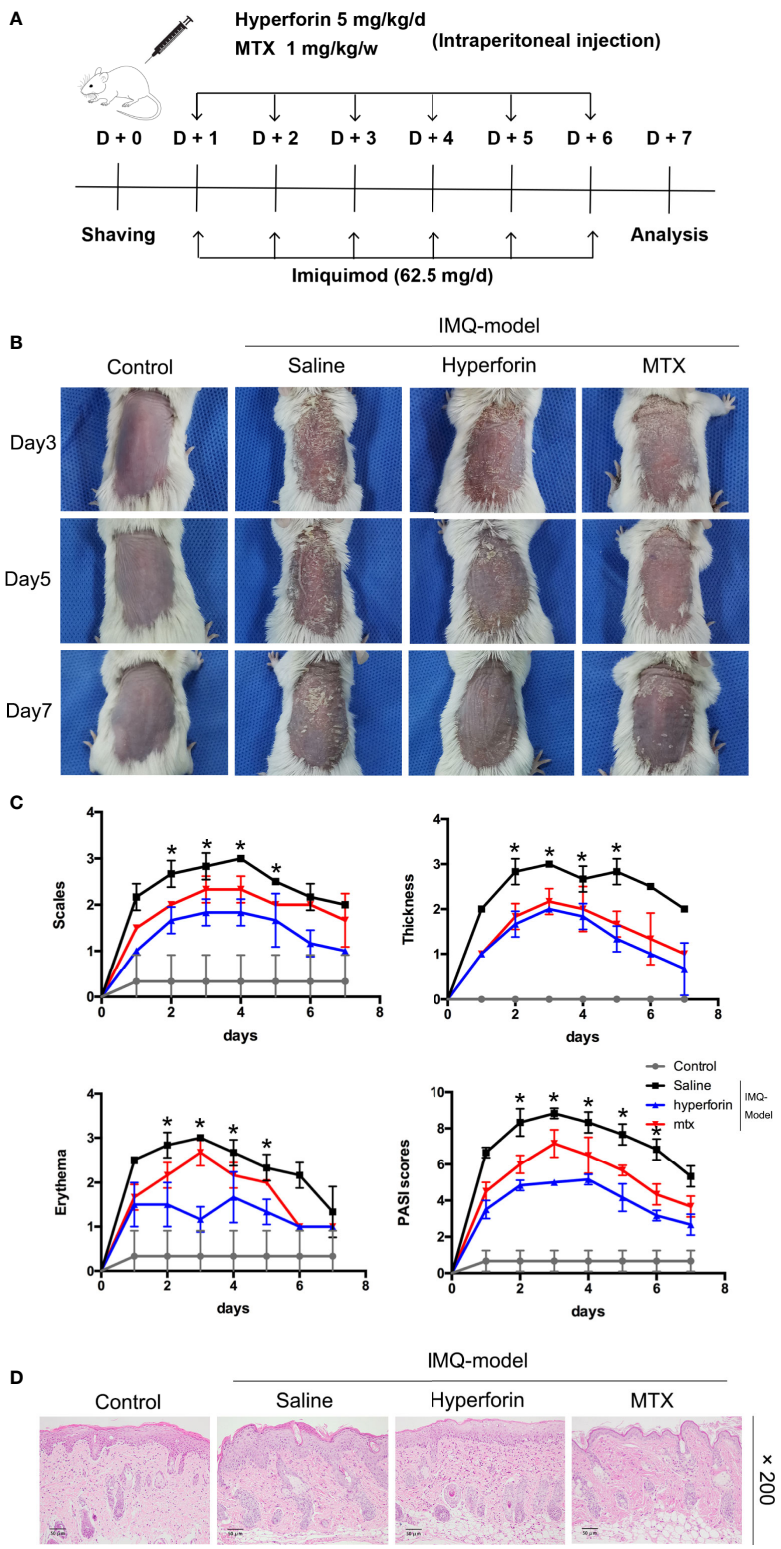

FIGURE 1 | Hyperforin ameliorates psoriatic symptoms and skin inflammation in IMQ-induced psoriatic mice. **(A)** Flow chart of this experiment. **(B)** The back-skin photos of mice were taken at 3th, 5th, 7th day after IMQ painting. **(C)** PASI scores in all groups of mice were evaluated daily and the statistical difference between all groups at 8th day was indicated. **(D)** H&E staining of the dorsal skin with the original magnification of 200×. Data are expressed as mean ± SD (n = 5 mice/group, *$p < 0.05$ *vs*. Model). One representative of three separate experiments is shown while all results were similar among these three experiments.

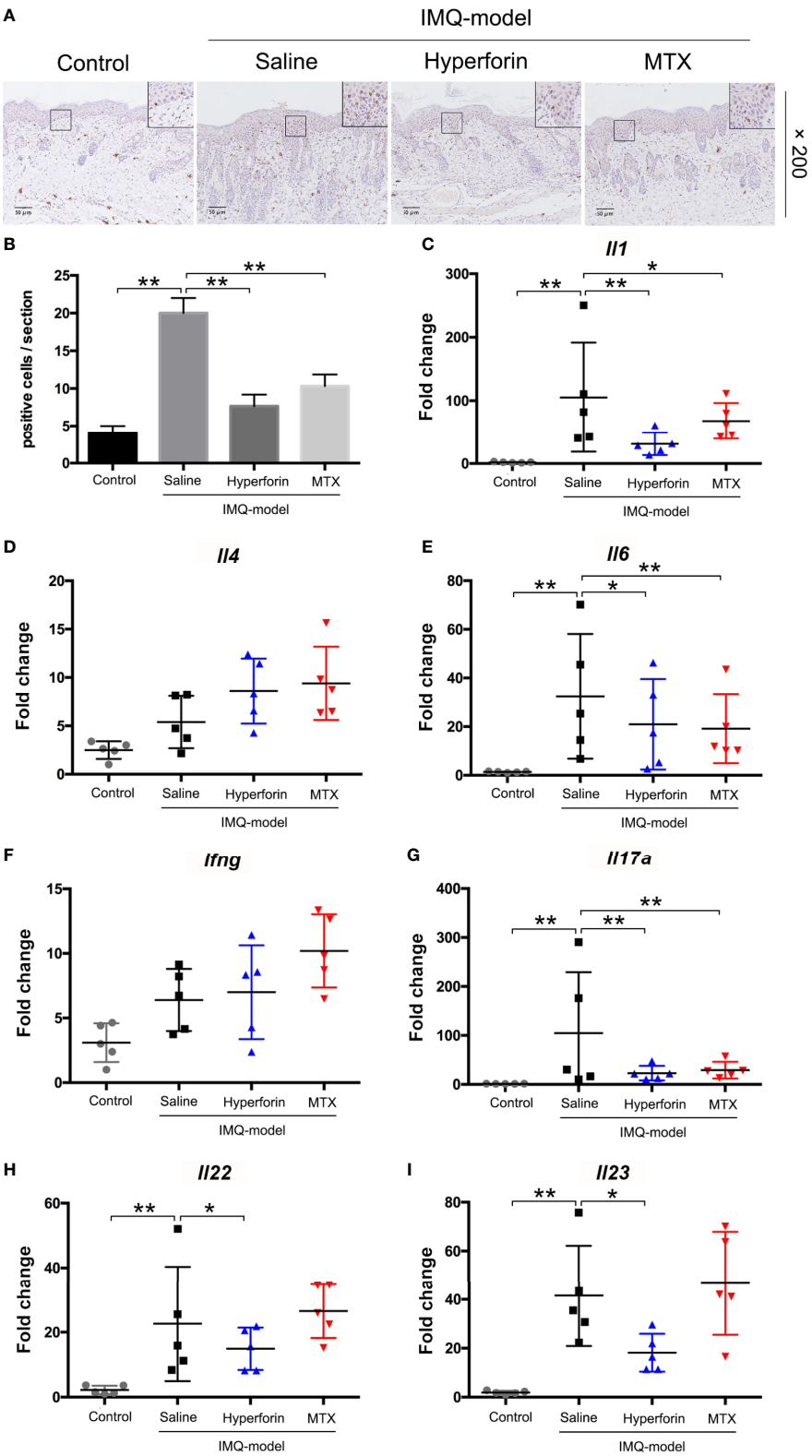

FIGURE 2 | Hyperforin inhibited inflammatory cell infiltration and inflammatory cytokines releasing in skin of IMQ-induced mice model. **(A)** Immunohistochemical staining of $CD3^+$ cells in skin lesion. **(B–I)** The mRNA expression of psoriasis associated inflammatory cytokines in the skin lesion of mice model. Data are shown as mean ± SD(A–E). n=5 mice. *P < 0.05 and **P < 0.01 *vs* Model group.

To further investigate the effect of hyperforin on the infiltration of γδ T cell and CCR6$^+$ γδ T cells (the IL-17 A–producing cells), we tested the percentage of γδ T cells and CCR6$^+$ γδT cells in spleen and axillary lymph node by utilizing flow cytometry. The ratio of γδ T cells was notably higher in the model group compared to the control group, the ratio of CCR6$^+$ γδT cells also accumulated remarkably (**Figures 4A–C**). Cell mass in spleen of the model group shifted toward the right compared to the control group (**Figure 4A**). Lower ratios of γδ T cells and CCR6$^+$ γδT cells were presented in the spleen (**Figures 4A–C**) and lymph nodes (**Figures 4D–F**) of hyperforin-treated mice compared to the model group. In general, both hyperforin and MTX inhibited the infiltration of splenic and lymphatic γδ T cells and CCR6$^+$ γδT cells.

Hyperforin Downregulated the mRNA Expression of Antimicrobial Peptides (AMPs) in skin of IMQ-Induced Mouse Model and TNF-α Stimulated HaCaT Cells

Previous study reported that hyperforin has effects on bactericidal and were often used to treat infection (10). Recently, antimicrobial peptides and proteins (AMPs) such as cathelicidin, β-defensins, and S100 proteins, secreted by keratinocytes are inferred to be related with severity of psoriasis lesions (21), and excessive

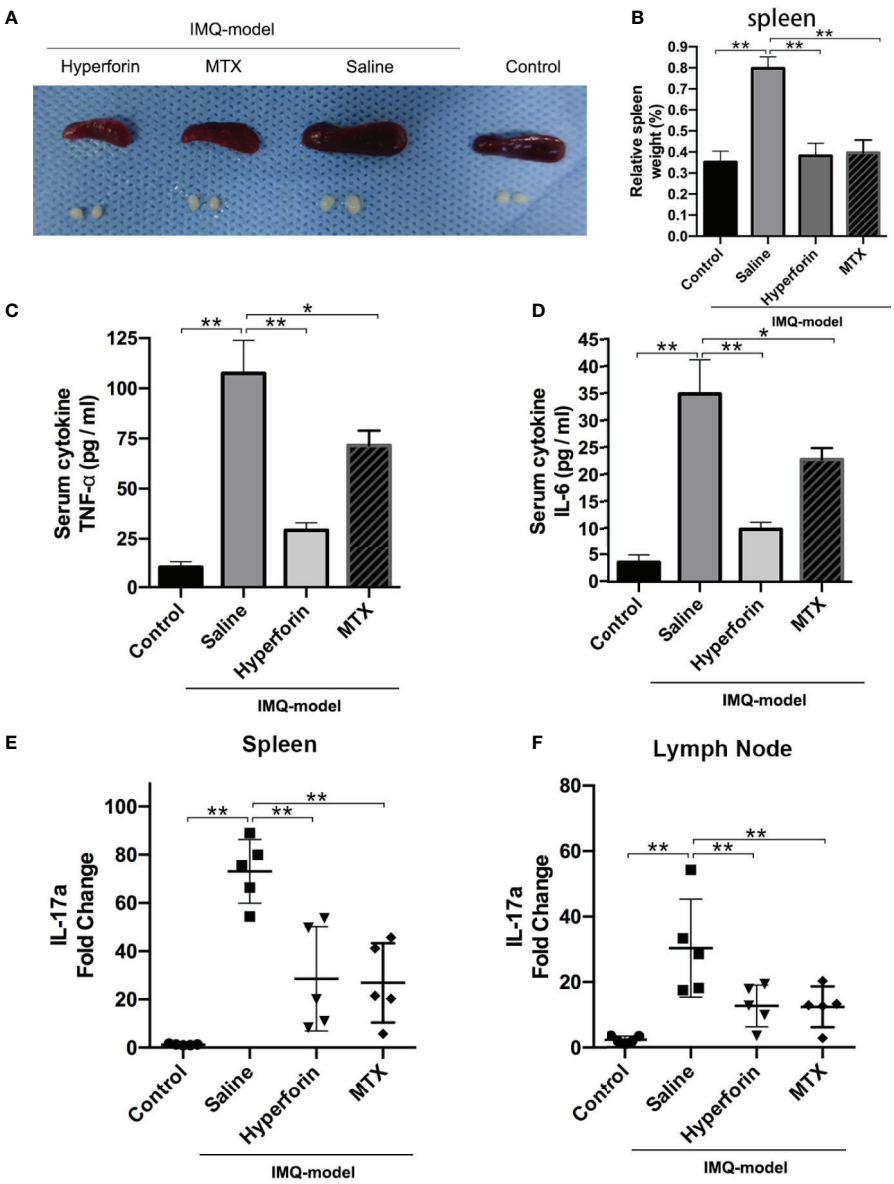

FIGURE 3 | Hyperforin suppressed imiquimod-induced systemic inflammation **(A)** The photos of spleen and skin draining lymph nodes taken at 7th day after IMQ painting. **(B)** Relative spleen weight of the control and test groups. **(C, D)** Serum levels of TNF-α and IL-6. **(E, F)** The mRNA levels of Il17a in spleen and axillary lymph nodes. (n = 5 mice). *P < 0.05 and **P < 0.01 *vs* model group.

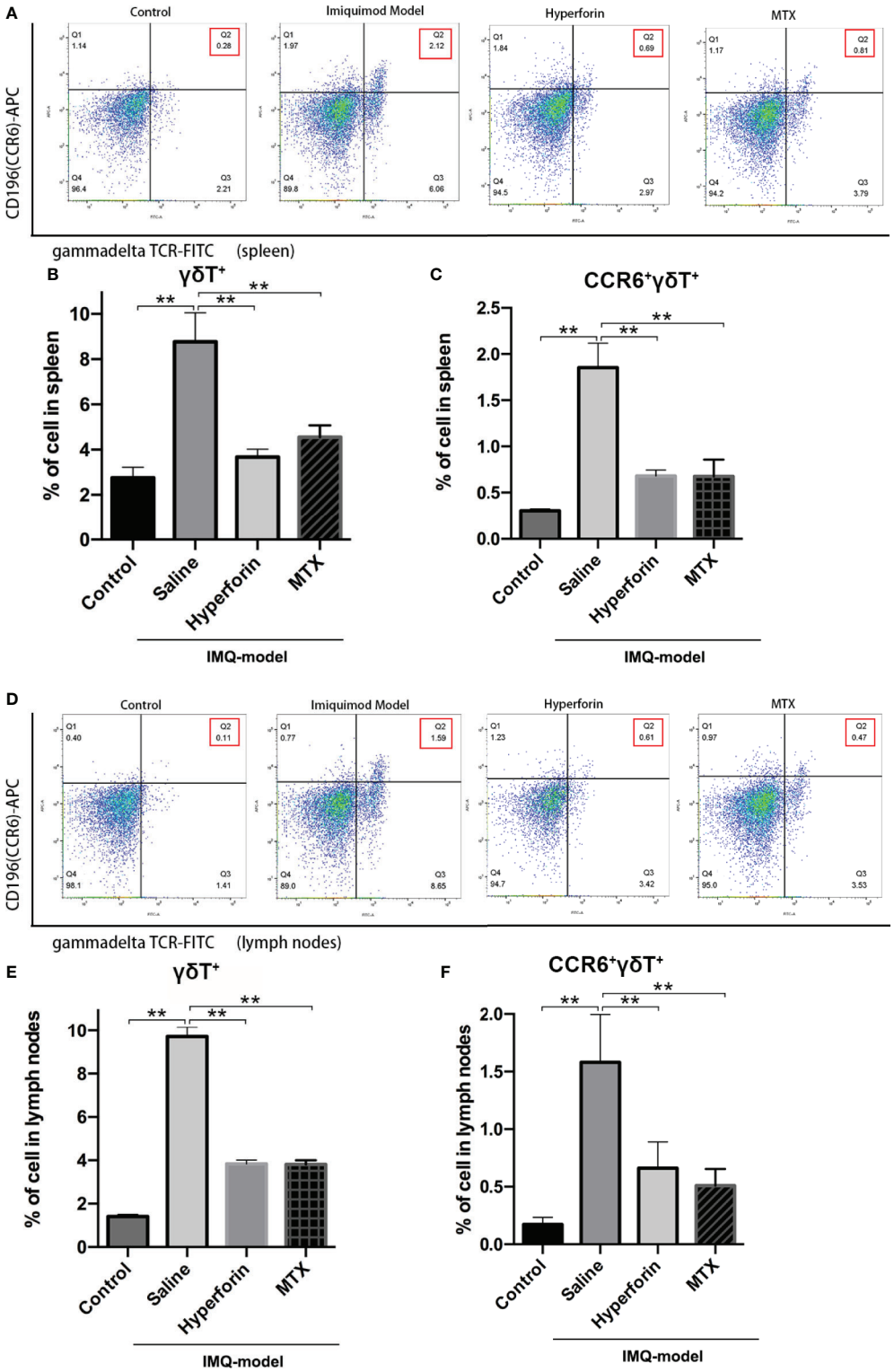

FIGURE 4 | Hyperforin reduced the abundance of γδ T cells in the spleen and lymph nodes of psoriasis-like mouse model. The percentage of γδ T cells and CCR6$^+$ γδ T cells in the total live cells in spleen **(A–C)** and axillary lymph nodes **(D–F)**. (n=5 mice). *P < 0.05 and **P < 0.01 *vs* model group.

production of AMPs are widely confirmed in psoriasis lesions (21–23). Previous studies have indicated that imiquimod increase the production of AMPs (24–26). Therefore, our experiments were exerted to demonstrate the effect of hyperforin on the expression of AMPs. Data showed a result of remarkably enhanced expression of S100A7, S100A8, S100A9 and CRAMP by imiquimod. In the hyperforin treated group, expression of S100A7, S100A8, S100A9 and CRAMP was highly reduced (**Figures 5A–D**).

To investigate whether hyperforin could directly inhibit the productions of AMPs from *in vitro* cultured keratinocytes, HaCaT cells were stimulated with TNF-α and RT-qPCR was used to detect the effects of hyperforin on the mRNA expression of LL37, S100A7, S100A8, and S100A9. Data showed a result of remarkedly enhanced expression of S100A7, S100A8, S100A9, and LL37 by TNF-α. In the hyperforin treated group, expression of LL-37, S100A7, S100A8, and S100A9 was highly reduced (**Figures 5E–H**).

Effects of Hyperforin in γδ T Cells on IMQ-Induced Psoriasis-Like Skin Inflammation

Figure 6A showed that the IMQ treated *Tcrd*$^{-/-}$mice transferred with γδ-vehicle T cells emerged typical psoriasis-like inflammatory responses on back, such as erythema, scaling and thickening, compared to the control group. However, *Tcrd*$^{-/-}$ mice transferred with γδ-Hyperforin T cells had notably alleviated IMQ-induced skin lesions. H&E staining suggested that IMQ induced typical pathological characteristics of psoriasis in *Tcrd*$^{-/-}$ mice transferred with γδ-vehicle T cells, such as epidermal parakeratosis, thickening of acanthosis cell layer, and downward epidermal extension of in-depth dermis (**Figure 6B**). However, the pathological characteristics in γδ-Hyperforin group showed reduced epidermal parakeratosis, thinning of the acanthosis cell layer and reduction of the downward epidermal extension of in-depth dermis (**Figure 6B**). *Tcrd*$^{-/-}$ mice transferred with γδ-Hyperforin notably alleviated the severity of IMQ induced psoriasis compared to the γδ-vehicle T cells group according to the scores of erythema, scaling, thickness and PASI score on day 7 (**Figure 6C**).

In order to detect the transferred γδ T cells in skin, we prepared the single-cell suspensions of the mice trunk skin tissues, and performed the cytofluorimetric analysis of CD3 and γδTCR antigens. The γδ-vehicle group and the γδ-Hyperforin group showed elevated percentages of dermal γδ T cells infiltration (**Figure 6D**). To further observe the infiltration of γδ T17 cells in dermis, we performed the cytofluorimetric analysis of CD3, γδTCR, IL-17A, and IFN-γ antigens. The γδ-vehicle group showed elevated percentage of IL-17A$^+$ cells in dermal γδ T cells (gated on CD3$^+$ γδTCR$^+$ T cells) compared to the γδ-Hyperforin group (**Figure 6E**). The percentage of transferred γδ T cells in skin and IL-17A$^+$ cells in dermal γδ T cells (gated on CD3$^+$ γδTCR$^+$ T cells) were showed in **Figure 6F** and **Figure 6G**. In summary, these findings proved that the abnormal differentiation of γδ T17 cells induced by hyperforin may play an important role in psoriasis.

Hyperforin Reduced the Expression and Secretion of IL-17A in γδ T Cell *In Vitro*

To investigate whether hyperforin has an effect on the function of γδ T cells, we cultured murine splenic γδ T cell *in vitro*. By using the MTT assay, we found that hyperforin, at the concentration of 0.1 to 10 μM, did not affected the viability of cultured γδ T cell (Data not shown). Therefore, we use the concentration range of 0.1 to 10 μM in the subsequent *in vitro* experiments. **Figure 7A** showed that the mRNA level of *Il7a* was increased in the model group compared to the control group, while hyperforin decreased the mRNA level of *Il7a*, and this inhibitory effect was gradually enhanced as the dose increases. Also, we detected the secreted IL-17A in the supernatant by ELISA. **Figure 7B** showed that the supernatant of γδ T cell incubated with hyperforin secreted less IL-17A than the model group.

Hyperforin Reduced the Phosphorylation of MAPK and STAT3 Pathways in γδ T Cell *In Vitro*

MAPK/STAT3 activation plays an important role in the pathogenesis of psoriasis (27, 28). The expression and phosphorylation of p38, ERK, JNK and STAT3 in the *in vivo* cultured γδ T cells were detected by Western Blot. **Figure 8** showed that the expressions of p-p38, p-ERK, p-JNK and p-STAT3 were increased in the model group compared to the control group, while the expression of p38, ERK, JNK and STAT3 were not changed. Furthermore, hyperforin, especially at the dosage of 10 μM, reduced the expressions of p-p38, p-ERK, p-JNK and p-STAT3.

Hyperforin Suppressed Phosphorylation of MAPK and STAT3 Pathways in TNF-α Stimulated HaCaT Cells

To investigate whether hyperforin has an effect on keratinocytes, we cultured TNF-α stimulated HaCaT cells. The expression and phosphorylation of p38, ERK, JNK, and STAT3 in the *in vivo* cultured HaCaT cells were detected by Western blot. **Figure 9** showed that the expressions of p-p38, p-ERK, p-JNK, and p-STAT3 were increased in the TNF-α-stimulated HaCaT cells compared to the control group, while the expression of p38, ERK, JNK, and STAT3 were not changed. Furthermore, hyperforin, especially at the dosage of 10 μM, reduced the expressions of p-p38, p-ERK, p-JNK, and p-STAT3.

DISCUSSION

In this study, we demonstrated that hyperforin alleviated IMQ induced psoriasiform dermatitis in mice. These mice treated with hyperforin showed lower cumulative scores, epidermal thickening, inflammatory cell infiltration and inflammatory cytokines released in IMQ-induced psoriasis-like mouse model. In addition, hyperforin also reduced enlargement of spleen. Moreover, hyperforin inhibited γδ T cells infiltration in spleen and lymph nodes and showed a similar effect on suppression of epidermal thickening and inhibition of systemic inflammation without obvious side effects compared to MTX which is commonly used for psoriasis (1). *In vitro* study, we found that hyperforin reduced the expression and secretion of IL-17A in γδ T cell. Moreover, we demonstrated that hyperforin significantly inhibited the phosphorylation of MAPK/STAT3 signaling in *in vitro* cultured γδ T cell and TNF-α-stimulated HaCaT

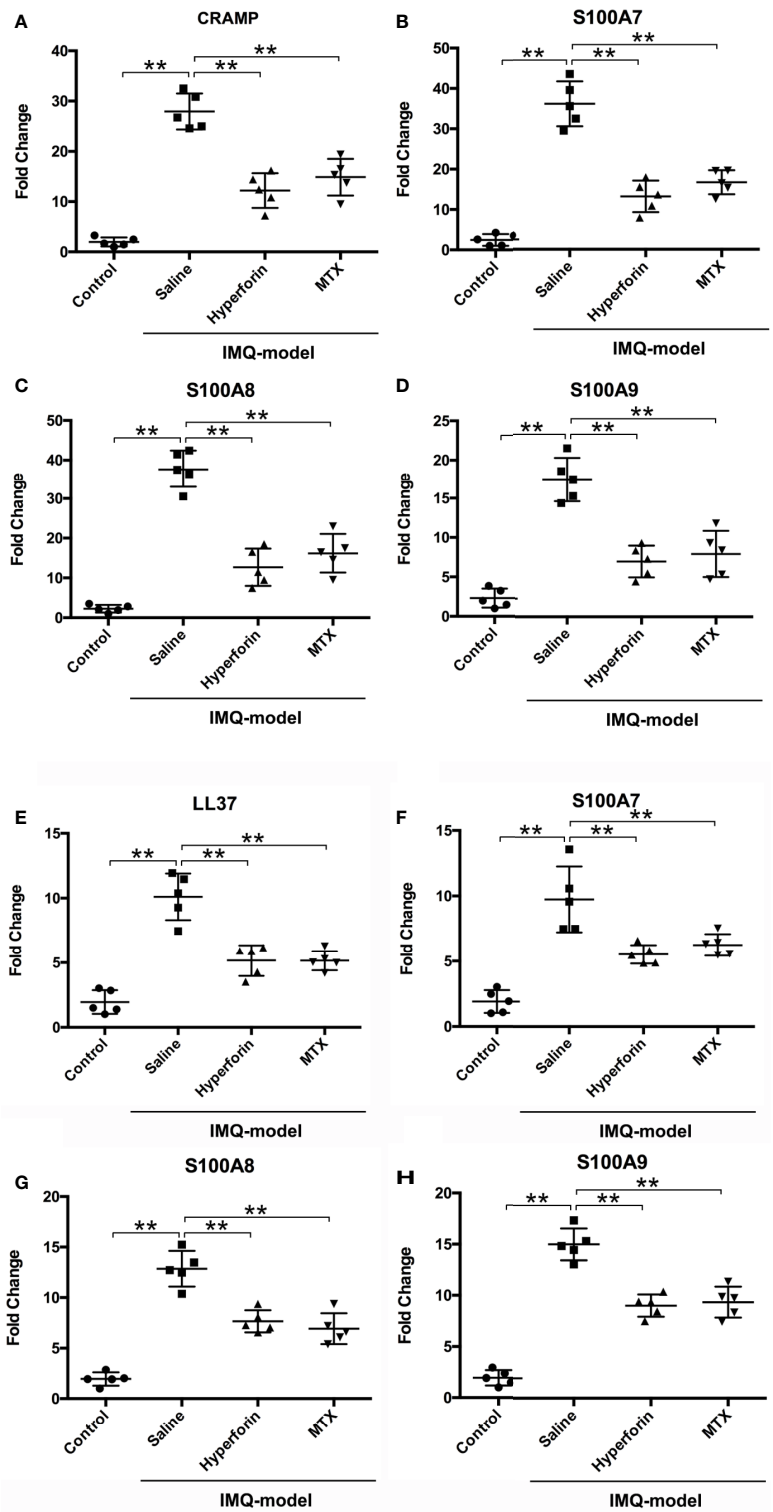

FIGURE 5 | Hyperforin downregulated the mRNA expression of AMPs in in skin of IMQ-induced mouse model and TNF-α stimulated HaCaT cells. The mRNA expression of AMPs in the skin lesion of mice model, CRAMP **(A)**, S100A7 **(B)**, S100A8 **(C)**, and S100A9 **(D)**. After pre-treatment with hyperforin, HaCaT cells were stimulated with TNF-α, and RT-qPCR was used to detect the effects of hyperforin on the mRNA expression of LL37 **(E)**, S100A7 **(F)**, S100A8 **(G)**, and S100A9 **(H)**. Data are expressed as fold induction of relevant mRNA sequences compared to untreated controls. Data represent mean ± SEM from at least three independent experiments performed in triplicates; $^{**}P < 0.01$ *vs* model group.

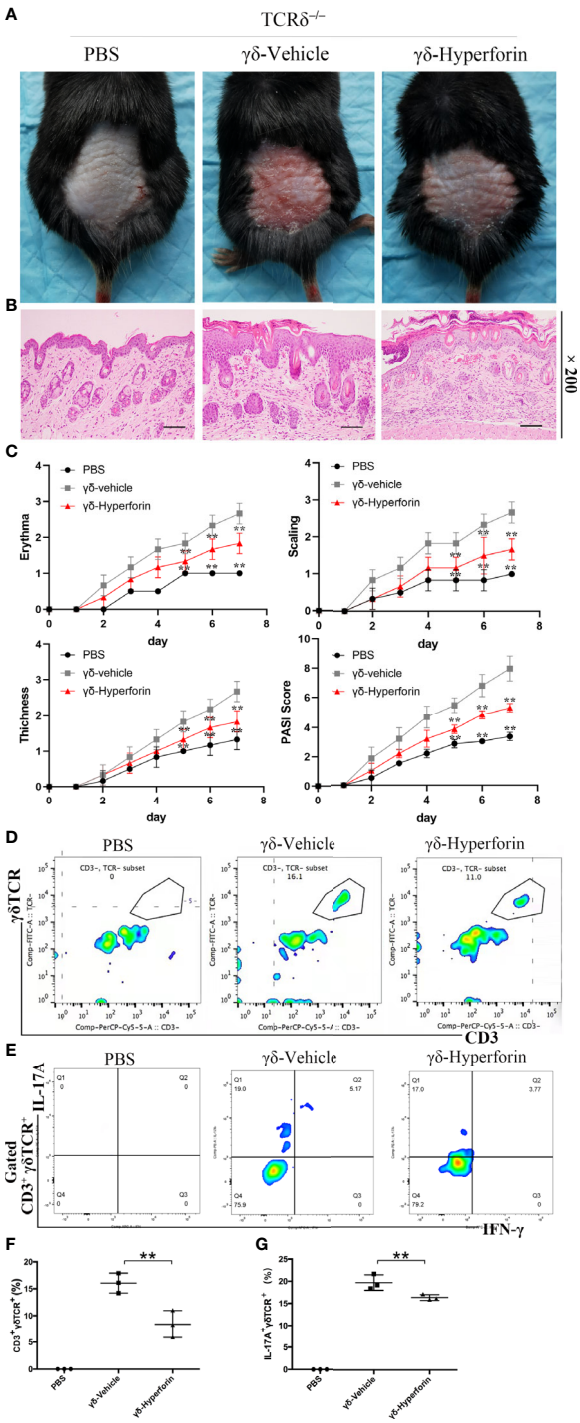

FIGURE 6 | Effects of hyperforin in γδ T cells on IMQ-induced psoriasis-like skin inflammation. *Tcrd*$^{-/-}$ mice were transferred with PBS or naive γδ T cells pretreated with hyperforin (γδ-Hyperforin) or not (γδ-Vehicle). The mice were then subjected to IMQ-induced psoriasis as part of the psoriasis-like mouse model. The back-skin photos of mice **(A)** and H&E staining of skin lesions **(B)** (scale bars: 100 μm), and PASI scores **(C)**. n = 3. **(D, F)** Detection of the transferred γδ T cells in skin. **(E, G)** Percentage of IL-17A$^+$ cells in dermal γδ T cells (gated on CD3$^+$ γδTCR$^+$ T cells). n = 3. Data are presented as the mean ± SD. (**P < 0.01).

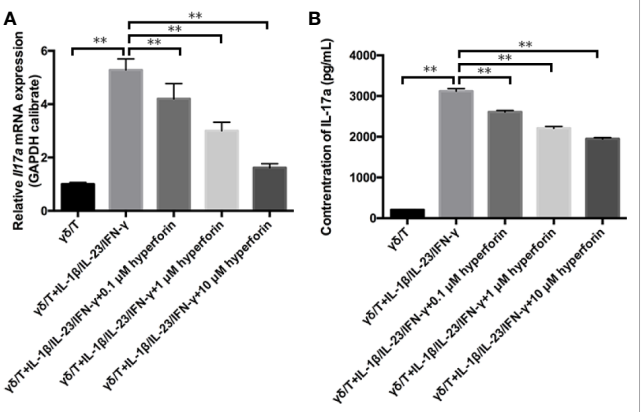

FIGURE 7 | Hyperforin reduced the expression and secretion of IL-17A by γδ T cell *in vitro*. **(A)** The expression of *Il17a* mRNA was significantly lower than that in the model group. **(B)** The level of IL-17 A in the culture supernatant was measured by ELISA to determine the effects of hyperforin on the secretion of IL-17 A by cultured γδ T cells (n=8). **P < 0.01 *vs* model group.

cells. In this study, it is the first time we elucidated the reduction effect of hyperforin on γδ T cells. The interplay of immune cells and skin-resident keratinocytes participates in establishing and sustaining inflammatory and immune responses in psoriasis (29). Therefore, our study showed the dual regulation of hyperforin in the keys cells of psoriasis. In conclusion, our results presented a novel mechanism of hyperforin in psoriasis and provided a potential effective approach for psoriasis therapy.

As a specific TRPC6 activator, hyperforin is a phytochemical produced by parts of the members of the plant genus Hypericum (St John's wort) (9). Previous study reported the expression levels of TRPC6 channel on mRNA and protein levels are significantly reduced both in cultured psoriatic keratinocytes and psoriasis plaques (30). Recently, other group detected mRNA expression levels of TRP channels in PBMCs of 30 patients with psoriasis, data showed that in the patient group, the TRPC6 expression levels were lower compared to controls (31). We have also used the data in the GEO database to perform differential gene analysis to clarify the exact changes of mRNA levels of TRPC6 channels in psoriasis skin lesions. Results elucidated that the mRNA level of TRPC6 in psoriasis skin lesions decreased compared with normal people, however, the statistical difference needs further verification (data not show). Hyperforin is also well-known for its anti-inflammatory, anti-tumor, anti-bacterial, antioxidant, and stabilize skin barrier properties (9–13). Studies had reported the anti-inflammatory effects of hyperforin in pancreatic β cells, microglia, vascular endothelial cells, and neuronal cells (17–19). Previous studies had focused on the modulatory effect of hyperforin in kerationcytes. Topical ointment (5%) in which hyperforin is the main active ingredients, was reported to have anti-inflammatory and anti-psoriatic dermatitis effects (14, 15, 32–34). In our study, it is the first time we applied hyperforin for systemic use to treat psoriasis.

In this study, hyperforin inhibited immune cell activation in psoriasis-like mouse model. To further confirm the valid effect of hyperforin, we examined the mRNA levels of inflammatory

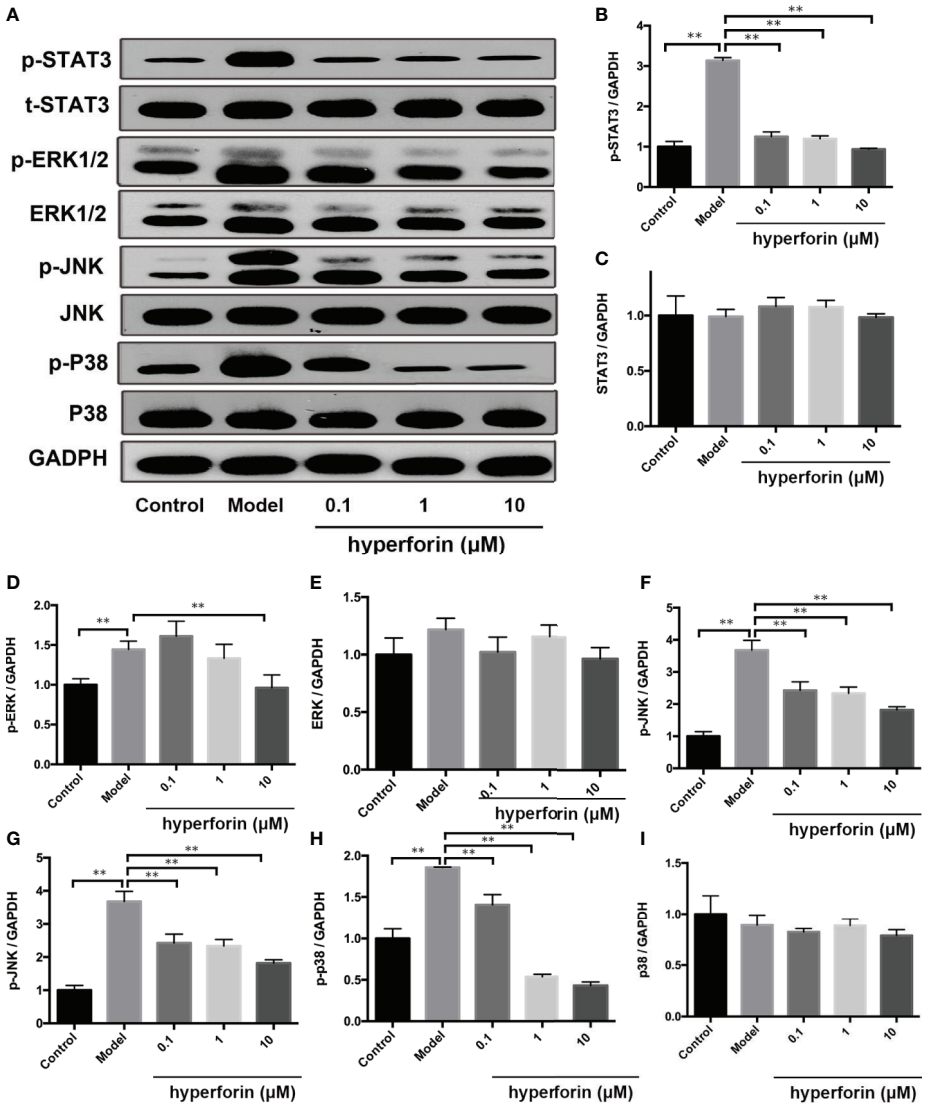

FIGURE 8 | Hyperforin inhibits phosphorylation of MAPK and STAT3 pathway components in *in vitro* cultured γδ T cells. **(A)** Representative images of Western blot. **(B–I)** quantification of the Western blot data by densitometric analysis and normalization to GAPDH (n = 3 independent experiments). **P < 0.01 *vs* model group.

cytokine in skin lesions by RT-PCR, the data showed that mRNA levels of *Il1*, *Il6*, *Il23p40*, *Il17a*, and *Il-22* were declined, especially *Il17a*. Recent studies have found that γδ T cells in dermis may be the main source of IL-17A in the skin of imiquimod murine models (5, 6). Moreover, the abundance of γδ T cell and $CCR6^{+}$γδ T were also discovered to change in the spleen of hyperforin treated mice. γδ T cells in spleen performed highly expressed CCR6 and transcriptional factor RORγt (5). Furthermore, we cultured murine splenic γδ T cell *in vivo*. Data showed that hyperforin decreased the mRNA level of *Il7a*, suggesting that hyperforin possibly suppressed γδ T-associated inflammation.

In serum and skin lesions of psoriasis patients, IL-17 and IL-22 were showed to promote the expression of antimicrobial peptides in keratinocytes, such as β-defensin-2 (BD-2), S100A7 (psoriasin), cathelicidin (LL37), and S100A8/9 (calprotectin), all of which may lead to the development of psoriasis in individuals with a higher resistance to skin infections (35, 36). In this study, results showed that hyperforin pretreatment downregulated the mRNA levels of AMPs both in skin lesions of imiquimod-mouse model and *in vitro* cultured HaCaT cells. In psoriasis, decrease of antimicrobial peptides may lead to an increased risk of skin infections (37). In atopic dermatitis, the AMPs expression in the skin lesions are down-regulated, the patients are more susceptible to Staphylococcus aureus skin infections (38). As hyperforin has an anti-bacterial effect, therefore, it may offset the infection risk caused by the decrease in antimicrobial peptides.

Previous studies have suggested that MAPK kinases were involved in the pathogenesis of psoriasis (28). The MAPK kinases constitute three signaling pathways, named mitogen-activated protein kinases p38 (p38 MAPKs), extracellular signal-

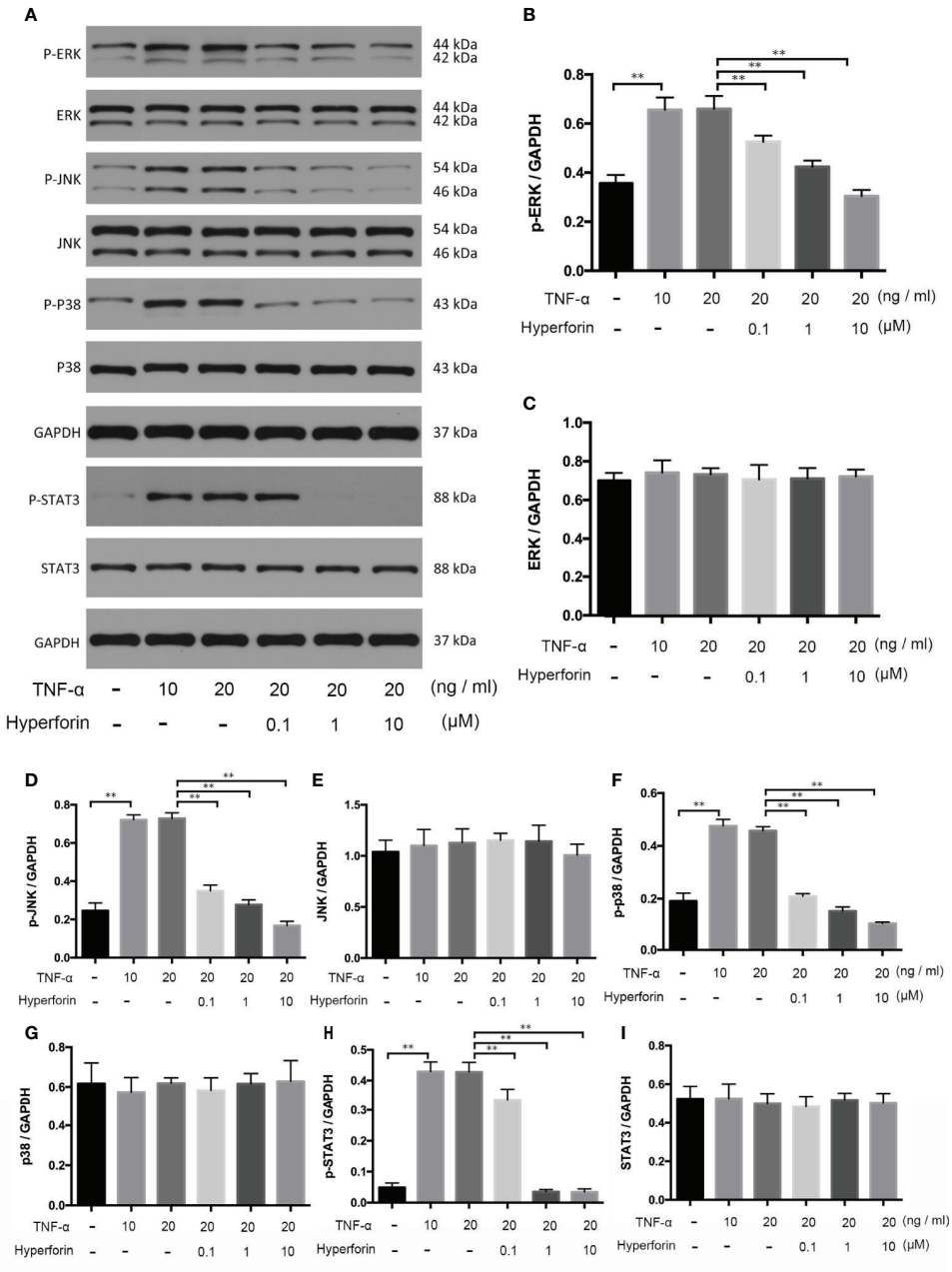

FIGURE 9 | Hyperforin inhibits phosphorylation of MAPK and STAT3 pathway components induced by TNF-α in HaCaT cells. HaCaT cells were pretreated with different doses of hyperforin, and stimulated with TNF-α. The total protein was extracted from the cells and associated protein expression was determined *via* western blotting **(A)**. The quantification data are shown in the right panel **(B–I)**. (n = 3 independent experiments). **P < 0.01 *vs* model group.

regulated kinase 1/2 (ERK1/2), and c-Jun N-terminal kinase (JNK) which modulate important functions such as cell proliferation, differentiation, gene expression, and apoptosis within cells (39). Phosphorylation of STAT-3 and of STAT-1 at serine 727 induced by p38 has also been demonstrated in psoriatic lesion (27, 40). Thus, keratinocytes in the psoriatic epidermis are characterized not only by abnormal proliferation and apoptosis but also increased expression of inflammatory cytokines (41). This phenomenon seems to be regulated by the same signal arising from the activation of MAPK signaling cascades of p38 and ERK1/2 (35, 42).

In conclusion, our work demonstrates that hyperforin alleviates IMQ-induced inflammation in psoriasis through suppressing the immune responses exerted by IL-17 A–producing γδ T cells and related cytokines by modulating MAPK/Stat3 pathways. Together with its effectiveness and safety, the current study provides the

evidence to support hyperforin as a promising therapeutic tragedy for treatment of psoriasis.

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 the USUHS Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

SZ, JZ, and JY performed experiment, analyzed data, and wrote the paper. FZ, WW, LZ, and WC performed experiment, analyzed the data, and prepared the images. XC, YW, and NL designed, conducted the research, interpreted data, and wrote the paper. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Boehncke W-H, Schön MP. Psoriasis. *Lancet* (2015) 386:983–94. doi: 10.1016/S0140-6736(14)61909-7
2. Prinz I, Sandrock I, Mrowietz U. Interleukin-17 Cytokines: Effectors and Targets in Psoriasis-a Breakthrough in Understanding and Treatment. *J Exp Med* (2020) 217:e20191317. doi: 10.1084/jem.20191397
3. Lowes MA, Kikuchi T, Judilyn FD, Cardinale I, Zaba LC, Haider AS, et al. Psoriasis Vulgaris Lesions Contain Discrete Populations of Th1 and Th17 T Cells. *J Investig Dermatol* (2008) 128:1207–11. doi: 10.1038/sj.jid.5701213
4. Fitch E, Harper E, Skorcheva I, Kurtz SE, Blauvelt A. Pathophysiology of Psoriasis: Recent Advances on IL-23 and Th17 Cytokines. *Curr Rheumatol Rep* (2007) 9:461–7. doi: 10.1007/s11926-007-0075-1
5. Cai Y, Shen X, Ding C, Qi C, Li K, Li X, et al. Pivotal Role of Dermal IL-17-producing γδ T Cells in Skin Inflammation. *Immunity* (2011) 35:596–610. doi: 10.1016/j.immuni.2011.08.001
6. Francisco RV, Gray EE, Cyster JG. Inflammation Induces Dermal Vγ4+ γδt17 Memory-Like Cells That Travel to Distant Skin and Accelerate Secondary IL-17-driven Responses. *Proc Natl Acad Sci USA* (2015) 112:8046–51. doi: 10.1073/pnas.1508990112
7. Calautti E, Avalle L, Poli V. Psoriasis: A Stat3-Centric View. *IJMS* (2018) 19:171. doi: 10.3390/ijms19010171
8. Schön MP. Adaptive and Innate Immunity in Psoriasis and Other Inflammatory Disorders. *Front Immunol* (2019) 10:1764. doi: 10.3389/fimmu.2019.01764
9. Shelton RC, Keller MB, Gelenberg A, Dunner DL, Hirschfeld R, Thase ME, et al. Effectiveness of St John's Wort in Major Depression: A Randomized Controlled Trial. *JAMA* (2001) 285:1978–86. doi: 10.1001/jama.285.15.1978
10. Fiebich BL, Heinrich M, Langosch JM, Kammerer N, Lieb K. Antibacterial Activity of Hyperforin From St John's Wort. *Lancet* (1999) 354:777. doi: 10.1016/S0140-6736(05)76019-0
11. Meinke MC, Schanzer S, Haag SF, Casetti F, Müller ML, Wölfle U, et al. In Vivo Photoprotective and Anti-Inflammatory Effect of Hyperforin is Associated With High Antioxidant Activity In Vitro and Ex Vivo. *Eur J Pharm Biopharm* (2012) 81:346–50. doi: 10.1016/j.ejpb.2012.03.002
12. Schempp CM, Winghofer B, Lüdtke R, Simon-Haarhaus B, Schöpf E, Simon JC. Topical Application of St John's Wort (*Hypericum Perforatum* L.) and of its Metabolite Hyperforin Inhibits the Allostimulatory Capacity of Epidermal Cells. *Br J Dermatol* (2000) 142:979–84. doi: 10.1046/j.1365-2133.2000.03482.x
13. Chen WT, Chen YK, Lin SS, Hsu FT. Hyperforin Suppresses Tumor Growth and NF-κb-Mediated Anti-apoptotic and Invasive Potential of Non-small Cell Lung Cancer. *Anticancer Res* (2018) 38:2161–7. doi: 10.21873/anticanres.12457
14. Najafizadeh P, Hashemian F, Mansouri P, Farshi S, Surmaghi MS, Chalangari R. The Evaluation of the Clinical Effect of Topical St Johns Wort (*Hypericum Perforatum* L.) in Plaque Type Psoriasis Vulgaris: A Pilot Study. *Australas J Dermatol* (2012) 53:131–5. doi: 10.1111/j.1440-0960.2012.00877.x
15. Mansouri P, Mirafzal S, Najafizadeh P, Safaei-Naraghi Z, Salehi-Surmaghi MH, Hashemian F. The Impact of Topical Saint John's Wort (Hypericum Perforatum) Treatment on Tissue Tumor Necrosis Factor-Alpha Levels in Plaque-Type Psoriasis: A Pilot Study. *J Postgrad Med* (2017) 63:215–20. doi: 10.4103/0022-3859.201423
16. Müller M, Essin K, Hill K, Beschmann H, Rubant S, Schempp CM, et al. Specific TRPC6 Channel Activation, a Novel Approach to Stimulate Keratinocyte Differentiation. *J Biol Chem* (2008) 283:33942–54. doi: 10.1074/jbc.M801844200
17. Novelli M, Menegazzi M, Beffy P, Porozov S, Gregorelli A, Giacopelli D, et al. St. John's Wort Extract and Hyperforin Inhibit Multiple Phosphorylation Steps of Cytokine Signaling and Prevent Inflammatory and Apoptotic Gene Induction in Pancreatic β Cells. *Int J Biochem Cell Biol* (2016) 81:92–104. doi: 10.1016/j.biocel.2016.10.017
18. Zhang J, Yao C, Chen J, Zhang Y, Yuan S, Lin Y. Hyperforin Promotes Post-Stroke Functional Recovery Through Interleukin (IL)-17A-mediated Angiogenesis. *Brain Res* (2016) 1646:504–13. doi: 10.1016/j.brainres.2016.06.025
19. Wang H, Shao B, Yu H, Xu F, Wang P, Yu K, et al. Neuroprotective Role of Hyperforin on Aluminum Maltolate-Induced Oxidative Damage and Apoptosis in PC12 Cells and SH-SY5Y Cells. *Chem Biol Interact* (2019) 299:15–26. doi: 10.1016/j.cbi.2018.11.016
20. Gan L, Duan J, Zhang S, Liu X, Poorun D, Liu X, et al. Cold Atmospheric Plasma Ameliorates Imiquimod-Induced Psoriasiform Dermatitis in Mice by Mediating Antiproliferative Effects. *Free Radical Res* (2019) 53:269–80. doi: 10.1080/10715762.2018.1564920
21. Morizane S, Gallo RL. Antimicrobial Peptides in the Pathogenesis of Psoriasis. *J Dermatol* (2012) 39:225–30. doi: 10.1111/j.1346-8138.2011.01483.x
22. Lai Y, Gallo RL. Amped Up Immunity: How Antimicrobial Peptides Have Multiple Roles in Immune Defense. *Trends Immunol* (2009) 30:131–41. doi: 10.1016/j.it.2008.12.003
23. Takahashi T, Gallo RL. The Critical and Multifunctional Roles of Antimicrobial Peptides in Dermatology. *Dermatol Clin* (2017) 35:39–50. doi: 10.1016/j.det.2016.07.006
24. Eckert RL, Broome A-M, Ruse M, Robinson N, Ryan D, Lee K. S100 Proteins in the Epidermis. *J Invest Dermatol* (2004) 123:23–33. doi: 10.1111/j.0022-202X.2004.22719.x
25. Nomura I, Goleva E, Howell MD, Hamid QA, Ong PY, Hall CF, et al. Cytokine Milieu of Atopic Dermatitis, as Compared to Psoriasis, Skin Prevents Induction of Innate Immune Response Genes. *J Immunol* (2003) 171:3262–9. doi: 10.4049/jimmunol.171.6.3262
26. Salamah MF, Vallance TM, Kodji X, Ravishankar D, Williams HF, Brain SD, et al. The Antimicrobial Cathelicidin CRAMP Augments Platelet Activation During Psoriasis in Mice. *Biomolecules* (2020) 10:1–13. doi: 10.3390/biom10091267

27. Johansen C, Vinter H, Soegaard-Madsen L, Olsen LR, Steiniche T, Iversen L, et al. Preferential Inhibition of the mRNA Expression of p38 Mitogen-Activated Protein Kinase Regulated Cytokines in Psoriatic Skin by Anti-Tnfα Therapy. *Br J Dermatol* (2010) 163:1194–204. doi: 10.1111/j.1365-2133.2010.10036.x
28. Andrés RM, Hald A, Johansen C, Kragballe K, Iversen L. Studies of Jak/STAT3 Expression and Signalling in Psoriasis Identifies STAT3-Ser727 Phosphorylation as a Modulator of Transcriptional Activity. *Exp Dermatol* (2013) 22:323–8. doi: 10.1111/exd.12128
29. Cristina A, Stefania M, Paolo G, Giampiero G. The Interplay Between Keratinocytes and Immune Cells in the Pathogenesis of Psoriasis. *Front Immunol* (2018) 9:1549. doi: 10.3389/fimmu.2018.01549
30. Kristina L, Margarethe K, Ute W, Heike B, Christian H, Boehncke W-H, et al. Reduced TRPC Channel Expression in Psoriatic Keratinocytes Is Associated With Impaired Differentiation and Enhanced Proliferation. *PloS One* (2011) 6 (2):e14716. doi: 10.1371/journal.pone.0014716
31. Özcan S S, Gürel G, Çakır M. Gene Expression Profiles of Transient Receptor Potential (TRP) Channels in the Peripheral Blood Mononuclear Cells of Psoriasis Patients. *Hum Exp Toxicol* (2021) 2:960327121991911. doi: 10.1177/0960327121991911
32. Haag SF, Tscherch K, Arndt S, Kleemann A, Gersonde I, Lademann J, et al. Enhancement of Skin Radical Scavenging Activity and Stratum Corneum Lipids After the Application of a Hyperforin-Rich Cream. *Eur J Pharm Biopharm* (2014) 86:227–33. doi: 10.1016/j.ejpb.2013.06.016
33. Takada H, Yonekawa J, Matsumoto M, Furuya K, Sokabe M. Hyperforin/HP-β-Cyclodextrin Enhances Mechanosensitive Ca (2+) Signaling in HaCaT Keratinocytes and in Atopic Skin Ex Vivo Which Accelerates Wound Healing. *BioMed Res Int* (2017) 2017:8701801. doi: 10.1155/2017/8701801
34. Papotto PH, Ribot JC, Silva-Santos B. Il-17(+) γδ T Cells as Kick-Starters of Inflammation. *Nat Immunol* (2017) 18:604–11. doi: 10.1038/ni.3726
35. Yu XJ, Li CY, Dai HY, Cai DX, Wang KY, Xu YH, et al. Expression and Localization of the Activated Mitogen-Activated Protein Kinase in Lesional Psoriatic Skin. *Exp Mol Pathol* (2007) 83:413–8. doi: 10.1016/j.yexmp.2007.05.002
36. Wang S, Uchi H, Hayashida S, Urabe K, Moroi Y, Furue M. Differential Expression of Phosphorylated Extracellular Signal-Regulated Kinase 1/2, Phosphorylated p38 Mitogen-Activated Protein Kinase and Nuclear Factor-Kappab p105/p50 in Chronic Inflammatory Skin Diseases. *J Dermatol* (2009) 36:534–40. doi: 10.1111/j.1346-8138.2009.00696.x
37. Haase I, Hobbs RM, Romero MR, Broad S, Watt FM. A Role for Mitogen-Activated Protein Kinase Activation by Integrins in the Pathogenesis of Psoriasis. *J Clin Invest* (2001) 108:527–36. doi: 10.1172/JCI12153
38. Takahashi H, Ibe M, Nakamura S, Ishida-Yamamoto A, Hashimoto Y, Iizuka H. Extracellular Regulated Kinase and C-Jun N-terminal Kinase are Activated in Psoriatic Involved Epidermis. *J Dermatol Sci* (2002) 30:94–9. doi: 10.1016/s0923-1811(02)00064-6
39. Kyriakis JM, Avruch J. Mammalian MAPK Signal Transduction Pathways Activated by Stress and Inflammation: A 10-Year Update. *Physiol Rev* (2012) 92:689–737. doi: 10.1152/physrev.00028.2011
40. Hald A, Andrés RM, Salskov-Iversen ML, Kjellerup RB, Iversen L, Johansen C. STAT1 Expression and Activation is Increased in Lesional Psoriatic Skin. *Br J Dermatol* (2013) 168:302–10. doi: 10.1111/bjd.12049
41. Arthur JSC, Darragh J. Signaling Downstream of p38 in Psoriasis. *J Invest Dermatol* (2006) 126:1689–91. doi: 10.1038/sj.jid.5700280
42. Guan Z, Buckman SY, Pentland AP, Templeton DJ, Morrison AR. Induction of Cyclooxygenase-2 by the Activated MEKK1 –> SEK1/MKK4 –> p38 Mitogen-Activated Protein Kinase Pathway. *J Biol Chem* (1998) 273:12901–8. doi: 10.1074/jbc.273.21.12901

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HVEM Promotes the Osteogenesis of allo-MSCs by Inhibiting the Secretion of IL-17 and IFN-γ in Vγ4T Cells

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Bone defects are a common orthopaedic concern, and an increasing number of tissue-engineered bones (TEBs) are used to repair bone defects. Allogeneic mesenchymal stem cells (allo-MSCs) are used as seed cells in many approaches to develop TEB constructs, but the immune response caused by allogeneic transplantation may lead to transplant failure. V gamma 4 T (Vγ4T) cells play an important role in mediating the immune response in the early stage after transplantation; therefore, we wanted to verify whether suppressing Vγ4T cells by herpesvirus entry mediator (HVEM)/B and T lymphocyte attenuator (BTLA) signalling can promote MSCs osteogenesis in the transplanted area. *In vitro* experiments showed that the osteogenic differentiation of MSCs and Vγ4T cells was weakened after co-culture, and an increase in interleukin-17 (IL-17) and interferon-γ (IFN-γ) levels was detected in the culture supernatant. HVEM-transfected MSCs (MSCs-HVEM) still exhibited osteogenic differentiation activity after co-culture with Vγ4T cells, and the levels of IL-17 and IFN-γ in the co-culture supernatant were significantly reduced. *In vivo* experiments revealed that inflammation in the transplanted area was reduced and osteogenic repair was enhanced after Vγ4T cells were removed. MSCs-HVEM can also consistently contribute to reduced inflammation in the transplanted area and enhanced bone repair in wild-type (WT) mice. Therefore, our experiments verified that HVEM can promote the osteogenesis of allo-MSCs by inhibiting IL-17 and IFN-γ secretion from Vγ4T cells.

Keywords: HVEM-BTLA, Vγ4T cells, MSc, Tissue engineered bone, IL-17, immunomodulatory

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INTRODUCTION

Bone defects are common diseases treated in orthopaedic clinics, and most bone defects are caused by bone tuberculosis, osteoarthritis, bone tumour resection and severe fracture (1, 2). Autogenous bone grafts are the gold standard for the treatment of bone defects, but the lack of an autogenous bone graft source and risks of donor site infection and bleeding are major obstacles. Therefore, allogeneic bone transplantation has become the other choice of treatment, but it has the

disadvantages of high cost and strong rejection (2). The development of tissue-engineered bone (TEB) derived from mesenchymal stem cells (MSCs) as seed cells have begun to emerge in the medical and scientific communities. Through tissue engineering, autologous bone marrow-derived MSCs (BMMSCs) were implanted into the scaffold material of TEB. This structure was found to actively assist in bone regeneration (2–5). The proliferation and differentiation ability of BMMSCs *in vitro* decreased with increasing donor age. In addition, it takes too long to construct autologous TEB grafts *in vitro*, and it is difficult for autologous MSCs to meet the needs of individual and large-scale clinical applications. Therefore, allo-MSCs have been used as seed cells by many researches aiming to develop ideal TEB constructs (6).

The success of allogeneic TEB depends on the strength of the immunogenicity induced by the transplanted cells. Although MSCs have low immunogenicity, they can still induce natural immune rejection when transplanted in animals (7–9). The inflammatory response induced by immune rejection will seriously affect the osteogenic differentiation of implanted MSCs and cause transplantation failure. In allogeneic transplantation, γδT cells modulate the size and productivity of pre-immune peripheral B cell populations and mediate early graft versus host disease (GVHD) (10, 11). V gamma 4 T cells (Vγ4T cells, a subtype of γδT cells) are the main producers of IL-17 during early GVHD; this production induces an early inflammatory response and plays a key role in the early innate immune response (4-96 hours) (11–15). IL-17 can inhibit the osteogenic differentiation of MSCs (16). Thus, we hypothesize that inhibiting the function of Vγ4T cells to reduce IL-17 secretion promotes the osteogenesis of TEB arising from MSCs.

B and T lymphocyte attenuator (BTLA) is an inhibitory receptor of the Ig superfamily that plays an important role in negative immune regulation (17). BTLA plays an active role in alleviating GVHD (18, 19). The BTLA protein structure is similar to that of programmed death 1 (PD-1) and cytotoxic T lymphocyte associated antigen 4 (CTLA-4) and comprises an extracellular domain, a cytoplasmic domain and a transmembrane domain (20). The unique ligand of BTLA is herpesvirus entry mediator (HVEM), which belongs to the tumour necrosis factor (TNF) receptor superfamily and is also widely expressed in B cells, T cells, macrophages, DCs, and endothelial cells. The discovery of HVEM and its receptor BTLA provided the third immunosuppressive pathway similar to PD-1 and CTLA-4. Studies have shown that BTLA signalling inhibits donor versus host T cell responses and improves GVHD *via* successful implantation of donor haematopoietic cells. Activation of the HVEM-BTLA signalling pathway can overcome lymphopenia and T cell proliferation after haematopoietic stem cell transplantation, thus preventing GVHD and host versus graft reaction (HVGR) without global immunosuppression and reducing the acute inflammatory response (18, 19). As the osteogenic differentiation ability of allo-MSCs was inhibited by IL-17 and IFN-γ (16, 21), the early source of IL-17 in the transplant area was identified as Vγ4T cells (12); thus, we investigated whether HVEM-BTLA signalling could inhibit the function of Vγ4T cells to promote allo-MSC-based TEB repair of bone fractures. First, we implanted TEB constructed with MSCs into wild-type (WT) mice and a Vγ4T cell-free femoral defect mouse model (Vγ4-D) to observe the osteogenic ability of the cells *in vivo*. Then, the osteogenic differentiation potential of MSCs or HVEM-expressing MSCs was measured when these cells were co-cultured with Vγ4T cells *in vitro*.

MATERIALS AND METHODS

The Extraction, Proliferation and Identification of MSCs

The animal experiments were approved by the Animal Ethics Committee of the Third Military Medical University. Four-week-old male C57BL/6 mice (Experimental Animal Centre of Third Military Medical University, Chongqing, China) were killed, and their femurs and tibias were harvested under sterile conditions and rinsed with phosphate-buffered saline (PBS) before removal of all soft tissues still attached. The ends of the bones were cut off, and the bone marrow was washed out with C57BL/6 mouse MSC complete medium (Cyagen Biosciences, China). The resulting cell suspension was transferred to a 15 ml centrifuge tube (Nest, China) and centrifuged for 10 minutes at 200 ×g, after which the supernatant was discarded. The cell suspension was resuspended in MSC medium at a density of 1×10^6 cells/ml, seeded into six-well plates and cultured at 37°C and 5% CO_2. The medium was first changed the next day before shifting to a schedule of every 3 days until the cells reached 90% confluence. The cells were washed with sterile PBS three times, digested with 0.25% EDTA trypsin (HyClone, USA) at 37°C for 3 minutes, treated with medium to terminate the digestion, gently pipetted several times, and centrifuged for 10 minutes at 200 ×g; the supernatant was then discarded. The cells were resuspended in medium at a density of 1×10^5 cells/ml and seeded in a six-well plate (NEST, China). MSCs were identified after the 3rd passage (P3). P3 cells were digested, centrifuged and resuspended in PBS. MSCs were identified by flow cytometry with antibodies against CD11b, CD29, CD31, CD44, CD45 and CD90 (eBioscience, BioLegend, Abcam). P3 MSCs were infected with adenovirus carrying HVEM-EGFP and EGFP (kindly donated by Professor He Weifeng, Burn Research Institute of Southwest Hospital). The transfection efficiency was observed by fluorescence microscopy.

Isolation, Culture and Identification of Vγ4T Cells

Eight-week-old female BALB/c mice were anaesthetized and sacrificed. After the mice were immersed in 75% alcohol for 3 minutes, the spleen was removed and ground into pulp in sterile PBS by glass sliding before the tissue was centrifuged for 10 minutes at 200 ×g; the supernatant was discarded. Two millilitres of sterile red cell lysis buffer was added for 1 minutes to lyse residual red blood cells before 10 ml of 1640 medium was added to terminate the reaction. The samples were centrifuged at 200 ×g for 10 minutes, and the supernatant was discarded. Vγ4T cell

culture medium comprised 1640 medium (Hyclone, USA), CD28 antibody (BioXcell, USA), rIL-2 (R&D Systems, USA), β-mercaptoethanol (Sigma, USA), and 10% foetal bovine serum (Gibco, USA), penicillin-streptomycin solution (Beyotime, China). Vγ4T cell culture medium was used to resuspend cells to a density of 2.5×10^6 cells/ml, cells were seeded on the preplate with Vγ4 antibody (BioXcell, USA) for 2 days. Then Vγ4T cells were changed the plate and cultured in Vγ4T cells medium without CD28 antibody and Vγ4 antibody. The cells were activated by treatment with anti-CD3 (1 μg/ml, BioXcell, USA) for 12h could be used for identification and use on the 7th day after purification. Flow cytometry with antibodies targeting CD3, γδ and Vγ4 (Invitrogen, BioLegend, USA) were used to determine the purity of the cell population.

Osteogenic Differentiation of allo-MSCs or HVEM-Expressing allo-MSCs Co-Cultured With Vγ4T Cells *In Vitro*

Vγ4T cells were stimulated with 1 μg/ml CD3 and co-cultured with allogeneic MSCs or HVEM-MSCs at a ratio of 4:1 for 72 hours. Morphological changes in MSCs were observed during co-culture. C57BL/6 mouse MSC-specific osteogenic differentiation complete medium (Cyagen Biosciences China) was used instead of culture medium. The medium was changed every 3 days, and cells were cultured for 12 days. Reverse transcription polymerase chain reaction (RT-PCR) and Western blotting were conducted as follows: Runx2 and osterix (OSX) levels were measured after osteogenic differentiation culture for 3 days; Col I levels were measured on day 6, and osteocalcin (OCN) levels were measured on day 9. MSCs were co-cultured with Vγ4T cells at ratios of 1:0, 1:1, 1:2, and 1:4, and osteogenic differentiation was observed on the 9th day of co-culture. Alkaline phosphatase (ALP) staining was evaluated using the BCIP/NBT alkaline phosphatase assay kit (Beyotime, China), and on the 12th day after co-culture, Alizarin Red S staining (Cyagen, China) was used for evaluation. ImageJ software (National Institutes of Health, USA) was used to analyse the images of stained cells.

RT-PCR

mRNA was extracted from WT MSCs and MSCs expressing HVEM using TRIzol reagent (Invitrogen, USA) at designated time points after osteoinduction. According to the manufacturer's instructions, the total mRNA was isolated and purified using the RNeasy Mini Kit (Qiagen, USA) and then quantified using a Beckman Coulter DU-600 (USA). cDNA was synthesized from 2 μg of total RNA using a ThermoScript RT-PCR system (Invitrogen, USA). PCR was performed using ReverTra -Plus-™ (TOYOBO) and Mir-X miRNA qRT-PCR TB Green® Kit (Takara) and run on an ABI7500 qPCR system (Applied Biosystems, USA). Gene-specific primer pairs: COLI: 5′—3′(forward) CAGAGGCG AAGGCAACA 5′—3′(reverse) GTCCAAGGGAGCCACATC; Runx2: 5′—3′(forward) GCACA AACATGGCCAGATTCA, 5′—3′(reverse) AAGCCATGGTGCCCGTTAG; Osterix: 5′—3′(forward) CCCCTC GCTCTCTCCTATT 5′—3′(reverse) TAGGCACTGGCAAAGGC; OCN: 5′—3′(forward) AGCAGG AGGGCA ATAAGGT 5′—3′(reverse) GCTTTAGGGCAGCA CAGG; GAPDH: 5′—3′(forward) TGTTTCCTCGTCCC GTAGA 5′—3′(reverse) ATCTCCACTTTGCCACTGC. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as a housekeeping gene. The gene expression level was quantified from the standard curve and normalized to GAPDH gene expression.

Western Blotting Analysis

Total protein was extracted by RIPA lysis buffer (1 ml cold lysis buffer with 10 μl phosphatase inhibitor, 1 μl protease inhibitor and 5 μl 100 mm PMSF). The protein content was determined by the BCA method. Equal amounts of protein (20 μg) per sample were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) through 10% gels and transferred to a 0.22 μm polyvinylidene fluoride (PVDF) membrane. The membrane was blocked for 1-2 hours in 5% bovine serum albumin (BSA) in Tris-buffered saline containing 0.1% Tween-20 (TBST). Then, antibodies targeting HVEM (1:1000, Santa Cruz Biotechnology, USA), COL I (1:500, Bioss, China), Runx2 (1:1000, Cell Signaling Technology, USA), OCN (1:1000, Bioworld, USA), OSX (1:1000, Abcam, USA) and GAPDH (1:2000, BioLegend, USA) were incubated with the membranes overnight at 4°C. After a series of washes, the membranes were incubated with secondary antibodies (anti-rabbit IgG or anti-mouse IgG, 1:5000) for 1 hour at room temperature. The signals on the membranes were visualized with an enhanced chemiluminescence kit (UK) and Quantity One software, and quantitative analysis of gray value of WB band was performed by ImageJ software.

ELISA

The supernatant of cells co-cultured for 3 days was processed to detect the expression of IL-17a and IFN-γ with respective ELISA kits (Dakewe, China).

Construction of Mutilation Fracture Models With TEBs

The BMMSCs obtained above were inoculated onto both sides of demineralized bone matrix (DBM) that presented complete decalcification. Eight-week-old WT BALB/c female mice weighing 23-25 g were selected, 21 of which were treated with neutralizing antibody against Vγ4 (BioXcell, USA) at a dose of 200 μg/mouse (22). Three days later, usable mice lacking Vγ4T cells (Vγ4-D) were obtained (**Figure 1A**). Took out the spleen, ground it into a pulp, and lysed the remaining red blood cells, and the clearance of Vγ4T cells was detected by CD3, γδ and Vγ4 (Invitrogen, BioLegend, USA) levels (**Figure 1A**).

The mice were divided into groups according to mouse model and treatment as follows: WT/MSCs+DBM, Vγ4-D/MSCs+DBM, WT/MSCs-EGFP+DBM, WT/MSCs-HVEM+DBM and Vγ4-D/MSCs-HVEM+DBM, with 10 in each group. After mice were subjected to anaesthesia with 1% pentobarbital sodium, the right femur was exposed. A femoral shaft 2 mm long was cut off with a high-speed motor. The bone defect area was filled with constructed TEB, and the fracture area was fixed with an internal fixation pin to establish the mutilation fracture model (**Figure 1B**). Grafts were collected from each group at three

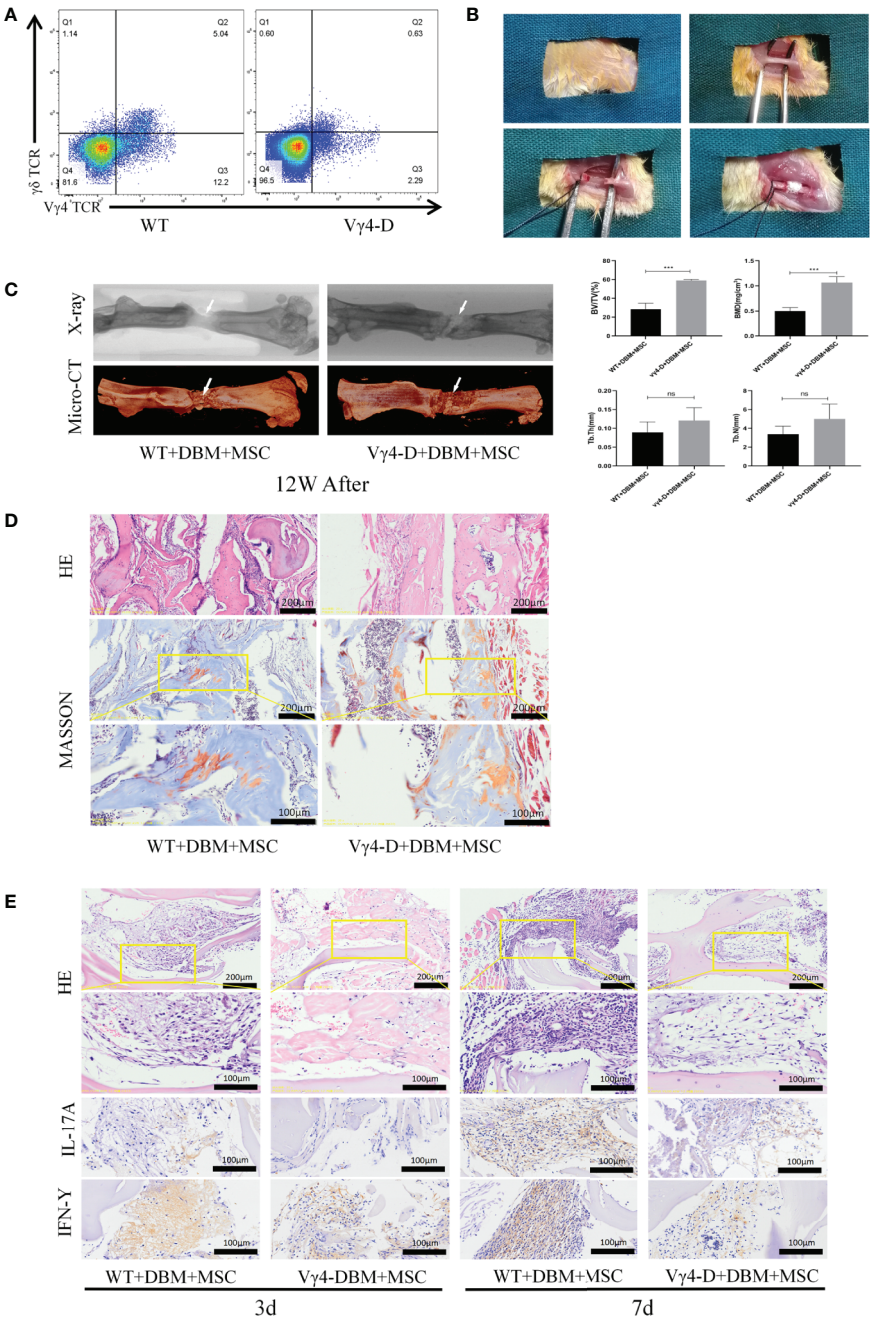

FIGURE 1 | Establishment of femoral nonunion fracture model and Osteogenesis of TEB in vivo of femal Balb/c mice aged 8 weeks old. **(A)** Constructed Vγ4T cells free Balb/c mice (Vγ4-D). **(B)** Process for careting the nonunion fracture models in mice. **(C)** Morphological analysis of bone formation in 2 groups mice femur defect at 12 weeks after operation and Microarchitecture analyses of bone volume fraction (BV/TV) ,bone mineral density (BMD) , trabecular number of bone formation area (Tb.N) and trabecular thickness (Tb.Th) by X-ray and 3D Reconstruction . Error bars represent mean ± SD. One-way ANOVA test was used to calculated P value (***P < 0.01, ns P > 0.05). **(D)** Histological analysis of newly formed bone by H&E and Masson's Trichrome staining. **(E)** Infiltration of inflammatory cells and expressions of IL-17A and IFN-γ detected by immunohistochemistry at 3 days and 7 days post implantation.

days and seven days after surgery to histologically examine the inflammation and infiltration of the graft. The growth of new bone within the defect was evaluated by X-ray examination, micro-computed tomography (micro-CT) and histological analysis at 12 weeks post-operation.

Micro-CT Measurement and Histological Assessment

Twelve weeks after the procedure, the femurs were removed for micro-CT scanning (quantum FX micro-CT imaging system, PerkinElmer, Ma, USA), and the three-dimensional images were

reconstructed in the regions of interest (ROIs), which were defined as cylinders in the new bone in the non-union fracture area. New bone formation (bone volume/tissue volume ratio, BV/TV), bone mineral density (BMD), trabecular number (Tb.N) and trabecular thickness (Tb.Th), which were calculated with CTAn software (Bruker, Belgium), were recorded to evaluate bone regeneration and microstructure in bone defects.

The femurs of mice were fixed with 4% neutral-buffered paraformaldehyde, decalcified at 4°C for 4 weeks in 10% EDTA, embedded in paraffin, and sectioned at 5 μm. H&E and Masson's trichrome staining was used to evaluate the tissue morphology.

Immunohistochemistry

Immunohistochemistry was performed using SABC IHC kits (Zhongshan Corporation) with primary antibodies targeting IL-17a and IFN-γ (1:500, Abcam, USA) according to the manufacturer's instructions, and nuclei were counterstained with haematoxylin. We captured the images with a Leica Microsystems microscope (DFC300 FX, Heerbrugg, Switzerland). The brief procedure for integrated optical density (IOD) analysis was performed as follows: an ROI for a positively stained area was analysed, and the average signalling intensity was quantified by ImageJ software.

CCK8 Analysis

MSCs (1×10^4 cells/well) or MSCs-HVEM (1×10^4 cells/well) were cultured with or not with Vγ4T cells (4×10^4 cells/well) in a Flat bottom 96-well plate for 3 days. The proliferation of cell was measured by using Cell Counting Kit-8 (CCK-8, Beyotime, China). Gently transferred the co-cultured medium into a new well,washed the 96-well plate with MSCs attachment 3 times with PBS added 100 μl new medium without FBS and also added 10 μl CCK8 solution to all wells, followed by incubation for 2 hours in the cell incubator. Finally, the absorbance value of the solution in each well was detected at the wavelength of 450 nanometers by a microplate reader (Type3001, Thermo Fisher Scientific, USA).

Treatment of MSCs Co-Culture With Vγ4T Cells With IFN-γ, IFN-γ Antibody, IL-17A and IL-17A Antibody

In order to explore the possible mechanism that HVEM inhibits the secretion of IL-17A and IFN-γ in Vγ4T cells and promotes the osteogenesis of allo-MSCs, MSCs were planted in 6-well plates (1×10^5 cells/well) and co-cultured with or without Vγ4T cells (4×10^5 cells/well). Some wells added IFN-γ, IFN-γ antibody, IL-17A and IL-17A antibody (50 ng/ml, R&D Systems, USA), they were co-cultured or treated for 3d and incubated with osteogenic differentiation medium for another 9d, the expression of osteogenic markers was checked by Western blot analysis.

Statistical Analysis

Data are presented as the means ± standard deviation (SD). Statistical analysis was performed with one-way analysis of variance (ANOVA) using the GraphPad Prism 8.0 statistical software package (GraphPad software, USA). The data conformed to the normal distribution when checked by Normality and Lognormality Tests, so we chose the ordinary ANOVA test or Brown-Forsythe and Welch ANOVA tests according to whether the data had the same SDs. $P < 0.05$ was considered statistically significant.

RESULTS

Osteogenic Effect of TEBs Constructed by MSCs *In Vivo* in Vγ4T Cell-Free Mice

The methods of constructing TEB with MSCs were based on our previous studies (16), and TEB was transplanted into sites of femoral-amputated defects created by a high-speed motor. After morphological analysis of the mouse femur and TEB by micro-CT, the scan image and X-ray image at 12 weeks after surgery were reconstructed in three dimensions using CTVox software (**Figure 1B**). Nascent bone in the WT and Vγ4-D groups was observed in the fracture area. Upon assessment, we found that the quality of new bone in the Vγ4-D group was better than that in the WT group according to the BMD, BV/TV, Tb.N, and Tb.Th values (**Figure 1C**).

Twelve weeks after transplantation, H&E and Masson trichrome staining were used to further observe the degree of new bone formation in the transplanted area. More nascent recombinant woven calli formed in the Vγ4-D group than in the WT group. These results suggested that the osteogenic efficiencies of transplanted TEB were strengthened when Vγ4T cells were eliminated (**Figure 1D**). Additionally, at 3 days and 7 days after implantation, we observed the infiltration of inflammatory cells around the grafts with H&E staining and detected the expression of IL-17 and IFN-γ by immunohistochemical methods. The Vγ4-D group had less inflammatory cell infiltration and expressed fewer inflammatory mediators than did the WT group; therefore, we suggest that Vγ4T cells inhibit the osteogenic differentiation of grafts by secreting IL-17 and IFN-γ and subsequently inducing inflammation (**Figure 1E**).

Osteogenic Differentiation of MSCs After Co-Culture With Vγ4T Cells *In Vitro*

We co-cultured MSCs and Vγ4T cells at ratios of 1:0, 1:1, 1:2, and 1:4 for 3 days before we removed the supernatant, washed the cells 3 times with PBS and added osteoinduction medium, which was changed every 3 days. Cells were stained for ALP 9 days after osteoinduction and with Alizarin Red 12 days after osteoinduction. The experimental results showed that Vγ4T cells can inhibit the osteogenic differentiation of MSCs, and as

the proportion of Vγ4T cells increased, the inhibitory effect was also strengthened (**Figure 2A**). To evaluate the osteogenic differentiation potential of MSCs after co-culture with Vγ4T cells (ratio of 1:4), RT-PCR and Western blot were used to measure the mRNA and protein expression, respectively, of osteogenic genes such as COL I, Runx2, OSTERIX, and OCN. We found that the mRNA and protein expression levels of osteogenic genes in MSCs co-cultured with Vγ4T cells in osteogenic induction medium were lower than those in MSCs cultured with osteogenic induction medium alone but higher than those in MSCs cultured with complete medium (**Figure 2B**). However, we do not know why Vγ4T cells could inhibit the osteogenic differentiation of MSCs *in vitro*. After detecting IL-17 and IFN-γ in the co-culture supernatant, we discovered that IL-17 and IFN-γ in the co-culture supernatant was suspected to downregulate the osteogenic differentiation of MSCs (**Figure 2C**). We also wanted to know whether inhibiting the function of Vγ4T cells would promote MSC bone formation.

The Expression of BTLA (CD272) in Vγ4T Cells and Changes in Surface Marker Expression in HVEM-Expressing MSCs

FACS analysis showed of BTLA (CD272) expression in 10.9% of activated Vγ4T cells but only in 1.69% of inactivated Vγ4T cells (**Figure 2D**). Twenty-four hours after transfection of MSCs with Ad-EGFP-HVEM recombinant adenovirus, we used flow cytometry to analyse the expression of surface markers on MSCs, EGFP-MSCs cells, and HVEM-MSCs, and there was no difference in the expression of CD11b, CD29 and CD34 among the three groups (**Figure 2E**).

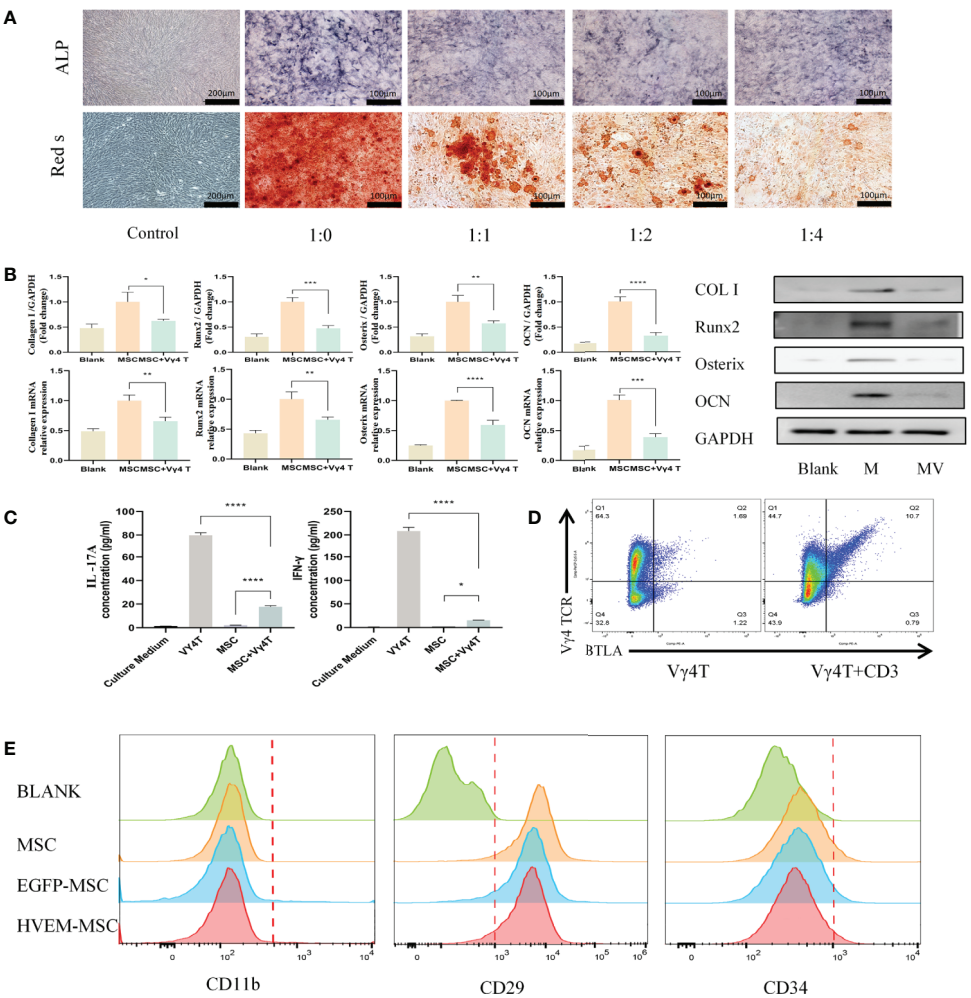

FIGURE 2 | Vγ4T cells inhibite the osteogenic differentiation of MSCs. **(A)** ALP staining and alizarin red staining show various concentrations of Vγ4T cells inhibited the osteogenic differentiation of MSCs. **(B)** Expression of markers of osteogenesis by MSCs with or not with Vγ4T cells(the ratio is 1:4) were measured by RT-PCR and Western blotting analyses, relative protein expression of COL I, Runx2, Osterix, and OCN by Western blotting, mRNA expression levels were tested by RT-PCR. **(C)** The concentration of IL-17A and IFN-γ was measured in the supernatant of co cultured cells for 3 days. **(D)** BTLA expression in Vγ4T cells. **(E)** Changes of surface markers of MSCs or HVEM- expressing MSCs (MSCs-HVEM). Error bars represent mean ± SD. One-way ANOVA test was used to calculated P value (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001).

Osteogenic Differentiation of HVEM-MSCs After Co-Culture With Vγ4T Cells *In Vitro*

According to the ALP and Alizarin Red staining results, the osteogenic differentiation ability of MSCs transfected with adenovirus carrying the HVEM gene was roughly the same as that of MSCs transfected with empty virus and non-transfected MSCs. The presence of HVEM could resist the inhibitory effect of Vγ4T cells on MSC osteogenesis(**Figure 3**). The co-cultured supernatant was removed and tested for IL-17A and IFN-γ levels by ELISA; the results indicate that HVEM significantly inhibited the secretion of IL-17A and IFN-γ by Vγ4T cells (**Figure 4A**). This suggested that HVEM promotes MSCs osteogenic differentiation because HVEM inhibited the function of Vγ4T cells and reduced the secretion of IL-17a and IFN-γ. Subsequently, we used Western blot and RT-PCR to detect the expression of MSC-related osteogenic genes after osteoinduction and found that the osteogenic expression in MSCs and EGFP-MSCs co-cultured with Vγ4T cells was weakened, while the expression in HVEM-MSCs co-cultured with Vγ4T cells returned to normal levels (**Figures 4B–D**).

Effects of MSCs-HVEM on Proliferation of MSCs and Vγ4T Cells

To determine the effect of HVEM on proliferation of MSCs and Vγ4T cells, MSCs or MSCs-HVEM were co-cultured with or without Vγ4T cells (ratio was 1:4). The result of CCK8 test showed that MSCs proliferation was obviously inhibited when co-cultured with Vγ4T cells and HVEM-expressing MSCs could reverse this trend. Compared with MSCs and MSCs-EGFP, MSCs-HVEM could also inhibit the proliferation of Vγ4T cells (**Figure 5A**).

IL-17 and IFN-γ Inhibited Osteogenesis of MSCs *In Vitro*

We co-cultured the MSCs and Vγ4T cells (ratio was 1:4) and with the treatment of IFN-γ, IFN-γ antibody (IFN-γ Ab), IL-17A and IL-17A antibody (IL-17A Ab) for 3 days, added osteoinduction medium, which was changed every 3 days. Western blot was used to detect the expression of MSC-related osteogenic genes, we found that treatment inhibited osteogenic differentiation of MSCs *in vitro*, it was the same as treatment with Vγ4T cells, added the antibody of IL-17 or IFN-γ into the co-culture system, the osteogenic differentiation of MSCs was better than those co-cultured with Vγ4T cells (**Figure 5B**).

Osteogenesis Repair Function of MSCs-HVEM in Bone Defect Areas *In Vivo*

Based on morphological analysis of mouse femur and TEB by micro-CT, 3D reconstruction of scan image and X-ray image using CTVox software, new bones could be seen in each group in the fracture area 12 weeks after surgery (except for the samples with bending fractures). Comparing the HVEM-expressing MSCs WT mouse group (WT+MSCs-HVEM) and HVEM-expressing MSCs Vγ4T cell-free mouse group (Vγ4-D+MSCs-HVEM), there were similar osteogenesis performances in terms of new bone volume, new bone density, number of bone trabeculae, and average trabecular bone thickness; any differences were not statistically significant.

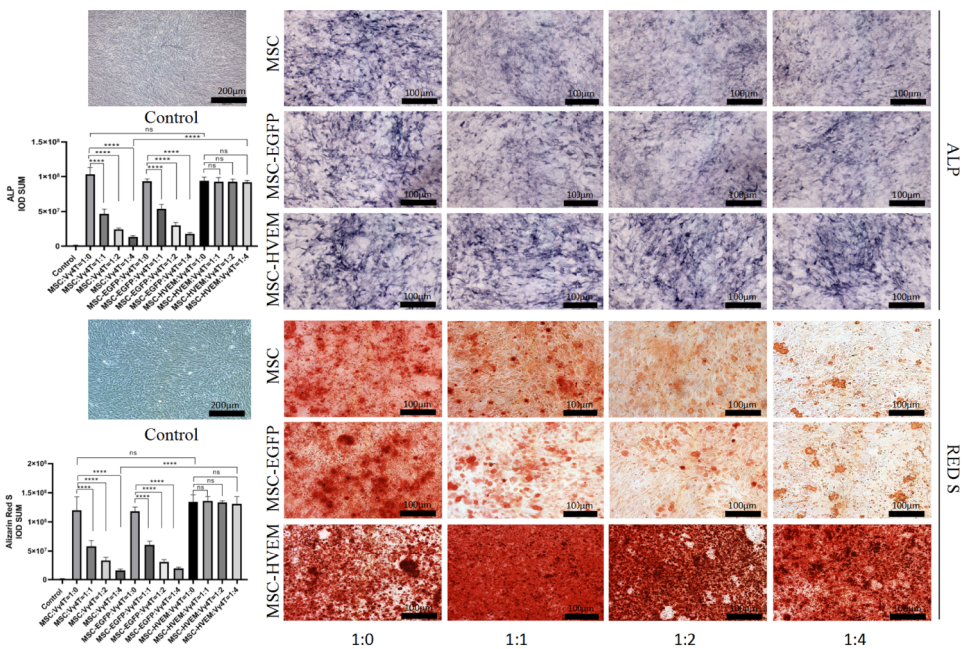

FIGURE 3 | Vγ4T cells co-culture with MSCs or MSCs-HVEM then induced in osteogenic medium and ALP staining and alizarin red staining show various concentrations of Vγ4T cells inhibited the osteogenic differentiation of MSCs but MSCs-HVEM could reverse the inhibition of Vγ4T cells. Error bars represent mean ± SD. One-way ANOVA test was used to calculated P value (***P < 0.0001, ns P > 0.05).

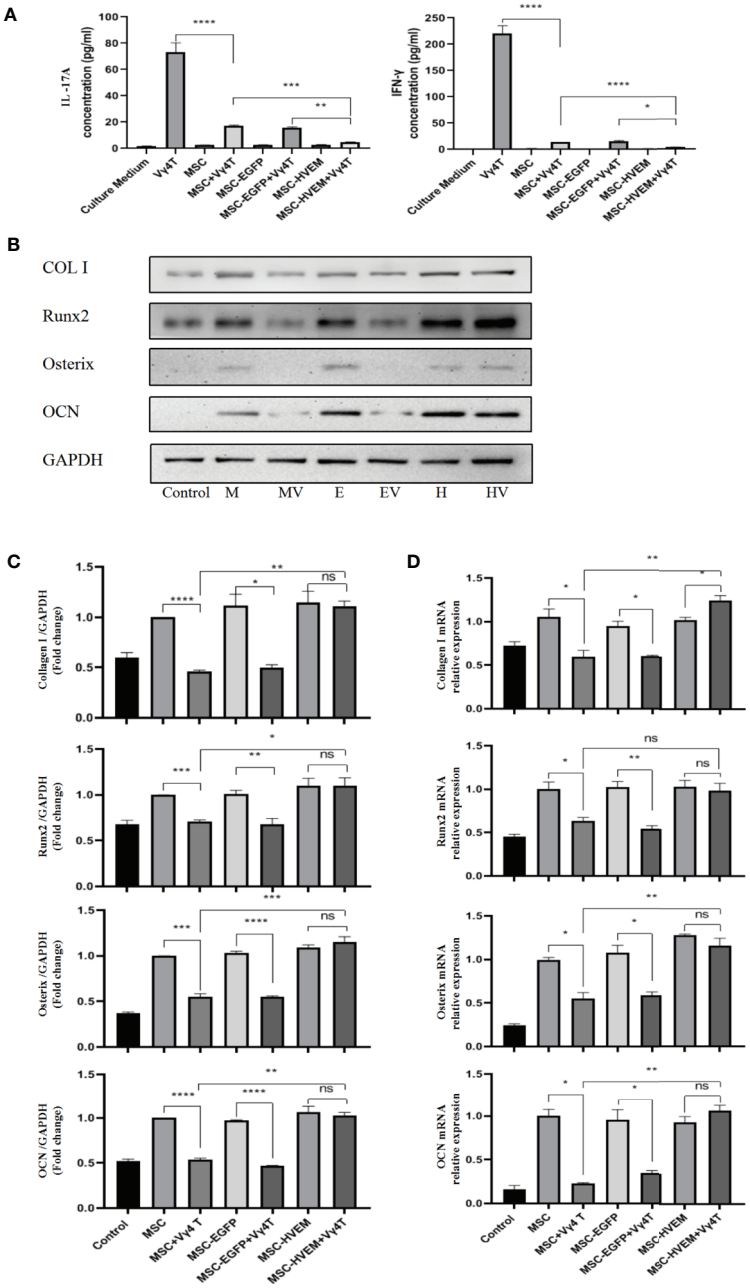

FIGURE 4 | Vγ4T cells co-culture with MSCs or MSCs-HVEM then induced in osteogenic medium. **(A)** MSC can inhibit IL-17A and IFN-γ secretion from purified Vγ4T cells cells, the inhibition ability of MSCs-HVEM was stronger than that of MSC cells. **(B, C)** The Expression of markers of osteogenesis such as COL I, Runx2, Osterix, and OCN by MSCs or MSCs-HVEM with or not with Vγ4T cells(the ratio is 1:4) were measured by Western blotting analyses, the osteogenic differentiation ability of MSC decreased when co-culture with Vγ4T cells but MSCs-HVEM can counter or even reverse that trend .MSCs (M),MSCs + Vγ4T cells (MV), MSCs-EGFP (E), MSCs-EGFP + Vγ4T cells (EV), MSCs-HVEM (H), MSCs-HVEM + Vγ4T cells (HV). **(D)** mRNA expression levelsof COL I, Runx2, Osterix, and OCN were tested by RT-PCR after 9 days of MSCs or MSCs-HVEM with or not with Vγ4T cells (the ratio is 1:4, relative to expression in MSCs showed the same trend. Error bars represent mean ± SD. One-way ANOVA test was used to calculated P value (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, ns P > 0.05).

Bothgroups showed better osteogenesis performance than did the EGFP-MSCs WT mouse group with respect to the same variables (**Figure 6A**). This meant that in the presence of Vγ4T cells, TEB osteogenesis was weakened, while HVEM expression on MSCs or clearance of Vγ4T cells reduced inflammation and restored TEB osteogenesis. The levels of bone repair in the TEB area were analysed by immunohistochemical analysis using H&E and Masson's trichrome staining at 12 weeks after implantation, which showed the same tendency (**Figure 6B**).

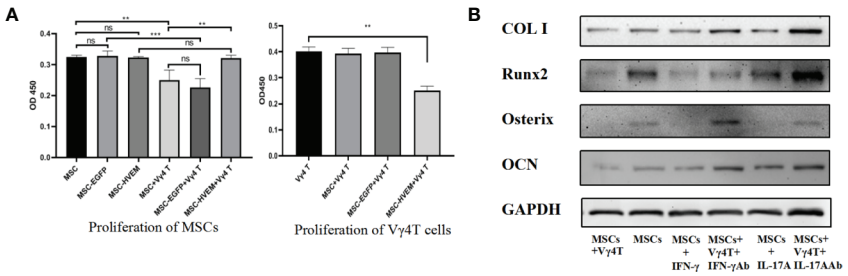

FIGURE 5 | The effect of Vγ4T cells on MSCs proliferation and osteogenic differentiation. **(A)** The proliferation of cell was measured by using CCK-8 assay on 3rd day. Absorbance values at 450 nm for each group are shown when MSCs or MSCs-HVEM co-culture with or not with Vγ4T cells. Data are reported as means ± SD. * $0.01 \le P \le 0.05$, ** $P \le 0.01$, *** $P \le 0.001$ (One Way ANOVA). **(B)** The Expression of markers of osteogenesis such as COL I, Runx2, Osterix, and OCN by MSCs with Vγ4T cells (the ratio is 1:4) treated with IFN-γ, IFN-γ antibody (IFN-γ Ab) , IL-17A, IL-17A antibody (IL-17A Ab) are shown. Error bars represent mean ± SD. One-way ANOVA test was used to calculated P value (**$P < 0.01$, ***$P < 0.001$, ns $P > 0.05$).

We also harvested bone grafts 3 and 7 days after implantation and performed H&E staining after pathological sectioning. The numbers of inflammatory cells in the HVEM-MSCs WT mouse groups and HVEM-MSCs Vγ4T cell-free mouse groups were both lower than those in the EGFP-MSCs WT mouse group, which indicated that HVEM-transformed MSCs could inhibit early inflammation, possibly by controlling Vγ4T cell function. The HVEM-MSCs WT mouse group and HVEM-MSCs Vγ4T cell-free mouse group expressed lower levels of inflammatory mediators than did the EGFP-MSCs WT mouse group based on immunohistochemical detection of IL-17A and IFN-γ expression (**Figure 6C**).

DISCUSSION

TEB repair of bone defects requires efficient seed cells as a core. At present, many seed cell studies involve MSCs with osteogenic differentiation abilities (23, 24). Unfortunately, autologous MSCs (auto-MSCs) are short in number, have a long preparation time, and cause donor area-related disease. By contrast, allo-MSCs provide the ability of pluripotent differentiation and the ability to overcome the abovementioned difficulties. Many scholars have shown that allo-MSCs from sheep, rats and mice are immunogenic *in vivo*, which may lead to graft failure without immunosuppressive therapies (7–9, 25, 26). Therefore, due to potential immune rejection, the clinical use of allo-MSCs is still controversial. The balance of inflammation is essential to regulate the osteogenesis of TEB to repair bone defects. Our previous studies have found that HVEM-modified MSCs promote TEB bone formation *in vivo* (16), but the relevant mechanism is still unclear. Vγ4T cells can promote acute rejection through IL-17 signalling and reduce the survival time of mice with transplanted hearts and skin (13). We speculate that HVEM inhibits the function of Vγ4T cells to promote MSCs bone formation *in vitro* and *in vivo*. In this study, we tried to determine the role of Vγ4T cells in TEB osteogenesis by eliminating Vγ4T cells and explored whether HVEM-modified MSCs can inhibit the function of Vγ4T cells and promote osteogenesis.

Our results showed that TEB osteogenesis was enhanced after Vγ4T cells were eliminated. In the early stage of implantation in Vγ4-D mice, the infiltration of inflammatory cells and the secretion of inflammatory factors in the implant area were reduced. Compared with allogeneic MSCs alone, allogeneic MSCs co-cultured with Vγ4T cells showed significantly reduced expression levels of the osteogenic markers COLI, Runx2, OSX, and OCN as well as lower numbers of mineralized nodules and reduced ALP activity when cultured in osteogenic medium. The concentrations of IL-17 and IFN-γ in the co-culture supernatant were higher than those in the MSCs monoculture supernatant. IL-17A and IFN-γ treatment inhibited osteogenesis of MSCs *in vitro*. These results confirmed our speculation that Vγ4T cells could inhibit MSCs osteogenic differentiation, possibly by secreting IL-17 and IFN-γ. These results are consistent with the findings of Spaggiari et al. (27–29). By contrast, compared with allogeneic MSCs under the same conditions, MSCs expressing HVEM maintained the expression of osteogenic markers, ALP activity and calcium nodule formation, thereby maintaining their osteogenic differentiation ability. HVEM-transfected MSCs inhibited the secretion of IL-17 and IFN-γ from Vγ4T cells and enhanced the osteogenic ability of TEB seed cells. These results are consistent with the findings of Dai et al., who confirmed that CTLA4- or HVEM-modified MSCs showed good heterotopic osteogenesis after subcutaneous implantation or transplantation into the femoral muscle pocket of rats as well as excellent repair effects on large orthotopic bone defects after xenotransplantation (1, 16, 30).

The mechanism by which HVEM suppresses the immune response and promotes osteogenesis has not been fully elucidated. Our previous study found that IL-17 treatment of MSCs can inhibit their osteogenic differentiation, but the presence of HVEM on MSCs can reverse this trend (16). Vγ4T cells, a cell type that plays an important role in immune diseases, is the main source of IL-17 in the early stage of the immune response (12, 31), and we observed that MSCs expressing HVEM significantly inhibited IL-17 and IFN-γ secretion from Vγ4T cells

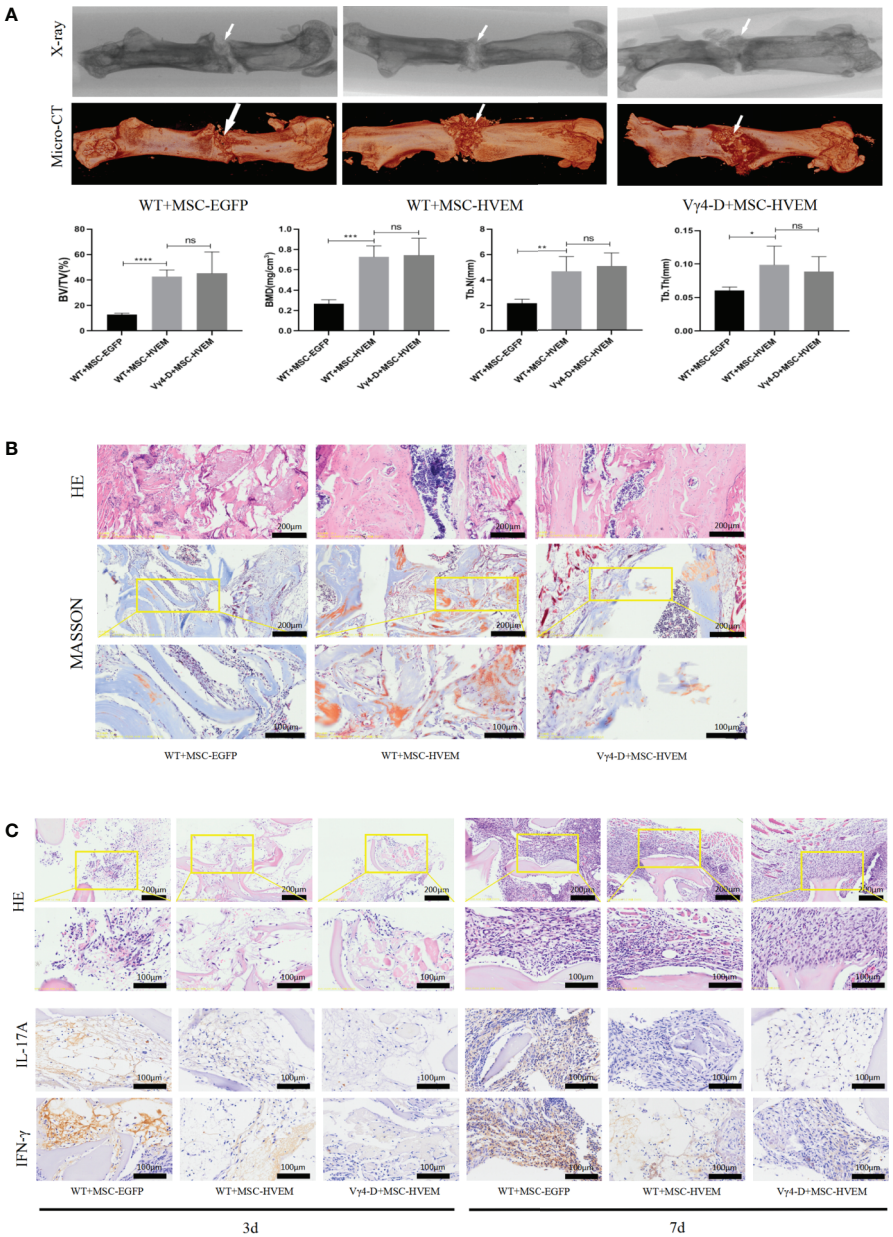

FIGURE 6 | Osteogenic ability and Local immune response of TEB constructed by MSC^{HVEM} in vivo. **(A)** Both WT+MSC^{HVEM} groups and VY4-D+MSC^{HVEM} groups show higher quality of bone formation than that of WT+MSC in BV/TV, BMD, Tb.N, Tb.Th, but the quality of bone formation of between WT+MSC^{HVEM} groups and VY4-D+ MSC^{HVEM} groups had no statistics difference ($P > 0.05$). **(B)** Histological analysis of newly formed bone by H&E and Masson's Trichrome staining in 3 groups. **(C)** Infiltration of inflammatory cells and expressions of IL-17A and IFN-γ detected by immunohistochemistry at 3 days and 7 days post implantation. Error bars represent mean ± SD. One-way ANOVA test was used to calculated P value (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, ns $P > 0.05$).

and the proliferation of Vγ4T cells when co-cultured, and also released the inhibitory effect of Vγ4T cells on MSCs proliferation during co-cultured. IL-17 not only is an effective stimulator of RANKL expression but also induces the synthesis of matrix-degrading enzymes to reduce the bone formation of MSCs (32, 33). Kim et al. found that rat calvarial cells express IL-17-related receptor subtypes (34). IFN-γ can enhance not only the immunogenicity of MSCs by increasing the expression of the major histocompatibility complex (MHC-II) (35) but also TNF-α signalling, leading to MSCs apoptosis and a decrease in osteogenic differentiation (27, 36). BTLA-HVEM, CTLA4 and PD1 are all negative immune cell regulators. Among them, only BTLA is highly expressed on γδT cells, thereby inhibiting the proliferation and function of T cells and thus playing an important role in the immune response (37). HVEM can induce inhibitory signal transduction of lymphocytes and

innate immune cells through BTLA (38). Our study showed that Vγ4T cells with CD3 stimulation could significantly enhance the expression of BTLA (**Figure 2D**). Through interaction between HVEM on MSCs and BTLA on Vγ4T cells, HVEM-expressing MSCs could inhibit Vγ4T cells to secrete IL-17 and IFN-γ, resulting in decreased inflammation and increased bone formation. It's reasonable to assume that the activation of BTLA *via* HVEM results in the consequent down-regulation of the NF-κB signalling pathway of Vγ4T cells (39, 40), thereby inhibiting IL-17A and IFN-γ secretion to promote effective bone formation. Additional studies by scholars have shown that the STAT3 and nuclear factor (NF)-κB signalling pathways and the key transcription factor RORγt strictly regulate the secretion of IL-17 (40). Chang et al. stated that the proinflammatory cytokine IL-17 stimulated the IκB kinase (IKK)-NF-κB signalling axis, thereby reducing the osteogenic differentiation of MSCs. By contrast, inhibiting IKK-NF-κB (IKKVI) signal transduction could significantly enhance MSC-mediated bone formation (41). The infiltration of inflammatory cells mediated by the proinflammatory factor IFN-γ was also negatively correlated with the late ossification of the grafts (42). Therefore, in allogeneic transplantation, after Vγ4T cells were cleared, the level of early inflammation, which promoted the effective formation of bone, was reduced. Of course, our study just demonstrated that Vγ4T cells were deeply involved in HVEM-mediated pro-osteogenesis of MSCs. The precise roles of Vγ4T cells in HVEM-mediated pro-osteogenesis of MSCs need to be further clarified by using animals with conditional depletion of BTLA in Vγ4T cells. Furthermore, Vγ4T cells as early major source of IL-17A and IFN-γ played an important role in initiating inflammation. However, the exactly effects of IL-17A and IFN-γ on MSCs osteogenesis had not been confirmed *in vivo*. IL-17A $^{-/-}$ or IFN-γ $^{-/-}$ mice should be used to answer this question in future study.

In conclusion, the current research shows that clearing Vγ4T cells in mice can reduce inflammation in the graft area and significantly improve graft osteogenesis. In addition, allo-MSCs expressing HVEM can also inhibit the function of Vγ4T cells *in vitro*, inhibit the proliferation of Vγ4T cells *in vitro*, release the inhibitory effect of Vγ4T cells on MSCs proliferation during co-culture, thereby reducing localized inflammation and enhancing graft osteogenesis. Our results provide a solid foundation for exploring HVEM-expressing MSCs as an ideal source of seed cells for xenogeneic bone transplantation in tissue engineering methods.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Ethics Committee of the Third Military Medical University.

AUTHOR CONTRIBUTIONS

Conceptualization: FD and WH. Methodology: LH, JX, LS, ZR, and RZ. Validation: LH, JX, LS, ZR, and RZ. Formal analysis: LH, JX, and LS. Investigation: LH and ZR. Resources: LH, JX, LS, ZR, and RZ. Writing-original draft: LH and JX. Writing-reviews and editing: FD and WH. Funding acquisition: FD. Supervision: FD and WH. All authors contributed to the article and approved the submitted version.

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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.2021.689269/full#supplementary-material

REFERENCES

1. Dai F, Shi D, He W, Wu J, Luo G, Yi S, et al. hCTLA4-Gene Modified Human Bone Marrow-Derived Mesenchymal Stem Cells as Allogeneic Seed Cells in Bone Tissue Engineering. *Tissue Eng* (2006) 12(9):2583–90. doi: 10.1089/ten.2006.12.2583
2. Tang D, Tare R, Yang L, Williams D, Ou K, Oreffo R. Biofabrication of Bone Tissue: Approaches, Challenges and Translation for Bone Regeneration. *Biomaterials* (2016) 83:363–82. doi: 10.1016/j.biomaterials.2016.01.024
3. Niemeyer P, Fechner K, Milz S, Richter W, Suedkamp N, Mehlhorn A, et al. Comparison of Mesenchymal Stem Cells From Bone Marrow and Adipose Tissue for Bone Regeneration in a Critical Size Defect of the Sheep Tibia and the Influence of Platelet-Rich Plasma. *Biomaterials* (2010) 31(13):3572–9. doi: 10.1016/j.biomaterials.2010.01.085
4. Xi J, Yan X, Zhou J, Yue W, Pei X. Mesenchymal Stem Cells in Tissue Repairing and Regeneration: Progress and Future. *Burns Trauma* (2013) 1 (1):13–20. doi: 10.4103/2321-3868.113330
5. Hu P, Yang Q, Wang Q, Shi C, Wang D, Armato U, et al. Mesenchymal Stromal Cells-Exosomes: A Promising Cell-Free Therapeutic Tool for Wound Healing and Cutaneous Regeneration. *Burns Trauma* (2019) 7:38. doi: 10.1186/s41038-019-0178-8
6. Ben-Ari A, Rivkin R, Frishman M, Gaberman E, Levdansky L, Gorodetsky R. Isolation and Implantation of Bone Marrow-Derived Mesenchymal Stem Cells With Fibrin Micro Beads to Repair a Critical-Size Bone Defect in Mice. *Tissue Eng Part A* (2009) 15(9):2537–46. doi: 10.1089/ten.tea.2008.0567
7. Coathup MJ, Kalia P, Konan S, Mirza K, Blunn GW. A Comparison of Allogeneic and Autologous Mesenchymal Stromal Cells and Osteoprogenitor Cells in Augmenting Bone Formation Around Massive Bone Tumor Prostheses. *J Biomed Mater Res Part A* (2013) 101(8):2210–8. doi: 10.1002/jbm.a.34536
8. Tasso R, Augello A, Boccardo S, Salvi S, Carida M, Postiglione F, et al. Recruitment of a Host's Osteoprogenitor Cells Using Exogenous Mesenchymal Stem Cells Seeded on Porous Ceramic. *Tissue Eng Part A* (2009) 15(8):2203–12. doi: 10.1089/ten.tea.2008.0269

9. Chatterjea A, LaPointe VL, Alblas J, Chatterjea S, van Blitterswijk CA, de Boer J. Suppression of the Immune System as a Critical Step for Bone Formation From Allogeneic Osteoprogenitors Implanted in Rats. *J Cell Mol Med* (2014) 18(1):134–42. doi: 10.1111/jcmm.12172
10. Huang Y, Getahun A, Heiser R, Detanico T, Aviszus K, Kirchenbaum G, et al. γδ T Cells Shape Preimmune Peripheral B Cell Populations. *J Immunol (Baltimore Md 1950)* (2016) 196(1):217–31. doi: 10.4049/jimmunol.1501064
11. Vermijlen D, Ellis P, Langford C, Klein A, Engel R, Willimann K, et al. Distinct Cytokine-Driven Responses of Activated Blood Gammadelta T Cells: Insights Into Unconventional T Cell Pleiotropy. *J Immunol (Baltimore Md 1950)* (2007) 178(7):4304–14. doi: 10.4049/jimmunol.178.7.4304
12. Li Y, Huang Z, Yan R, Liu M, Bai Y, Liang G, et al. Vγ4 γδ T Cells Provide an Early Source of IL-17A and Accelerate Skin Graft Rejection. *J Invest Dermatol* (2017) 137(12):2513–22. doi: 10.1016/j.jid.2017.03.043
13. Kimura N, Nakae S, Itoh S, Merk D, Wang X, Gong Y, et al. Potential Role of γδ T Cell-Derived IL-17 in Acute Cardiac Allograft Rejection. *Ann Thorac Surg* (2012) 94(2):542–8. doi: 10.1016/j.athoracsur.2012.03.049
14. Li Y, Wang Y, Zhou L, Liu M, Liang G, Yan R, et al. Vgamma4 T Cells Inhibit the Pro-healing Functions of Dendritic Epidermal T Cells to Delay Skin Wound Closure Through IL-17a. *Front Immunol* (2018) 9:240. doi: 10.3389/fimmu.2018.00240
15. Song Y, Li Y, Xiao Y, Hu W, Wang X, Wang P, et al. Neutralization of Interleukin-17A Alleviates Burn-Induced Intestinal Barrier Disruption Via Reducing Pro-Inflammatory Cytokines in a Mouse Model. *Burns Trauma* (2019) 7:37. doi: 10.1186/s41038-019-0177-9
16. Rong Z, Zhang F, Wang Z, He W, Dong S, Xu J, et al. Improved Osteogenesis by HVEM-Expressing Allogenic Bone Marrow-Derived Mesenchymal Stem Cells in an Immune Activation Condition and Mouse Femoral Defect Model. *Tissue Eng Part A* (2018) 24(15-16):1167–78. doi: 10.1089/ten.tea.2017.0354
17. Watanabe N, Gavrieli M, Sedy J, Yang J, Fallarino F, Loftin S, et al. BTLA is a Lymphocyte Inhibitory Receptor With Similarities to CTLA-4 and PD-1. *Nat Immunol* (2003) 4(7):670–9. doi: 10.1038/ni944
18. Albring J, Sandau M, Rapaport A, Edelson B, Satpathy A, Mashayekhi M, et al. Targeting of B and T Lymphocyte Associated (BTLA) Prevents Graft-Versus-Host Disease Without Global Immunosuppression. *J Exp Med* (2010) 207 (12):2551–9. doi: 10.1084/jem.20102017
19. Sakoda Y, Park J, Zhao Y, Kuramasu A, Geng D, Liu Y, et al. Dichotomous Regulation of GVHD Through Bidirectional Functions of the BTLA-HVEM Pathway. *Blood* (2011) 117(8):2506–14. doi: 10.1182/blood-2010-08-301325
20. Compaan D, Gonzalez L, Tom I, Loyet K, Eaton D, Hymowitz S. Attenuating Lymphocyte Activity: The Crystal Structure of the BTLA-HVEM Complex. *J Biol Chem* (2005) 280(47):39553–61. doi: 10.1074/jbc.M507629200
21. El-Zayadi A, Jones E, Churchman S, Baboolal T, Cuthbert R, El-Jawhari J, et al. Interleukin-22 Drives the Proliferation, Migration and Osteogenic Differentiation of Mesenchymal Stem Cells: A Novel Cytokine That Could Contribute to New Bone Formation in Spondyloarthropathies. *Rheumatology (Oxford England)* (2017) 56(3):488–93. doi: 10.1093/rheumatology/kew384
22. Li Y, Wang Y, Zhou L, Liu M, Liang G, Yan R, et al. Vγ4 T Cells Inhibit the Pro-healing Functions of Dendritic Epidermal T Cells to Delay Skin Wound Closure Through IL-17a. *Front Immunol* (2018) 9:240. doi: 10.3389/fimmu.2018.00240
23. Luu YK, Capilla E, Rosen CJ, Gilsanz V, Pessin JE, Judex S, et al. Mechanical Stimulation of Mesenchymal Stem Cell Proliferation and Differentiation Promotes Osteogenesis While Preventing Dietary-Induced Obesity. *J Bone Miner Res* (2009) 24(1):50–61. doi: 10.1359/jbmr.080817
24. Okada K, Kawao N, Tatsumi K, Ishida M, Takafuji Y, Kurashimo S, et al. Roles of Plasminogen in the Alterations in Bone Marrow Hematopoietic Stem Cells During Bone Repair. *Bone Rep* (2018) 8:195–203. doi: 10.1016/j.bonr.2018.04.005
25. Liu H, Kemeny DM, Heng BC, Ouyang HW, Melendez AJ, Cao T. The Immunogenicity and Immunomodulatory Function of Osteogenic Cells Differentiated From Mesenchymal Stem Cells. *J Immunol* (2006) 176 (5):2864–71. doi: 10.4049/jimmunol.176.5.2864
26. Li Z, Maitz P. Cell Therapy for Severe Burn Wound Healing. *Burns Trauma* (2018) 6:13. doi: 10.1186/s41038-018-0117-0
27. Liu Y, Wang L, Kikuiri T, Akiyama K, Chen C, Xu X, et al. Mesenchymal Stem Cell-Based Tissue Regeneration is Governed by Recipient T Lymphocytes Via IFN-γ and TNF-α. *Nat Med* (2011) 17(12):1594–601. doi: 10.1038/nm.2542
28. Yamaza T, Miura Y, Bi Y, Liu Y, Akiyama K, Sonoyama W, et al. Pharmacologic Stem Cell Based Intervention as a New Approach to Osteoporosis Treatment in Rodents. *PloS One* (2008) 3(7):e2615. doi: 10.1371/journal.pone.0002615
29. Spaggiari G, Capobianco A, Becchetti S, Mingari M, Moretta L. Mesenchymal Stem Cell-Natural Killer Cell Interactions: Evidence That Activated NK Cells Are Capable of Killing MSCs, Whereas MSCs Can Inhibit IL-2-Induced NK-Cell Proliferation. *Blood* (2006) 107(4):1484–90. doi: 10.1182/blood-2005-07-2775
30. Zhang F, Rong Z, Wang Z, Zhang Z, Sun D, Dong S, et al. Periostin Promotes Ectopic Osteogenesis of CTLA4-modified Bone Marrow Mesenchymal Stem Cells. *Cell Tissue Res* (2017) 370(1):143–51. doi: 10.1007/s00441-017-2655-3
31. Cai Y, Xue F, Fleming C, Yang J, Ding C, Ma Y, et al. Differential Developmental Requirement and Peripheral Regulation for Dermal Vγ4 and Vγ6T17 Cells in Health and Inflammation. *Nat Commun* (2014) 5:3986. doi: 10.1038/ncomms4986
32. Caetano-Lopes J, Canhão H, Fonseca J. Osteoimmunology–The Hidden Immune Regulation of Bone. *Autoimmun Rev* (2009) 8(3):250–5. doi: 10.1016/j.autrev.2008.07.038
33. Sato K, Suematsu A, Okamoto K, Yamaguchi A, Morishita Y, Kadono Y, et al. Th17 Functions as an Osteoclastogenic Helper T Cell Subset That Links T Cell Activation and Bone Destruction. *J Exp Med* (2006) 203(12):2673–82. doi: 10.1084/jem.20061775
34. Kim Y, Park J, Lee J, Suh J, Lee J, Chang B, et al. IL-17 Inhibits Osteoblast Differentiation and Bone Regeneration in Rat. *Arch Oral Biol* (2014) 59 (9):897–905. doi: 10.1016/j.archoralbio.2014.05.009
35. Chan J, Tang K, Patel A, Bonilla L, Pierobon N, Ponzio N, et al. Antigen-Presenting Property of Mesenchymal Stem Cells Occurs During a Narrow Window at Low Levels of Interferon-Gamma. *Blood* (2006) 107(12):4817–24. doi: 10.1182/blood-2006-01-0057
36. Wang L, Zhao Y, Shi S. Interplay Between Mesenchymal Stem Cells and Lymphocytes: Implications for Immunotherapy and Tissue Regeneration. *J Dental Res* (2012) 91(11):1003–10. doi: 10.1177/0022034512460404
37. Breloer M, Hartmann W, Blankenhaus B, Eschbach M, Pfeffer K, Jacobs T. Cutting Edge: The BTLA-HVEM Regulatory Pathway Interferes With Protective Immunity to Intestinal Helminth Infection. *J Immunol (Baltimore Md 1950)* (2015) 194(4):1413–6. doi: 10.4049/jimmunol.1402510
38. Steinberg M, Cheung T, Ware C. The Signaling Networks of the Herpesvirus Entry Mediator (TNFRSF14) in Immune Regulation. *Immunol Rev* (2011) 244 (1):169–87. doi: 10.1111/j.1600-065X.2011.01064.x
39. Shui J, Larange A, Kim G, Vela J, Zahner S, Cheroutre H, et al. HVEM Signalling at Mucosal Barriers Provides Host Defence Against Pathogenic Bacteria. *Nature* (2012) 488(7410):222–5. doi: 10.1038/nature11242
40. Kwok S, Cho M, Her Y, Oh H, Park M, Lee S, et al. TLR2 Ligation Induces the Production of IL-23/IL-17 Via IL-6, STAT3 and NF-kB Pathway in Patients With Primary Sjogren's Syndrome. *Arthritis Res Ther* (2012) 14(2):R64. doi: 10.1186/ar3780
41. Chang J, Liu F, Lee M, Wu B, Ting K, Zara J, et al. NF-κB Inhibits Osteogenic Differentiation of Mesenchymal Stem Cells by Promoting β-Catenin Degradation. *Proc Natl Acad Sci USA* (2013) 110(23):9469–74. doi: 10.1073/pnas.1300532110
42. Meyers C, Xu J, Zhang L, Asatrian G, Ding C, Yan N, et al. Early Immunomodulatory Effects of Implanted Human Perivascular Stromal Cells During Bone Formation. *Tissue Eng Part A* (2018) 24:448–57. doi: 10.1089/ten.tea.2017.0023

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