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Interactions between particulate matter and bacteria during cowshed PM_{2.5}-induced respiratory injury initiates GBP2/Caspase-11/NLRP3-mediated intracellular bacterial defense and pyroptosis

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Introduction: Fine particulate matter (PM_{2.5}) is an important factor in the induction of a variety of respiratory diseases and associated cellular damage. The composition of PM_{2.5} in the animal farm environments is complex, which poses a significant threat to the respiratory health of both workers and livestock, but the causative mechanisms are unclear.

Methods: In order to investigate targeted treatment options, this study focused on the role of microbial components in cowshed PM_{2.5}-induced respiratory damage. Utilizing the common pathogenic bacteria (*Pasteurella multocida*) in cowshed PM_{2.5} as a perspective, the intrinsic connection and interaction mechanism between PM_{2.5} particles and bacterial components were explored through *in vivo* and *in vitro* experiments. Bacterial components can interact with PM_{2.5} and are important factors in the respiratory toxicity of PM_{2.5} in farm animal environments by scanning electron microscopy (SEM), Fourier infrared spectroscopy (FTIR) and Zeta potential measurements.

Results: We demonstrate that Bacteria adhered to PM_{2.5} particles and modified the original surface functional groups characteristics, significantly enhanced toxic effects of PM_{2.5} on cells (including oxidative stress levels, release of inflammatory factors, etc.). Furthermore, PM_{2.5} particles significantly enhanced bacterial intracellular invasion, initiated the guanylate-binding protein 2 (GBP2)-mediated intracellular bacterial defense mechanism, further triggered the non-canonical NLRP3 pathway, and ultimately induced a cascade of inflammatory responses and pyroptosis. To explore therapeutic strategies, siRNA silencing of GBP2 and inhibition of NLRP3 were done; GBP2 silencing initially delayed cytotoxicity, but eventually increased the inflammatory response. However, inhibition of NLRP3 expression maintained cell viability and delayed pyroptosis, with potential as an effective solution for treatment of PM_{2.5}-induced lung injury in farm-animal environments.

Conclusion: In conclusion, the results of this study demonstrated the interaction between particulate matter and bacteria during cowshed PM_{2.5}-induced respiratory injury and clarified the signaling mechanisms among intracellular bacteria, GBP2, NLRP3, and pyroptosis. These findings provide a theoretical basis for developing therapeutic strategies against PM_{2.5}-related respiratory diseases in farm-animal environments.

KEYWORDS

the animal farm environment, PM_{2.5}, *Pasteurella multocida*, respiratory injury, GBP2, NLRP3

1 Introduction

Fine particulate matter (PM_{2.5}) is a global pollution in the air that affects human health (1). There is a clear association between PM_{2.5} and lung disease. According to the relevant statistics, the 3.7 million people in the same area, for every 10 µg/m³ increase in ambient PM_{2.5} concentration, physician visits for suspected pneumonia and number of respiratory infections increased by 6.32 and 4.72%, respectively (2). In addition to pneumonia, direct and indirect exposure to PM_{2.5} also contributes to chronic obstructive pulmonary disease (COPD) (3), asthma (4), pulmonary fibrosis (5) and even lung cancer (6). Therefore, elucidating mechanisms of PM_{2.5}-induced damage to the respiratory system and digging up effective strategies for treating PM_{2.5}-associated respiratory diseases based on this mechanism are crucial.

Composition, microscopic characteristics, and biological toxicity of PM_{2.5} vary among environments. Research has indicated that industrial activities, including vehicle exhaust and factory emissions, contribute to an increase in the presence of heavy metals and organic compounds in atmospheric PM_{2.5}. These components have been identified as the primary contributors to respiratory diseases caused by atmospheric PM_{2.5} (7). However, the composition of PM_{2.5} that induces respiratory diseases is also different in some specific environments. In the farm-animal environments, elevated stocking densities, poor ventilation, the equipment operation, and the frequent animal activity result in PM_{2.5} concentrations that are often maintained at high levels (6), whereas animal feed, feces, feathers, and bedding also affect PM_{2.5}, resulting in its higher microbial abundance (8). In addition, due to the smaller aerodynamic diameter of PM_{2.5}, it may transport disease-causing microorganisms to the end of the bronchi, thereby facilitating the transmission and induction of animal diseases. This is a major threat to staff health and animal health and production (9). At present, most studies focus on microbiological composition of PM_{2.5} in farm-animal environments. Some studies have found that poultry house PM_{2.5} contains pathogenic genera such as *Staphylococcus* and *Corynebacterium*, and harmful fungi such as *Aspergillus* and *Bombyx mori* (10). Bacterial aerosols in hog house also identified pathogenic bacterial genera such as *Streptococcus*, *Fusobacterium*, and *Pseudomonas* (11). In addition, Bacteria such as *Staphylococcus* and *Streptococcus* were also frequently detected in cowshed PM_{2.5} (12). The present studies have focused on the microbial composition of farmed environmental PM_{2.5}. However, the biotoxicity profile at higher PM_{2.5} microbial levels in inducing respiratory damage remains to be elucidated. Moreover, in the molecular mechanisms of respiratory damage caused by PM_{2.5} in animal farm environments, the intrinsic link between microbial components and PM_{2.5} particles are largely unknown.

In our previous study of the microbial diversity of ambient cowshed PM_{2.5} samples, *Pasteurella multocida* (*P. multocida*) was detected in all samples (13). *P. multocida* is a conditionally pathogenic bacterium, airborne transmission is an important means of its transmission, and it is a common causative agent of causing upper respiratory tract diseases (14). *P. multocida* has a wide range of hosts and can cause respiratory infections in a variety of domestic and wild animals, and it is the causative agent of bovine haemorrhagic

septicaemia (15), porcine pneumonic disease (16), avian cholera (17), and rabbit pasteurellosis (18), as well as bacteraemia, meningitis, etc., in humans (19, 20). However, there are doubts regarding the role of *P. multocida* in biological toxicity of PM_{2.5}, as well as interactions between *P. multocida* and PM_{2.5} and the specific mechanism of respiratory injury under the synergy between *P. multocida* and PM_{2.5}.

Organisms have complex self-defense mechanisms when faced with microbial invasion and infection. Among them, guanylate-binding proteins (GBPs), a conserved family of interferon-induced GTPases, which play a pivotal role in the immune system's defense against bacterial, viral and protozoan pathogens that infect the host (21). In our previous transcriptomics study by the research group, GBP2 was aberrantly expressed in alveolar macrophages by cowshed PM_{2.5} stimulation. GBP2, a member of the GBPs family, participate in host defense against intracellular pathogens (22). Studies have shown that GBP2 lyses vesicular membranes harboring pathogens, thereby facilitating host cell recognition and subsequent immune responses, exhibiting potent antimicrobial activity in both *in vivo* and *in vitro* models (23). Recent studies have suggested that GBP2 is closely linked to the activation of the NLRP3 inflammasome (24), but the mechanism is unclear. NLRP3 inflammasome, a member of the NOD-like receptor family, is widely distributed among various types of immune cells (25). As a key component of the innate immune system, NLRP3 can promote the generation of active Caspase-1, which drives maturation and secretion of inflammatory cytokines IL-1β and IL-18 (26). NLRP3 involves regulation and synergy of complex molecular signaling in cellular metabolic state, oxidative stress, pyroptosis and autophagy (27, 28). However, it is unknown whether GBP2 and NLRP3 have roles in cowshed PM_{2.5}-induced respiratory damage. In addition, mechanisms of activation of GBP2 and NLRP3 by cowshed PM_{2.5} and mechanisms of the transmission mechanism among the three are also unknown.

Given the many unknowns about the respiratory damage caused by cowshed PM_{2.5} as mentioned above, we hypothesized that microbial components are the primary reason of the observed cytotoxicity. Therefore, this study established an *in vivo* model of respiratory exposure to cowshed PM_{2.5} to investigate the specific mechanisms underlying the respiratory injury inflicted by cowshed PM_{2.5} on model animals (rats). *In vitro*, a model of synergistic infection of alveolar macrophages by *P. multocida* and PM_{2.5} was established, in order to investigate the intrinsic connections and interaction mechanisms between PM_{2.5} and microbial components. Furthermore, studies on the intrinsic association of GBP2 and NLRP3 were conducted, to determine mechanisms of respiratory damage and cellular defense in the presence of cowshed PM_{2.5}. These studies will inform treatment options for PM_{2.5}-induced respiratory system diseases in farm-animal environments.

2 Materials and methods

2.1 PM_{2.5} sample preparation

Based on previous studies by our research group (29), PM_{2.5} was collected using a multi-level flow particulate sampler on cattle farms

in Changchun, Jilin Province. These PM_{2.5} samples were analyzed for chemical and microbiological constituents (13). The PM_{2.5} standard (ERM-CZ110) was purchased from the JRC Science Hub and its main components are shown (Supplementary Table S1).

2.2 Animal model establishment and ethical statements

Experimental animals were selected 6-week-old specific pathogen free (SPF) grade Sprague Dawley (SD) rats (180–220 g) from Liaoning Changsheng Biotechnology Co, with ad libitum access to water and rat chow. After 1 week to acclimatize, exposure was done in an exposure box, as described in the group's previous studies (30, 31). During the experiment, rats were allocated into a Control group (exposed to clean air) and a PM_{2.5} exposure group (exposure to 4 times the cowshed environment PM_{2.5} concentration), 6 rats per group. The rats of the PM_{2.5} exposure group were exposed to PM_{2.5} for 6 h a day for 30 days, to be able to simulate the daily exposure patterns of animals in the cowshed. At the end of the experiment, to be able to simulate the daily exposure patterns of animals in the cowshed. Rats were anaesthetized by intraperitoneal injection of 3% sodium pentobarbital (40 mg/kg).

An ethical review of animal welfare was conducted by the Animal Experimentation Ethics Committee at Haihua Biotechnology Group Co., Ltd., who adhered to the ARRIVE guidelines and the Chinese National Standard Laboratory Animal Guidelines. The protocol was approved (Animal Experimentation Ethics Number: AUP-20231117-001).

2.3 Histopathological examination

Portions of lung were fixed in 4% paraformaldehyde, embedded in paraffin, cut into 5 µm sections, stained with hematoxylin and eosin (H&E), and observed with made a microscope (Phenix, China). Three samples were randomly selected from each group for histopathological assessment.

2.4 Lung wet/dry ratio

Immediately after death, a portion of the lung tissue of each mouse was excised and wet and dry, weights (after drying in an oven at 65°C for 24 h) were recorded and used to detect pulmonary edema (based on the W/D ratio of the lungs).

2.5 Cell model establishment and grouping

Rat alveolar macrophage cells (NR8383) were purchased from Shanghai Cell Bank and cultured in DMEM medium containing 10% fetal bovine serum and 1% penicillin–streptomycin (C0222). Cells were grown at 37°C in a 5% CO₂ environment and seeded into 6-well plates (10⁶ cells/well). Cells were cultured to 80% density and then required substances were added.

To determine effects of cowshed PM_{2.5} on NR8383 cells, we chose the concentration of cowshed PM_{2.5} based on the relevant literature (32, 33). These cells were divided into four groups (Control and low-,

medium- and high-concentrations of PM_{2.5} [0, 60, 120, and 240 µg/mL, respectively]), and stimulated for 12 h. To avoid other factors influencing the results, cells were infected with a mixture of *P. multocida* and PM_{2.5} standard to mimic the effects of microorganisms during cowshed PM_{2.5} infection. For this, cells were divided into Control group, PM_{2.5} standard group (120 µg/mL), *P. multocida* group (1 × 10⁷ CFU/mL) and Mixture group (PM_{2.5} standard + *P. multocida*, for intuitive comparison, half doses were used for both the PM_{2.5} standard and *P. multocida*), and stimulated for 12 h. In addition, referring to the relevant literature (34), an NLRP3 inhibitor (MCC950, HY-12815A, MCE, China) was used to validate the role of NLRP3 in cowshed PM_{2.5}-induced cellular damage. Cells were divided into the Control group, MCC950 group, PM_{2.5} group, and PM_{2.5} + MCC950 group, and stimulated for 12 h.

2.6 Bacterial strains and culture conditions

Pasteurella multocida strains were isolated from the cowshed PM_{2.5} samples. The strain was cultured and identified by streaking onto BHI agar plates and incubating at 37°C. Then, single colonies were picked and inoculated into the BHI liquid medium and incubated at 37°C with shaking. In the experiments, sterile and pyrogen-free BHI liquid medium was used to dilute the bacterial solution.

2.7 CCK-8

Cell viability was measured using the CCK-8 kit (C0037). For this, NR8383 cells were added to 96-well plates at a density of 5 × 10³ cells per well. After 12 h of stimulation under various conditions, 10 µL of CCK-8 was added to each well and incubated for 1 h. The OD values were read at 450 nm on an enzyme labeler. Cell viability was determined in accordance with the manufacturer's guidelines.

2.8 Oxidative stress indices

Kits were purchased from Nanjing Jiancheng Bioengineering Institute. Reactive oxygen species (ROS), malonic dialdehyde (MDA) and superoxide dismutase (SOD) were determined according to the manufacturer's instructions.

2.9 PM_{2.5} surface characterization

To investigate effects of *P. multocida* on surface properties of PM_{2.5}. Samples were divided into PM_{2.5} standard group, *P. multocida* group and PM_{2.5} standard + *P. multocida* group. Samples were fixed with 2% glutaraldehyde, dehydrated with anhydrous ethanol, and subsequently transferred to Shanghai Jiao Tong University for scanning electron microscopy acquisition, Zeta potential determination, and Fourier infrared spectroscopy.

2.10 Intracellular bacterial levels

Cells were seeded in 12-well plates at a density of 3 × 10⁵ cells/well using antibiotic-free medium and assigned to four groups: Control,

PM_{2.5} standard, *P. multocida*, and PM_{2.5} standard + *P. multocida*. Following 8 h of incubation at 37°C in 5% CO₂, the medium was replaced with DMEM containing 50 µg/mL gentamicin to eliminate extracellular bacteria. After 1 h, cells were lysed with 300 µL of 1% Triton X-100. Lysates were serially diluted 10-fold and plated on BHI agar for CFU enumeration.

2.11 siRNA transfection

GBP2 siRNA was transfected into NR8383 cells cultured in 6-well plates for 24 h using Lipofectamine 3000 (L3000015) following the manufacturer's protocol. Three individual siRNAs targeting GBP2 were used. After 12 h, the transfection medium was replaced with complete medium. Western blotting was performed 48 h post-transfection to validate GBP2 silencing efficiency. For experimental treatments, cells were divided into five groups: Control, NC-siRNA, GBP2-siRNA, PM_{2.5}, and PM_{2.5} + GBP2-siRNA.

2.12 ELISA

The levels of the inflammatory cytokine IL-1β were measured using ELISA kit (ml037361) according to the manufacturer's instructions.

2.13 LDH

The levels of the inflammatory cytokine LDH release were measured LDH cytotoxicity assay kit (C0016) according to the manufacturer's instructions.

2.14 Immunofluorescence

Immunofluorescence staining was performed to assess the expression of GSDMD-N in cells after receiving various stimuli. Cells were fixed with 4% paraformaldehyde for 20 min and permeabilized with 0.3% Triton-X 100 for 10 min. After blocking with BSA for 1 h at room temperature, cells were incubated with GSDMD-N antibody (1:100, ab215203) overnight at 4°C. CY3-coupled secondary antibody (1:100, SA00009-2) was incubated for 1 h at room temperature.

Following thorough washing, DAPI was used for nuclear staining, and images were captured under a fluorescence microscope (Olympus).

2.15 RT-qPCR

Total RNA was extracted from the cells using the Total RNA Extraction Kit (B511311) and reverse transcribed to cDNA using the PrimeScript™ RT Kit (RR047A). RT-qPCR was performed according to the instructions of the RT-qPCR kit. qPCR reactions were performed using TB Green Mix (RR820A). The target gene expression's CT value was then contrasted with that of the Control group. GAPDH was employed as a housekeeping control. The 2^{-ΔΔCT} method was used to conduct a relative quantitative analysis. The following primer sequences were used (Table 1).

2.16 Western blotting

RIPA lysate containing 1% PMSF (C500005) was used to lyse cultured cells to extract total protein. Sample protein concentrations were determined using a BCA protein quantification kit (P0010S), denatured and subjected to SDS-PAGE gel electrophoresis. Proteins were transferred to PVDF membranes (GVWP04700) and blocked with 5% skimmed milk powder for 2 h. Then, the membrane was incubated overnight with the corresponding primary antibody, and secondary antibodies were incubated for 2 h at room temperature. After sufficient washing using TBST solution, an ECL luminescence assay (PW30601S) was performed to assess protein expression. Protein bands were detected using an Amersham Imager 680. Image analysis was performed using ImageJ (Version: 1.54f). The details of the antibodies used are provided in Supplementary Table S2.

2.17 Statistical analysis

Each experiment was repeated at least three times and values expressed as the mean ± SD of the measurements, with plots using GraphPad Prism version 8.0 software (GraphPad Software, San Diego, CA, USA). Statistical comparisons were made using unpaired Student's *t*-tests. A one-way analysis of variance (ANOVA) was used to compare data between multiple groups, and the Tukey–Kramer post-test was used to locate differences. Statistical significance was defined as *p* < 0.05.

TABLE 1 qPCR primer sequence.

Gene	Forward primer (5' → 3')	Reverse primer (5' → 3')
<i>NLRP3</i>	GAGCTGGACCTCAGTGACAATGC	AGAACCAATGCGAGATCCTGACAAC
<i>IL-1β</i>	AGTGAGGAGAATGACCTGTTC	CGAGATGCTGCTGTGAGATT
<i>IL-18</i>	CGAACAGCCAACGAATCCCAGAC	TCACAGATAGGGTCACAGCCAGTC
<i>Caspase-1</i>	GCACAAGACTTCTGACAGTACCTTCC	GCTTGGGCACTTCAATGTGTTTCATC
<i>Caspase-11</i>	TTGGGCTATGATGTGGTGGTGAAG	TGCTGTCTGATGTTTGGTGCTCTG
<i>GAPDH</i>	CCTGCACCACCAACTGCTTA	CATCACGCCACAGCTTTCCA

3 Results

3.1 Cowshed PM_{2.5} induced rat lung and *in vitro* alveolar macrophage damage

To assess the effects of cowshed PM_{2.5} on the respiratory system, changes of *in vivo* rat pulmonary assessments and *in vitro* alveolar macrophages were independently evaluated after exposure to cowshed PM_{2.5}. *In vivo*, in comparison with the Control group, lung tissue obvious pathological damage, including alveolar hemorrhage, alveolar wall thickening, inflammatory cell infiltration, and alveolar interstitial exudates (Figure 1A). Moreover, comparison to the Control group, exposure of rats to cowshed PM_{2.5} significantly increased the lung W/D ratio and concentrations IL-1 β and IL-18 (Figures 1B–E).

Given the crucial role of alveolar macrophages in defending against PM_{2.5} pulmonary invasion (35), rat alveolar macrophages (NR8383) was selected for *in vitro* experiments. There was a notable decline in cell viability with as PM_{2.5} concentration increased (Figure 1F). Moreover, cell viability approached 50% at the exposure concentration of 240 μ g/mL. Thus, 120 μ g/mL was chosen for subsequent experiments. Regarding oxidative stress, there was a significant decrease in SOD and significant increases in MDA and ROS compared to the Control group, (Figures 1G–J). Furthermore, consistent with *in vivo* findings, exposure to cowshed PM_{2.5} significantly elevated IL-1 β and IL-18 (Figures 1K–M). In conclusion, the *in vivo* and *in vitro* fundamental data demonstrate that cowshed PM_{2.5} is respiratory toxic and poses a significant threat to animal respiratory health.

3.2 Microbiological components are important reason for cowshed PM_{2.5}-induced respiratory damage, and bacteria strongly amplified toxic effects of PM_{2.5} particles on cells

To determine the role of microbial components while minimizing alterations to cowshed PM_{2.5} composition, heat inactivation treatment was done and cellular toxicity assessed. Cell viability exhibited partial restoration (Figure 2A), concomitant with the restoration of ROS, MDA, and SOD contents in the cowshed PM_{2.5} inactivation group (Figures 2B,C,E,F), in comparison to the cowshed PM_{2.5} group. Furthermore, restoration of inflammatory factors IL-1 β and IL-18 concentrations was most prominent (Figures 2I–K). The results provide strong evidence that the presence of microorganisms is a significant cause of cowshed PM_{2.5}-induced respiratory damage.

The microbial species in cowshed PM_{2.5} are diverse, in order to explore the mechanisms and intrinsic links between microbial components in their induction of cytotoxicity, it is first necessary to clear components and control variables. In this regard, this study used PM_{2.5} standard with well-defined compositions, as well as *P. multocida*, a common pathogenic bacteria in cowshed environments to simulate cowshed PM_{2.5} and reevaluated cellular toxicity. The results showed that although both the PM_{2.5} standard group and the *P. multocida* group showed some cytotoxicity. However, the Mixture group at half the dose showed more significant cytotoxicity. Compared to the PM_{2.5} standard group, the Mixture group had significant decreases in cell viability and the antioxidant factor SOD (Figures 2D,P), significant

increases in the oxidant factors MDA and ROS, and a more pronounced increase in the inflammatory factors IL-1 β and IL-18 (Figures 2G–H,L,O). These results indicate that the bacterial component appears to strongly amplify the cytotoxic effect of PM_{2.5} during PM_{2.5} stimulation of cells, although at a much lower dose.

3.3 Interactions between bacteria and PM_{2.5} particles enhanced intracellular invasion and surface group modification

To clarify the mechanism of cowshed PM_{2.5}-induced respiratory damage, this study focused on the interaction between PM_{2.5} particles and bacteria. Firstly, with regard to effects of PM_{2.5} on bacteria, compared to the *P. multocida* single group, the PM_{2.5} standard + *P. multocida* group showed a significant increase in intracellular bacterial count (Figure 3A), indicating that PM_{2.5} enhances bacterial entry into the cytosol. The elevated viable bacteria in the cytosol may be a key factor for enhanced toxicity. Scanning electron microscopy further revealed that PM_{2.5} particles could adhere to and encapsulate bacteria (Figure 3B), directly explaining the increased intracellular bacterial load in the PM_{2.5} standard + *P. multocida* group.

In addition, whether the bacterial component also alters the surface properties of PM_{2.5} particles is unclear. To evaluate bacterial effects on PM_{2.5} particles, we first measured Zeta potential changes. The findings indicated a notable negative shift in zeta potential in the Mixture group in comparison with the PM_{2.5} standard group (Figure 3C). The significant changes in zeta potential indicate greater stability within the system (36). This suggests that the bacterium enhances the anti-aggregation and anti-deposition properties of PM_{2.5} particles. Subsequently, every group was subjected to analysis by FTIR. The results demonstrated that the absorption peaks of the PM_{2.5} standard included O–H and methyl stretching vibrations, etc., whereas absorption peaks of *P. multocida* included antisymmetric and symmetric stretching vibrations of amino N–H, and stretching vibrations of amide C=O, amongst others. In comparison to the PM_{2.5} standard group, the absorption peak at 3,385 cm^{−1} in the PM_{2.5} standard + *P. multocida* group was red-shifted towards 3,300 cm^{−1}. The change there suggests that bacterial cells induced elongation of O–H and N–H bonds in the PM_{2.5} standard + *P. multocida* group, leading to hydrogen bond association. The enhanced absorption peak at 1,650 cm^{−1} and the emergence of a new peak at 527 cm^{−1} suggest specific binding between bacterial organic groups and PM_{2.5} particles, which potentiates the biological toxicity of PM_{2.5} (Figure 3D). These alterations in the peaks of absorption indicated that *P. multocida* modified the surface groups of the PM_{2.5} standard. The results supported the hypothesis that interactions occurred between the bacterium and PM_{2.5} particles and indicated that the two were mutually promoting in terms of enhancing cytotoxicity.

3.4 Cowshed PM_{2.5} activates cellular GBP2 expression in response to defense against intracellular bacteria

Based on the finding that PM_{2.5} increases the probability of bacteria entering the cytosol as an important cause of cytotoxicity. This study focuses on digging into the signaling of alveolar

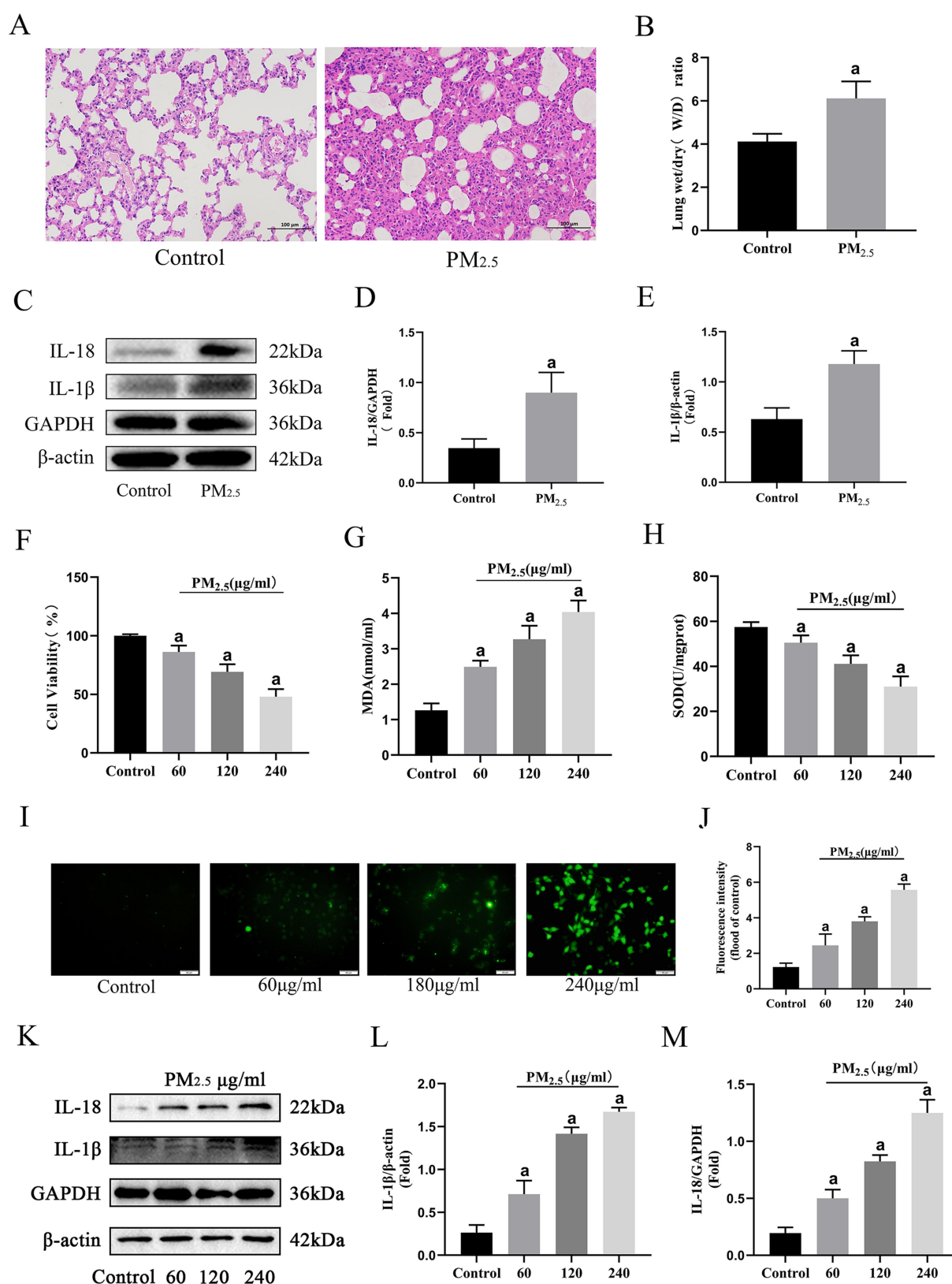


FIGURE 1

Cowshed PM_{2.5} induced rat lung and *in vitro* alveolar macrophage damage. (A) H&E stained sections of rat lungs after cowshed PM_{2.5} exposure treatment. Scale bar = 100 μ m. (B) W/D ratio in rat lung. (C–E) Expression levels of inflammatory factors in rat lungs. (F) Cell viability levels of NR8383 cells upon exposure to cowshed PM_{2.5}. (G–J) Oxidative stress in NR8383 cells during exposure to cowshed PM_{2.5}. (K–M) Inflammatory factor levels in NR8383 cells during exposure to cowshed PM_{2.5}. Results are expressed as mean \pm SD deviation of three determinations. ^a p < 0.05, compared to the Control group.

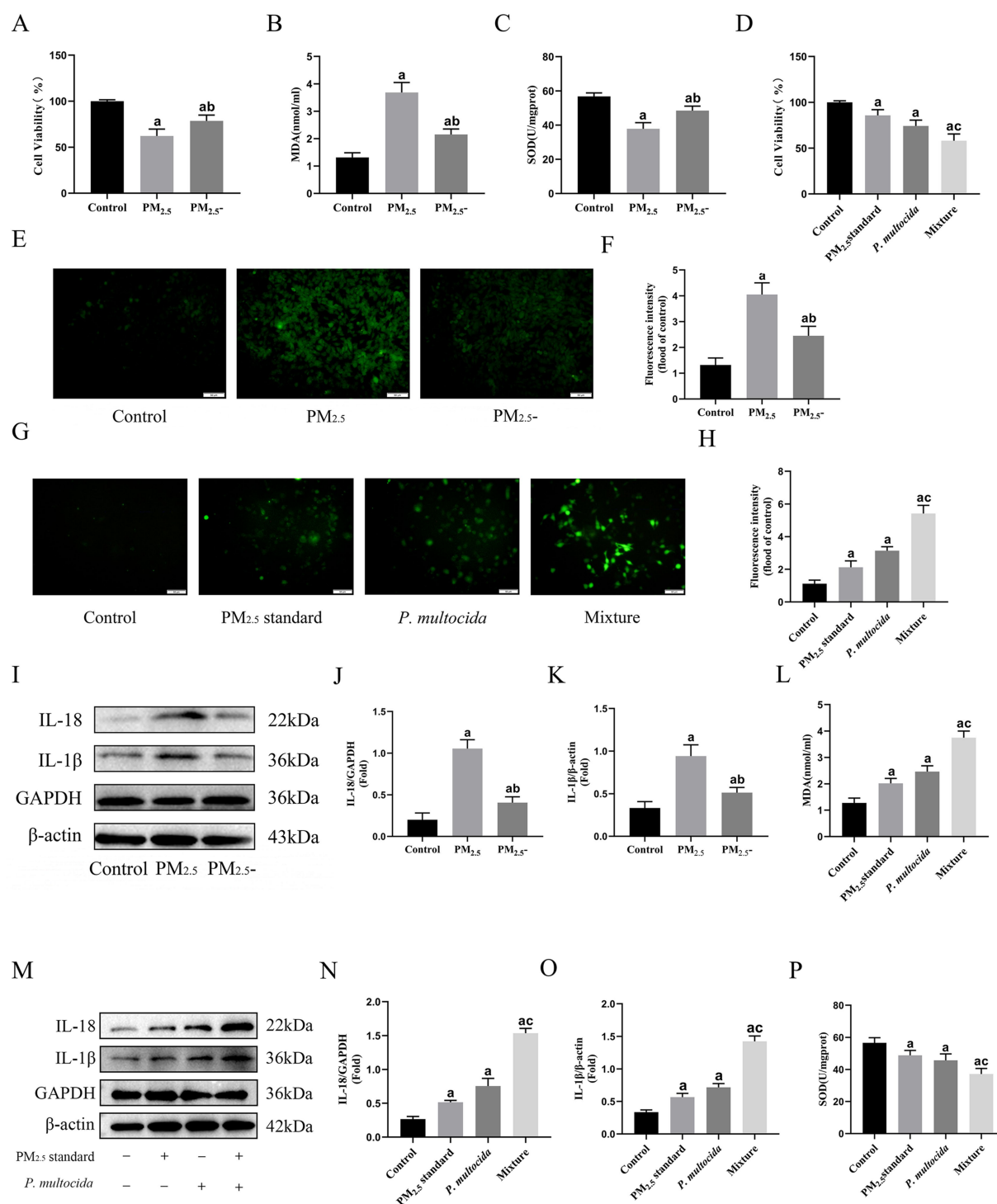


FIGURE 2

Microbiological components are important reason for cowshed PM_{2.5}-induced respiratory damage, and bacteria strongly amplified toxic effects of PM_{2.5} particles on cells. (A) Viability of NR8383 cells after inactivation of cowshed PM_{2.5}. (B,C,E,F) Oxidative stress on NR8383 cells after inactivation of cowshed PM_{2.5}. (D) Cell viability under the synergistic effect of PM_{2.5} standard and *P. multocida*. (G,H,L,P) Oxidative stress in NR8383 in response to the synergistic effect of PM_{2.5} standard and *P. multocida*. (I–K) Inflammatory factors in NR8383 cells after inactivation of cowshed PM_{2.5}. (M–O) Inflammatory factors in NR8383 cells under the synergistic effects of PM_{2.5} standard and *P. multocida*. Results are expressed as mean ± SD deviation of three determinations. **p* < 0.05, compared to the Control group. ***p* < 0.05, the PM_{2.5}-group compared to the PM_{2.5} group. ****p* < 0.05, the Mixture group compared to the PM_{2.5} standard group.

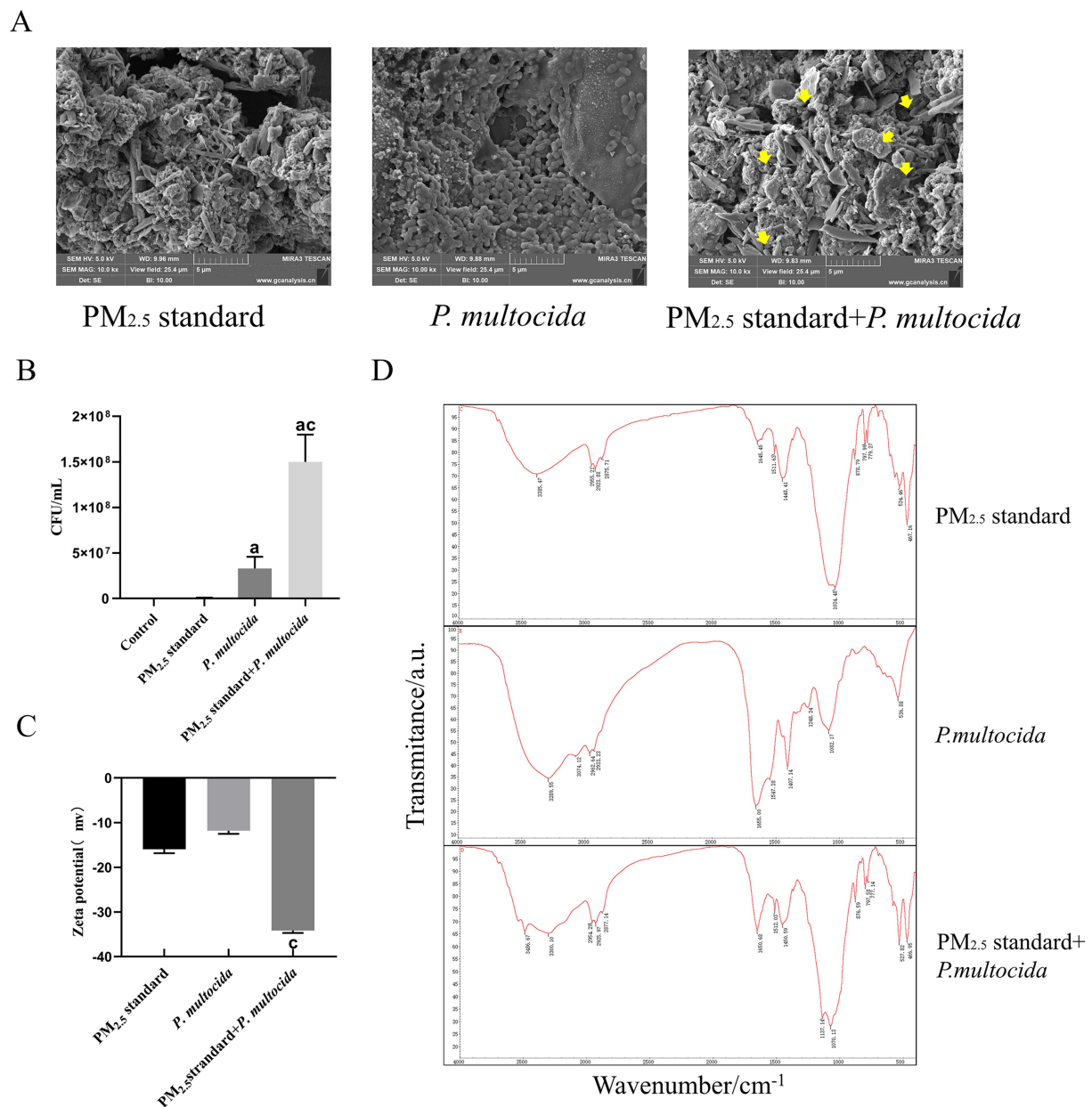


FIGURE 3

Interactions between bacteria and PM_{2.5} particles enhanced intracellular invasion and surface group modifications. **(A)** Scanning electron microscopy revealed *P. multocida* adhered to PM_{2.5} particles. **(B)** Significant increase in the number of intracellular bacteria after synergistic interaction of PM_{2.5} standard and *P. multocida*. **(C)** Zeta potential shifted significantly in the negative direction after synergistic interaction of PM_{2.5} standard and *P. multocida*. **(D)** Changes in PM_{2.5} surface groups after synergistic interaction of PM_{2.5} standard and *P. multocida*. Results are expressed as mean \pm SD deviation of three determinations. ^a $p < 0.05$, compared to the Control group. ^b $p < 0.05$, compared to the PM_{2.5} standard group.

macrophages to intracellular bacterial defenses to investigate the specific mechanisms of cowshed PM_{2.5} respiratory toxicity. Based on a bioinformatics screen for cowshed PM_{2.5}-induced cellular differential genes, there was a significant increase in guanylate binding protein 2 (GBP2) expression in the lung under the role of cowshed PM_{2.5}. Under the role of cowshed PM_{2.5}, the mRNA and protein expression levels of GBP2 are shown (Figures 4A–C). However, whether the high expression of GBP2 was induced by an increase in intracellular bacteria and whether it was involved in the host cell defense process against gram-negative cytoplasmic bacteria was not clear. Firstly, still used PM_{2.5} standard and *P. multocida* to simulate cowshed PM_{2.5}. In

comparison to the Control group, neither PM_{2.5} standard nor *P. multocida* groups effectively activated GBP2 expression. However, GBP2 expression was significantly higher in the Mixture group (Figures 4D,E). This result provides substantial evidence that, under the influence of PM_{2.5} in the cowshed, GBP2 expression is induced by an increase in intracellular bacteria.

In order to further validation of the above results, siRNA was used to silence GBP2 expression. First, the silencing effect was tested for three siRNAs. The results showed that all three siRNAs effectively inhibited GBP2 mRNA expression; however, as siRNA-447 had the best silencing effect (Figures 4F,G). Therefore,

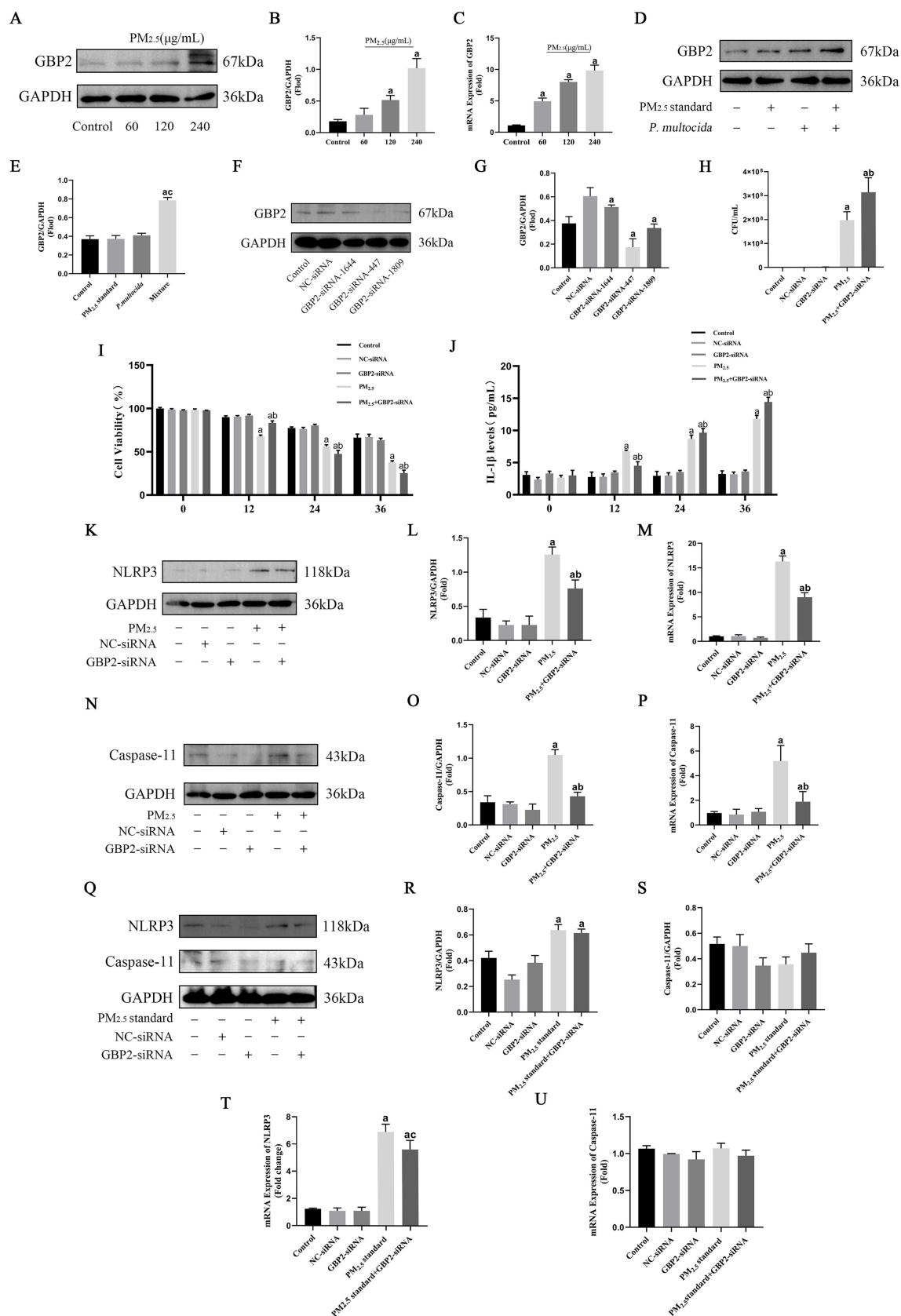


FIGURE 4

Cowshed PM_{2.5} activates cellular GBP2 expression in response to defenses against intracellular bacteria, whereas recognition of intracellular bacteria by GBP2 activates Caspase-11-mediated non-classical NLRP3. (A,B) Expression of GBP2 protein in NR8383 after cowshed PM_{2.5} treatment was detected

(Continued)

FIGURE 4 (Continued)

by Western blotting. (C) Expression of GBP2 mRNA in NR8383 after cowshed PM_{2.5} treatment was detected by qRT-PCR. (D,E) Expression of GBP2 under the synergistic effect of PM_{2.5} standard and *P. multocida*. (F,G) Silencing effect was verified for three siRNAs of GBP2. (H) The number of intracellular bacteria after interference with GBP2 under the influence of cowshed PM_{2.5}. (I) Changes in cell viability over time after interfering with GBP2 under the influence of cowshed PM_{2.5}. (J) Changes in IL-1 β over time after interfering with GBP2 under the influence of cowshed PM_{2.5}. (K–M) Decreased expression of NLRP3 after interference with GBP2 under the influence of cowshed PM_{2.5}. (N–P) Decreased expression of Caspase-11 after interference with GBP2 under the influence of cowshed PM_{2.5}. (Q–U) Interference with NLRP3 and Caspase-11 expression after interference GBP2 under the influence of PM_{2.5} standard. Results are expressed as mean \pm SD deviation of three determinations. ^a*p* < 0.05, compared to the Control group. ^b*p* < 0.05, compared to the PM_{2.5} group, ^c*p* < 0.05, compared to PM_{2.5} standard group.

siRNA-447 was selected for the following studies. Then, the number of intracellular bacteria was assessed after stimulation of normal and GBP2-silenced cells with cowshed PM_{2.5} for 24 h each. It was found that the number of intracellular bacteria was significantly increased in the GBP2-silenced group compared to the unsilenced group (Figure 5H). This suggests that GBP2 plays an important role in the intracellular bacterial defense of host cells against cowshed PM_{2.5} invasion. In addition, during the above process, cell viability of the silenced group was relatively high compared to the unsilenced group for 12 h. However, after 24 h, cell viability plummeted, and after 24 h, cell viability of the unsilenced group was higher than that of the silenced group (Figure 4I). The expression level of IL-1 β in the silenced group was initially lower than in the unsilenced group, but over time, expression of IL-1 β in the silenced group increased abruptly and was higher than in the unsilenced group (Figure 4J). These data suggest that GBP2 is able to intervene in the cowshed PM_{2.5}-induced cytotoxic response, but appears to have complex intrinsic mechanisms.

3.5 Recognition of intracellular bacteria by GBP2 activated Caspase-11-mediated non-classical NLRP3

IL-1 β is an important effector of NLRP3 expression, and in order to explain the phenomenon of the lag in changes in cell viability and IL-1 β expression after GBP2 silencing in the above study. This study focused on investigating the relationship between GBP2 silencing and changes in NLRP3 expression. The results showed that NLRP3 was significantly highly expressed under the effect of cowshed PM_{2.5}, whereas silencing GBP2 decreased expression of NLRP3 (Figures 4K–M). This suggests a link between GBP2 and NLRP3. The activation pathway of NLRP3 is relatively complex, and to further explore the relationship between GBP2 and NLRP3, this study focused on the intracellular Caspase-11-mediated non-classical NLRP3 activation pathway. The results showed a relative decrease in the expression of Caspase-11 in the silenced group compared to the unsilenced group (Figures 4N–P), suggesting that GBP2 was associated with the non-classical NLRP3 activation pathway mediated by Caspase-11. Furthermore, silencing or non-silencing of GBP2 had no significant effect on the expression of Caspase-11 and NLRP3 when cells were stimulated with PM_{2.5} standard (Figures 4Q–U). This strongly supports that under the effects of cowshed PM_{2.5}, GBP2 intervenes with the hypothesis that Caspase-11-mediated nonclassical NLRP3 activation is associated with the presence of intracellular bacteria. The phenomenon of the lag in changes in

cell viability and IL-1 β expression after GBP2 silencing may be influenced by the failure to activate Caspase-11 in time to mediate non-classical NLRP3.

3.6 PM_{2.5} enhanced intracellular invasion of *Pasteurella multocida*, initiated Caspase-11-mediated non-classical NLRP3 activation, and exacerbates pyroptosis

To further investigate in depth the mechanisms of respiratory toxicity induced by signal transduction in alveolar macrophages in response to intracellular bacterial defenses, similarly, PM_{2.5} standard and *P. multocida* were used to simulate cowshed PM_{2.5}. Although only half the dose was used in the Mixture group, in comparison to the PM_{2.5} standard group, expression levels of NLRP3, ASC, cleaved-Caspase-1, and Caspase-11 were elevated (Figures 5A–H). This suggests that during this process, the intracellular transport function of *P. multocida* due to PM_{2.5} particles plays a crucial role in Caspase-11-mediated non-classical NLRP3 activation. This data further elucidates why the Mixture group has an amplifying effect on cytotoxicity in the above results. Activation of the NLRP3 is a significant factor in the induction of pyroptosis, a major manifestation of cellular toxicity, during cellular physiological processes (37). Consequently, alterations in the level of pyroptosis were measured. The results showed that expression levels of GSDMD-N and LDH were significantly increased in the Mixture group compared to the PM_{2.5} standard group (Figures 5I–K). It is shown that pyroptosis is an effective form of cellular damage in the above process and is associated with enhanced intracellular invasion of *P. multocida* by PM_{2.5}.

3.7 Intervention of NLRP3 expression was an effective respond to cowshed PM_{2.5}-induced cellular damage

In this study, although GBP2 silencing initially preserved cell viability, the loss of intracellular bacterial control led to delayed cytotoxicity, indicating that targeting this Gram-negative bacterial defense protein is not a viable therapeutic strategy. Conversely, since cowshed PM_{2.5} triggers Caspase-11-mediated non-classical NLRP3 activation, NLRP3 inhibition was evaluated using an NLRP3 inhibitor (MCC950, MCE). As shown in Figures 6A–C, activation of NLRP3 was significantly inhibited in the PM_{2.5} + MCC950 group compared to the PM_{2.5} group. In addition, the expression levels of cleaved-Caspase-1, IL-1 β and IL-18 were also significantly reduced (Figures 6E–K). In terms of pyroptosis,

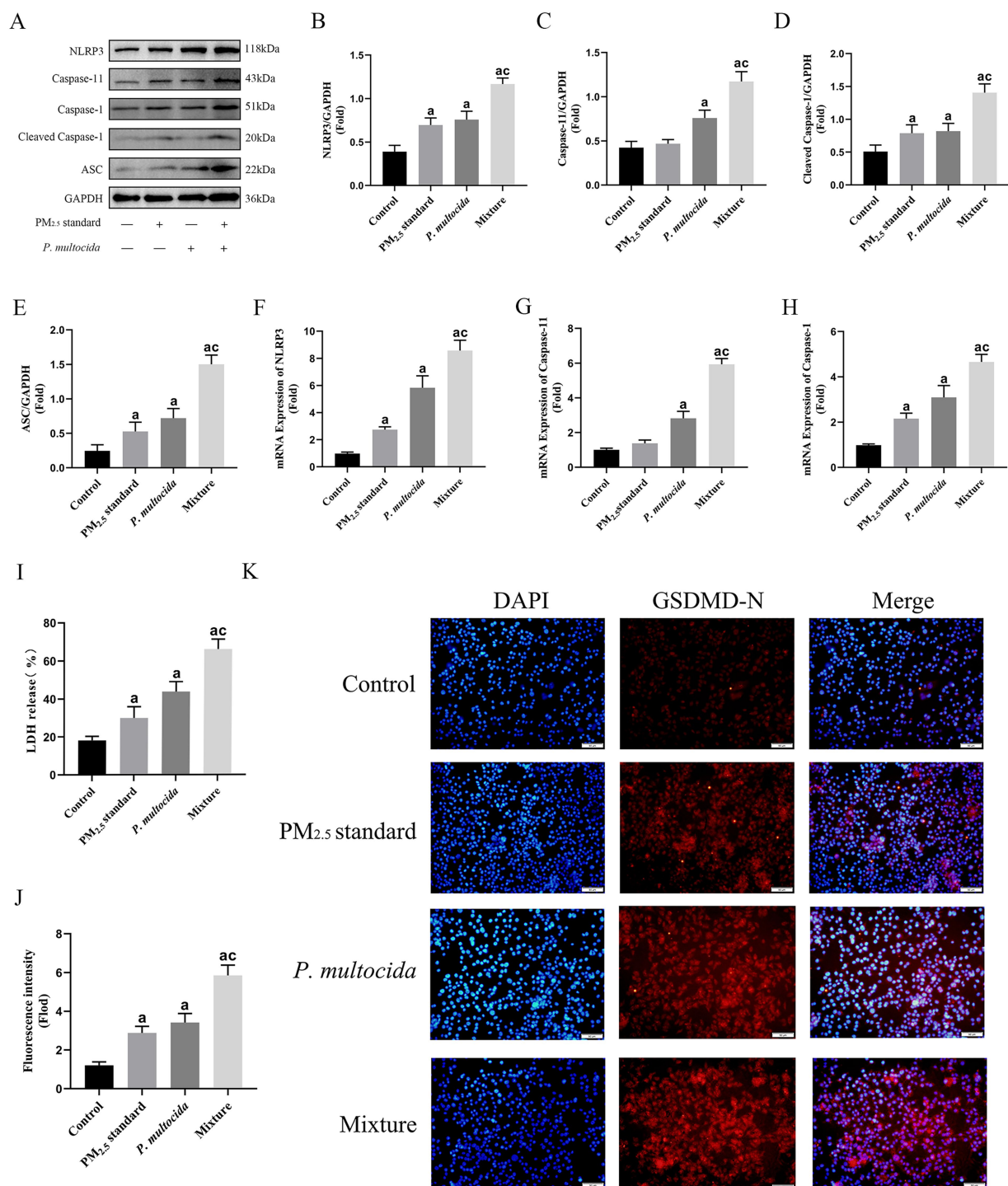


FIGURE 5

PM_{2.5} enhanced intracellular invasion of *P. multocida* and efficiently initiated Caspase-11-mediated non-classical NLRP3 activation and exacerbated pyroptosis. (A–E) Protein expression levels of NLRP3, Caspase-1, Caspase-11, and ASC under the synergistic effect of PM_{2.5} standard and *P. multocida*. (F–H) The mRNA expression levels of NLRP3, Caspase-1, Caspase-11 under the synergistic effect of PM_{2.5} standard and *P. multocida*. (I) LDH levels under the synergistic effect of PM_{2.5} standard and *P. multocida*. (J,K) GSDMD levels under the synergistic effect of PM_{2.5} standard and *P. multocida*. Results are expressed as mean ± SD deviation of three determinations. ^a*p* < 0.05, compared to the Control group. ^c*p* < 0.05, compared to the PM_{2.5} standard group.

the expression of GSDMD-N and LDH was significantly suppressed in the PM_{2.5} + MCC950 group compared to the cowshed PM_{2.5} group, and cell viability was maintained for an interval in the PM_{2.5} + MCC950 group (Figures 6D,L–N). These

data suggest that the use of NLRP3 inhibitors attenuates the onset of pyroptosis and maintains cell viability to some extent, which may be an effective means of treating cowshed PM_{2.5}-induced respiratory damage.

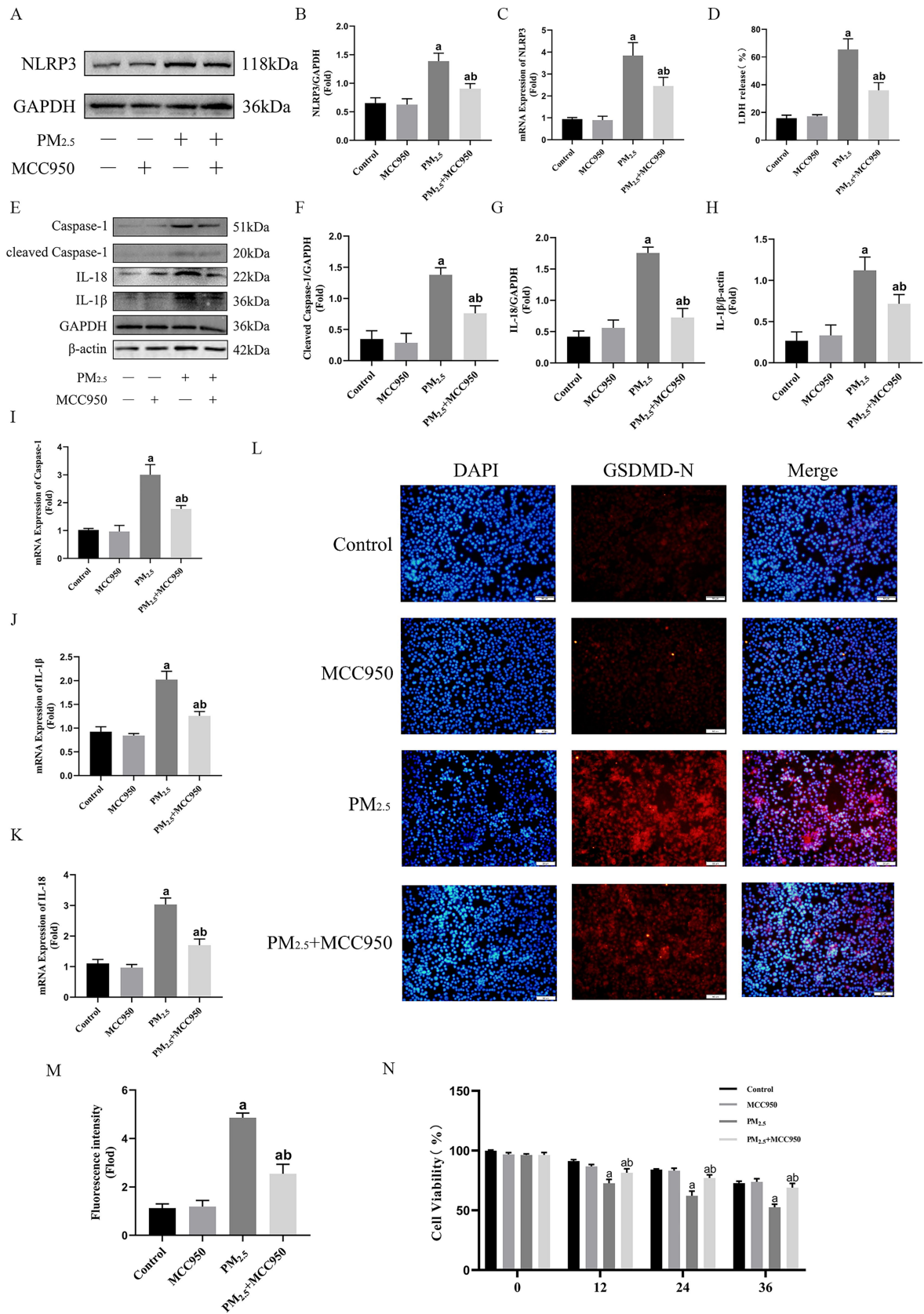


FIGURE 6
Intervention of NLRP3 expression was an effective respond to cowshed PM_{2.5}-induced cellular damage. **(A,B)** Protein levels of NLRP3 after addition of MCC950 under the influence of cowshed PM_{2.5}. **(C)** mRNA levels of NLRP3 after addition of MCC950 under the influence of cowshed PM_{2.5}. **(D)** LDH

(Continued)

FIGURE 6 (Continued)

levels in NR8383 after addition of MCC950 under the influence cowshed PM_{2.5}. (E–H) Caspase-1, IL-18, and IL-1 β protein expression levels after addition of MCC950 under the influence of cowshed PM_{2.5}. (I–K) Caspase-1, IL-18, and IL-1 β mRNA expression levels after addition of MCC950 under the influence of cowshed PM_{2.5}. (L–M) GSDMD levels in NR8383 after addition of MCC950 under the influence cowshed PM_{2.5}. (N) Changes in cell viability levels over time after addition of MCC950 under the influence of cowshed PM_{2.5}. Results are expressed as mean \pm SD deviation of three determinations. ^a $p < 0.05$, compared to the Control group. ^b $p < 0.05$, compared to the PM_{2.5} group.

4 Discussion

4.1 Microbial components are critical factors in cowshed PM_{2.5}-induced respiratory toxicity

The higher feeding densities and inefficient ventilation in animal farms results in elevated PM_{2.5} concentrations and prolongs exposure longer, thereby increasing the risk of respiratory illness and infection for both animals and workers (38). In the present study, there was substantial pathological damage to the lungs of the model animal (rat) after exposure to cowshed PM_{2.5}, accompanied by the release of inflammatory factors. Microorganisms, a major component of PM_{2.5} in the farm-animal environments, are widely present in all aspects of livestock production (39). Furthermore, physiological activities such as ruminating and flatulence are also sources of airborne microorganisms in cowsheds (40); they can utilize particulate matter as a medium for cultivation and transmission. This can result in the emergence of a range of airborne diseases, impairing animal growth, production and welfare, with potential for zoonotic diseases (41). Studies have shown that dairy cows kept for extended intervals in cowsheds with high PM_{2.5} concentrations are more prone to developing respiratory diseases (41). The presence of PM_{2.5} in piggeries has been demonstrated to induce oxidative stress and inflammatory responses in alveolar macrophages, which compromises pig immunity to a certain extent. Furthermore, this can significantly accelerate the course of disease when primary or secondary pathogenic infections are present (42).

Pathogenic bacteria, including *Pseudomonas aeruginosa*, *Shigella escherichii*, *Acinetobacter*, *Streptococcus*, and *Staphylococcus* have been detected in the air of cowsheds. These bacteria pose a serious risk to the organism's health since they can result in bacteremia or respiratory infections (43). Exposure to PM_{2.5} in the animal farm environment increases the susceptibility of livestock to microorganisms, and this synergistic effect leads to more severe respiratory damage and inflammatory responses (44). In our previous analysis of the composition of cowshed PM_{2.5}, bacteria accounted for 61.39% of the microbial composition of cowshed PM_{2.5} (13). Therefore, we speculated that microbiological components had an important role in cowshed PM_{2.5}-induced cytotoxicity. In this study, when cytotoxicity experiments were conducted following inactivation of cowshed PM_{2.5}, there was a significant decrease in toxicity compared to the not inactivated cowshed PM_{2.5}, confirming the above hypothesis. However, the present study employed high temperatures to inactivate cowshed PM_{2.5}, a method that is inherently limited in scope. On the one hand, high temperatures can only transform the microbiologically active components of cowshed PM_{2.5} into inactive components, and do not serve to remove the toxins. On the other hand, circulating high temperatures in localized air within the animal farmhouse is not a widespread practice. Nevertheless, UV light or microwave radiation

to address recirculating airflow within the environment has been demonstrated to be an efficacious approach for curbing airborne microbial activity. This may be a viable solution for regulating the biological toxicity of PM_{2.5} and the dissemination of pathogens within the farm-animal environment (45).

4.2 Interactions and toxicity amplification mechanisms between PM_{2.5} and bacteria

In addition to this, by analyzing how the microbial component of PM_{2.5} from livestock facilities enhances cytotoxicity, should inform treatment options for animals infected with respiratory diseases. In order to investigate the specific mechanism of microbial components in the induction of cellular damage by cowshed PM_{2.5}, we used *P. multocida* isolated from cowshed PM_{2.5} in synergy with PM_{2.5} standard of well-defined compositions to simulate cowshed PM_{2.5}. This combination not only excludes as much as possible the unknown effects of the other components, but also shows the characteristics of the cowshed PM_{2.5} as much as possible. Since during PM_{2.5} invasion of the lungs, alveolar macrophages have an important barrier role in the intake and processing of PM_{2.5} particles. Consequently, it is necessary to investigate the response of alveolar macrophages (NR8383) to cowshed PM_{2.5}. The experimental results obtained in this study demonstrate that when *P. multocida* is synergized with the PM_{2.5} standard, despite the fact that the stoichiometry is half that of the original, the cellular damage induced by the Mixture group is significantly increased. This also suggests that the microbiological component of PM_{2.5} may play a significant role in the effects on livestock.

In a study of measurements of biomass components in atmospheric PM_{2.5}, fungi were found to be the predominant bioactive substances in PM_{2.5}, due to the adsorption function of PM_{2.5} particles (46). Therefore, the exploration of the reasons for the strong amplification of PM_{2.5} cytotoxic effects by bacteria may be pivotal in elucidating the mechanism of cowshed PM_{2.5}-induced respiratory damage. In order to verify the aforementioned speculations, an examination was conducted of the surface characteristics of particle samples of synergizing PM_{2.5} standard with *P. multocida*, and it was confirmed that *P. multocida* was able to adhere to PM_{2.5} particles. It has been shown that the selenium yeast *A. brasiliense* of the genus *Azospirilla* can reduce selenite to basic selenium in the form of selenium nanospheres (47). Silver nanoparticles exhibit significant antimicrobial activity subsequent to interaction with bacterial extracellular polymers (48). This all suggests that there may be a degree of interaction between particulate matter and microorganisms. In this study, the Zeta potential shifted in a negative direction when the PM_{2.5} standard and *P. multocida* were mixed compared to the PM_{2.5} standard group. It has been demonstrated that negatively charged groups secreted by bacteria adsorbed onto the calcite surface induce

a shift in the Zeta potential towards a negative direction (49). In the case of *P. multocida*, it has certain surface characteristics and charge distribution itself. After interaction with PM_{2.5}, due to adsorption, binding, or other physicochemical processes, a change in the surface charge of PM_{2.5}. The shift of the Zeta potential in a negative direction resulted in a reduction in bacterial aggregation and sedimentation, while concurrently enhancing the stability of the PM_{2.5} standard and the *P. multocida* system (50). In the cowshed environment, this would serve to further enhance the spread, survival and pathogenicity of *P. multocida*. Moreover, it would facilitate the more easily formation of bioaerosols, thereby enhancing the toxic effects of PM_{2.5}. In the FTIR spectrum, compared to the PM_{2.5} standard group, the red shift of the absorption peak at 3,385 cm⁻¹ indicates that the O–H and N–H bond lengths have risen and the bond energy has reduced in the synergistic effect of PM_{2.5} standard and *P. multocida*. This suggests that the two form a stable complex through hydrogen bonding, binding PM_{2.5} to the bacteria. This lays a physical foundation for the subsequent enhancement of intracellular invasion efficiency. Enhancement of the absorption peak at 1,650 cm⁻¹ indicates an increase in C=O groups of bacterial origin within the mixture. This is direct evidence of bacterial components being adsorbed onto PM_{2.5} surfaces, thereby altering their chemical composition. This alteration may permit PM_{2.5} to carry more bacterial antigens or toxins (e.g., endotoxin LPS), which in turn enhances immune stimulation. The appearance of the 527 cm⁻¹ absorption peak provides further evidence to support that specific chemical binding of bacteria to PM_{2.5} results in the introduction of new toxic groups (e.g., organic acids, cellular metabolites such as carboxyl and hydroxyl groups in proteins) and it changes the PM_{2.5} surface charge distribution, which enhances the retention time of the complex in the respiratory tract and the cell adhesion ability. It has also been shown that in the animal farm environment, PM_{2.5} particles that carry *P. multocida* increase the biological toxicity of the particles themselves. Concurrently, PM_{2.5} particles carrying *P. multocida* are more prone to penetrate the organism's deeper layers, thereby elevating the probability of disease infection in animals.

4.3 Cowshed PM_{2.5} induces cellular pyroptosis and inflammatory responses through activation of the GBP2/Caspase-11/NLRP3 pathway

The present study investigated the effect on the level of intracellular infection of *P. multocida* in the presence of PM_{2.5} particles. It was found that PM_{2.5} greatly increased the probability of intracellular entry of *P. multocida*, which became a pivotal factor in amplifying PM_{2.5} cytotoxicity. However, after PM_{2.5} carries *P. multocida* into cells, the specific mechanism of causing toxicity remains to be elucidated. In this context, bioinformatics and transcriptomics screening revealed a significant up-regulation of GBP2 expression in the lungs in response to cowshed PM_{2.5}. GBP2 plays an important role in the immune response. Studies have shown that GBP2 is capable of recognizing and binding to pathogen-associated molecular patterns, thereby initiating immune defense mechanisms (51). Therefore, we speculate that the complex microbiological composition of cowshed PM_{2.5} may be a significant contributing factor to the elevated GBP2 expression. An increase in the number of intracellular bacteria, an enhanced effect of cowshed

PM_{2.5} on cell viability, and elevated expression of IL-1 β were detected for a period of time following interference with GBP2 expression. This further suggests that microbial components in PM_{2.5} induce high GBP2 expression, and suggests that GBP2 is associated with inflammatory responses induced by microbial components. However, after interfering with GBP2 expression, there was a lag in both cell viability and inflammatory factor expression under the influence of cowshed PM_{2.5}, which may be related to the failure of GBP2 to activate Caspase-11 in a timely manner. When GBP2 is silenced, *P. multocida* enters the cell. The clearance of the bacteria by the cell is diminished, and the bacteria continue to multiply intracellularly. However, they have not yet reached the threshold for triggering intense inflammation. This results in a transient increase in cell viability in the initial period (within 12 h). In the present study, it was also detected that after interfering with GBP2, there was no activation of Caspase-11 expression in the presence of cowshed PM_{2.5}. However, it has been demonstrated that persistent intracellular bacterial reproduction can ultimately trigger the lagging activation of the non-classical NLRP3 pathway, leading to a surge of inflammatory factors and, ultimately, triggering a sudden drop in cell viability. This phenomenon reveals the pivotal function of GBP2 in maintaining a balance between antimicrobial defense and inflammation control. Furthermore, these findings suggest that the silencing of GBP2, although protective in the short term, leads to more severe damage over time.

Many studies have shown that PM_{2.5} can induce a variety of programmed cell deaths, including apoptosis, autophagy and pyroptosis, and so on (52). However, the relationship and signaling mechanisms between PM_{2.5} and various forms of programmed cell death are complex. Among the various forms of programmed cell death described above, pyroptosis is capable of triggering a more intense inflammatory response, a process that helps to protect the host from microbial infections (53). However, the consequences of excessive pyroptosis are also evident, with excessive cellular pyroptosis leading to a series of inflammatory storms that can cause sepsis and autoimmune diseases (54). Therefore, it is crucial to explore the mechanism of pyroptosis induced by cowshed PM_{2.5}. Studies have shown that upon bacterial entry into cells, GBP2 cleaves pathogen-containing vesicles (PVs), thereby releasing bacteria and their associated LPS into the host cell cytoplasm and assembling a Caspase-11 activation platform on LPS-containing membranes as the first step of the inflammasome signaling, and activating the non-classical NLRP3 inflammasome (55). The mechanism of activation of the NLRP3 inflammasome is similarly intricate. Studies have shown that chemicals endocytosed into the cytoplasm by macrophages trigger lysosomal rupture and the release of histone B, leading to activation of the NLRP3 inflammasome (56). The rupture of the cytoplasmic membrane with K⁺ efflux caused by the PAHs component contained in PM_{2.5} may also be a significant reason for the activation of NLRP3 (57). In this study, the expression of NLRP3 and Caspase-11 did not change significantly after interfering with the expression of GBP2 under the role of PM_{2.5} standard. However, interference with GBP2 was found to have a substantial impact on the expression of NLRP3, cleaved-Caspase-1, Caspase-11, GSDMD, and LDH, under the synergistic effect of PM_{2.5} standard and *P. multocida*. These results provide validation for the upregulation of GBP2 expression upon the entry of cowshed PM_{2.5} into cells. Subsequently, GBP2 is able to recognize and bind to microbial components in

cowshed PM_{2.5}, further affecting the activation of the NLRP3 non-classical pathway mediated by Caspase-11.

4.4 Inhibition of NLRP3 can stably suppresses cowshed PM_{2.5}-induced respiratory toxicity

In this study, it was determined that GBP2 is not an effective target against cowshed PM_{2.5}-induced pyroptosis. Therefore, we focused our perspective on NLRP3, the activation of which is the central channel for pyroptosis caused by cowshed PM_{2.5} in the present study. In this regard, the present study attempted a therapeutic option to reduce cowshed PM_{2.5}-induced damage by inhibiting NLRP3. The development of cellular inflammation and pyroptosis was effectively controlled by using an NLRP3 inhibitor (MCC950) to inhibit NLRP3 expression. This demonstrates the effectiveness of this regimen. This also provides a valuable therapeutic target and dosing direction for the treatment of cowshed PM_{2.5}-induced lung injury. However, there are some limitations to this study. This study only assessed the interrelationships and mechanisms of action between PM_{2.5} and bacteria, and therefore does not reflect the full picture of the microbiological landscape within cowshed PM_{2.5}. This encompasses the potential involvement of disease-causing microorganisms (such as fungi, viruses, and parasites) in the process of damage to organisms. Further research is required to elucidate this aspect, which will be the focus of our subsequent studies.

5 Conclusion

In summary, the present study demonstrated that microbiological components were non-negligible and important factors in animal farm environment PM_{2.5}-induced lung injury. Bacterial components alter the surface characteristics of PM_{2.5} particles, whereas PM_{2.5} particles enabled bacteria to get inside the cells. The interaction between the two amplified the biological toxicity of PM_{2.5}. Intracellularly, recognition and defense of bacteria by GBP2 activated pyroptosis induced by non-classical NLRP3. The present study elucidated the relationship among intracellular bacteria, GBP2, and NLRP3 as a key process in cowshed PM_{2.5}-induced pyroptosis. In addition, inhibition of NLRP3 has potential for the treatment of PM_{2.5}-induced lung injury in a farm-animal environment.

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 approved by the Animal Experimentation Ethics Committee at Haihua Biotechnology Group Co. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

XD: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. ZM: Methodology, Supervision, Visualization, Writing – review & editing. YS: Methodology, Visualization, Writing – review & editing. YJ: Methodology, Writing – review & editing. XZ: Visualization, Writing – review & editing. CZ: Data curation, Writing – review & editing. XL: Visualization, Writing – review & editing. XY: Conceptualization, Formal analysis, Investigation, Resources, Validation, Writing – review & editing. YG: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Writing – review & editing.

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Conflict of interest

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

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fvets.2025.1631913/full#supplementary-material>

References

- Martins NR, Guilherme CDGA. Impact of PM2.5 in indoor urban environments: a review. *Sustain Cities Soc.* (2018) 42:S2210670718309272. doi: 10.1016/j.scs.2018.07.011
- Ge E, Lai K, Xiao X, Luo M, Fang Z, Zeng Y, et al. Differential effects of size-specific particulate matter on emergency department visits for respiratory and cardiovascular diseases in Guangzhou, China. *Environ Pollut.* (2018) 243:336–45. doi: 10.1016/j.envpol.2018.08.068
- Zhang K, Guo L, Wei Q, Song Q, Liu J, Niu J, et al. COPD rat model is more susceptible to cold stress and PM2.5 exposure and the underlying mechanism. *Environ Pollut.* (2018) 241:26–34. doi: 10.1016/j.envpol.2018.05.034
- Zhao L, Li B, Zhou L, Song C, Kang T, Xu Y, et al. PM2.5 exposure promotes asthma in aged Brown-Norway rats: implication of multiomics analysis. *Ecotoxicol Environ Saf.* (2023) 263:115393. doi: 10.1016/j.ecoenv.2023.115393
- Xu Z, Li Z, Liao Z, Gao S, Hua L, Ye X, et al. PM2.5 induced pulmonary fibrosis in vivo and in vitro. *Ecotoxicol Environ Saf.* (2019) 171:112–21. doi: 10.1016/j.ecoenv.2018.12.061
- Wang TH, Huang KY, Chen CC, Chang YH, Chen HY, Hsueh C, et al. PM2.5 promotes lung cancer progression through activation of the AhR-TMPRSS2-IL18 pathway. *EMBO Mol Med.* (2023) 15:e17014. doi: 10.15252/emmm.202217014
- Jia YY, Wang Q, Liu. Toxicity research of PM2.5 compositions in vitro. *IJERPH.* (2017) 14:232. doi: 10.3390/ijerph14030232
- Leenen KV, Joutet J, Demeyer P, Vermeir P, Pardon B. Particulate matter and airborne endotoxin concentration in calf barns and their association with lung consolidation, inflammation, and infection. *J Dairy Sci.* (2021) 104. doi: 10.3168/jds.2020-18981
- Wang M, Peng S, Liu D, Long D, Liu Z, Pu S. Characteristics and traceability analysis of microbial assemblage in fine particulate matter from a pig house. *Animals.* (2023) 13:5932–5947. doi: 10.3390/ani13061058
- Dai P, Shen D, Tang Q, Huang K, Li C. PM2.5 from a broiler breeding production system: the characteristics and microbial community analysis. *Environ Pollut.* (2020) 256:113368. doi: 10.1016/j.envpol.2019.113368
- Tang Q, Huang K, Liu J, Jin X, Li C. Distribution characteristics of bioaerosols inside pig houses and the respiratory tract of pigs. *Ecotoxicol Environ Saf.* (2021) 212:112006. doi: 10.1016/j.ecoenv.2021.112006
- Chmielowiec-Korzeniowska A, Trawińska B, Tymczyna L, Bis-Wencel H, Matuszewski. Microbial contamination of the air in livestock buildings as a threat to human and animal health – a review. *Ann Anim Sci.* (2021) 21:417–31. doi: 10.2478/aoas-2020-0080
- Zhang X, Ma Z, Hao P, Ji S, Gao Y. Characteristics and health impacts of bioaerosols in animal barns: a comprehensive study. *Ecotoxicol Environ Saf.* (2024) 278:116381. doi: 10.1016/j.ecoenv.2024.116381
- Peng Z, Wang X, Zhou R, Chen H, Wu B. *Pasteurella multocida*: genotypes and genomics. *Microbiol Mol Biol Rev.* (2019) 83. doi: 10.1128/MMBR.00014-19
- Prajapati A, Yogisharadhy R, Mohanty NN, Mendem SK, Nizamuddin A, Chanda MM, et al. Comparative genome analysis of *Pasteurella multocida* serogroup B: 2 strains causing haemorrhagic septicaemia (HS) in bovines. *Gene.* (2022) 826:146452. doi: 10.1016/j.gene.2022.146452
- Amass SF, Clark LK, Van Alstine WG, Bowersock TL, Murphy DA, Knox KE, et al. Interaction of mycoplasma hyopneumoniae and *Pasteurella multocida* infections in swine. *J Am Vet Med Assoc.* (1994) 204:102–7. doi: 10.2460/javma.1994.204.01.102
- Petruzzelli B, Rami ALR, Evans T, Pierson NP, Inzana FW, Biofilm TJ. Biofilm formation and avian immune response following experimental acute and chronic avian cholera due to *Pasteurella multocida*. *Vet Microbiol.* (2018) 222:114–23. doi: 10.1016/j.vetmic.2018.07.005
- Santiani F, Henker LC, Schwetz CI, Lorenzetti MP, Mendes RE, Casagrande RA. Pasteurellosis outbreak due to *Pasteurella multocida* type A in rabbits (*Oryctolagus cuniculus*). *Acta entiae Veterinariae.* (2019). doi: 10.22456/1679-9216.93928
- Nikolaos K, Sofia M, Irini D, Eleni M, Christina K, Eleni I. An unusual case of *Pasteurella multocida* bacteremic meningitis. *J Infect Public Health.* (2018) 12:95–96. doi: 10.1016/j.jiph.2018.05.012
- Piorunek M, Brajer-Luftmann B, Walkowiak J. *Pasteurella multocida* infection in humans. *Pathogens.* (2023) 12:8. doi: 10.3390/pathogens12101210
- Huang S, Meng Q, Maminska A, MacMicking JD. Cell-autonomous immunity by IFN-induced GBPs in animals and plants. *Curr Opin Immunol.* (2019) 60:71–80. doi: 10.1016/j.coi.2019.04.017
- Kirkby M, Tuipulotu ED, Feng S, Pilato LJ, Man SM. Guanylate-binding proteins: mechanisms of pattern recognition and antimicrobial functions. *Trends Biochem Sci.* (2023) 48:883–93. doi: 10.1016/j.tibs.2023.07.002
- Finethy R, Luoma S, Orench-Rivera N, Feeley EM, Haldar AK, Yamamoto M, et al. Inflammasome activation by bacterial outer membrane vesicles requires guanylate binding proteins. *MBio.* (2017) 8:10.1128/mbio.01188-17. doi: 10.1128/mbio.01188-17
- Huang W, Zhang Y, Zheng B, Ling X, Wang G, Li L, et al. GBP2 upregulated in LPS-stimulated macrophages-derived exosomes accelerates septic lung injury by activating epithelial cell NLRP3 signaling. *Int Immunopharmacol.* (2023) 124:111017. doi: 10.1016/j.intimp.2023.111017
- Fehervari Z. NLRP3 shapes immunity to *Leishmania*. *Nat Immunol.* (2015) 16:342. doi: 10.1038/ni.3135
- Xu J, Gabriel N. The NLRP3 inflammasome: activation and regulation. *Trends Biochem Sci.* (2022) 48:331–44. doi: 10.1016/j.tibs.2022.10.002
- Jin H, Zhu Y, Wang X-d, Luo E-f, Li Y-p, Wang B-l, et al. BDNF corrects NLRP3 inflammasome-induced pyroptosis and glucose metabolism reprogramming through KLF2/HK1 pathway in vascular endothelial cells. *Cell Signal.* (2021) 78:109843. doi: 10.1016/j.cellsig.2020.109843
- Li P, Li S, Wang L, Li H, Wang Y, Liu H, et al. Mitochondrial dysfunction in hearing loss: oxidative stress, autophagy and NLRP3 inflammasome. *Front Cell Dev Biol.* (2023) 11:9. doi: 10.3389/fcell.2023.1119773
- Ma Z, Du X, Sun Y, Sun K, Zhang X, Wang L, et al. RGS2 attenuates alveolar macrophage damage by inhibiting the Gq/11-Ca2+ pathway during cowshed PM2.5 exposure, and aberrant RGS2 expression is associated with TLR2/4 activation. *Toxicol Appl Pharmacol.* (2024) 487:11. doi: 10.1016/j.taap.2024.116976
- Ma Z, Du X, Sun Y, Jia Y, Liang X, Gao Y. Attenuation of PM2.5-induced lung injury by 4-phenylbutyric acid: maintenance of [Ca2+]i stability between endoplasmic reticulum and mitochondria. *Biomol Ther.* (2024) 14:1135. doi: 10.3390/biom14091135
- Wu Y, Pei C, Wang X, Wang Y, Huang D, Shi S, et al. Probiotics ameliorates pulmonary inflammation via modulating gut microbiota and rectifying Th17/Treg imbalance in a rat model of PM2.5 induced lung injury. *Ecotoxicol Environ Saf.* (2022) 244:114060. doi: 10.1016/j.ecoenv.2022.114060
- Sun Y, Sun K, Ma Z, Zhang X, Du X, Jia Y, et al. mi R-122–5p promotes cowshed particulate matter 2.5-induced apoptosis in NR8383 by targeting COL4A1. *Toxics.* (2024) 12:386. doi: 10.3390/toxics12060386
- Zeng Y, Li M, Zou T, Chen X, Xu H. The impact of particulate matter (PM2.5) on human retinal development in hESC-derived retinal organoids. *Front Cell Dev Biol.* (2021) 9:607341. doi: 10.3389/fcell.2021.607341
- Wang M, Zhao M, Yu J, Xu Y, Zhang J, Liu J, et al. MCC950, a selective NLRP3 inhibitor, attenuates adverse cardiac remodeling following heart failure through improving the cardiometabolic dysfunction in obese mice. *Front Cardiovasc Med.* (2022) 9:2022:727474. doi: 10.3389/fcvm.2022.727474
- Zhang Y, Ye F, Fu X, Li S, Wang L, Chen Y, et al. Mitochondrial regulation of macrophages in innate immunity and diverse roles of macrophages during cochlear inflammation. *Neurosci Bull.* (2024) 40:255. doi: 10.1007/s12264-023-01085-y
- Quast K. Literature review on the interaction of oleate with non-sulphide minerals using zeta potential. *Miner Eng.* (2016) 94:10–20. doi: 10.1016/j.mineng.2016.04.016
- Wang L, Qin X, Liang J, Ge P. Induction of pyroptosis: a promising strategy for cancer treatment. *Front Oncol.* (2021) 11:635774. doi: 10.3389/fonc.2021.635774
- Du L, Yang L, Yang C, Dominy R, Hu C, Du H, et al. Investigation of bio-aerosol dispersion in a tunnel-ventilated poultry house. *Comput Electron Agric.* (2019) 167:105043. doi: 10.1016/j.compag.2019.105043
- Guanliu Y, Yao W, Shouguo W, Changmin D, Liangmeng W, Jing G, et al. Effects of microbial aerosol in poultry house on meat ducks' immune function. *Front Microbiol.* (2016) 7:1245. doi: 10.3389/fmicb.2016.01245
- Salem E, Hägglund S, Cassard H, Corre T, Näslund K, Foret C, et al. Pathogenesis, host innate immune response, and aerosol transmission of influenza D virus in cattle. *J Virol.* (2019) 93:e01853-18. doi: 10.1128/JVI.01853-18
- Farrell CT, Hunter E, Wilson PB, White SJ. Genomic characterisation of bioaerosols within livestock facilities: a systematic review. *Sci Total Environ.* (2024) 918:170722. doi: 10.1016/j.scitotenv.2024.170722
- Tang Q, Huang K, Liu J, Wu S, Li C. Fine particulate matter from pig house induced immune response by activating TLR4/MAPK/NF-κB pathway and NLRP3 inflammasome in alveolar macrophages. *Chemosphere.* (2019) 236:124373. doi: 10.1016/j.chemosphere.2019.124373
- Yan X, Ma J, Chen X, Lei M, Li T, Han Y. Characteristics of airborne bacterial communities and antibiotic resistance genes under different air quality levels. *Environ Int.* (2022) 161:107127. doi: 10.1016/j.envint.2022.107127
- Cambra-López M, Aarnink AJA, Zhao Y, Calvet S, Torres AG. Airborne particulate matter from livestock production systems: a review of an air pollution problem. *Environ Pollut.* (2010) 158:1–17. doi: 10.1016/j.envpol.2009.07.011
- Ding L, Zhang Q, Wang C, Yao C, Shan F, Li Q. A clean and health-care-focused way to reduce indoor airborne bacteria in calf house with long-wave ultraviolet. *Microorganisms.* (2024) 12:1472. doi: 10.3390/microorganisms12071472
- Glikson M, Rutherford S, Simpson RW, Mitchell CA, Yago A. Microscopic and submicron components of atmospheric particulate matter during high asthma periods in Brisbane, Queensland, Australia. *Atmos Environ.* (1995) 29:549–62. doi: 10.1016/1352-2310(94)00278-S
- Tugarova AV, Mamchenkova PV, Dyatlova YA, Kamnev AA. FTIR and Raman spectroscopic studies of selenium nanoparticles synthesised by the bacterium *Azospirillum thioophilum*. *Spectrochim Acta A Mol Biomol Spectrosc.* (2017):458. doi: 10.1016/j.saa.2017.11.050

48. Dimkpa CO, Calder A, Gajjar P, Merugu S, Huang W, Britt DW, et al. Interaction of silver nanoparticles with an environmentally beneficial bacterium, *Pseudomonas chlororaphis*. *J Hazard Mater.* (2011) 188:428–35. doi: 10.1016/j.jhazmat.2011.01.118
49. Li Q, Dong F, Dai Q, Zhang C, Yu L. Surface properties of PM25 calcite fine particulate matter in the presence of same size bacterial cells and exocellular polymeric substances (EPS) of *Bacillus mucitagnosus*. *Environ Sci Pollut Res Int.* (2018) 25:22429–36. doi: 10.1007/s11356-017-0829-x
50. Lay ML, Wu HM, Huang CH. Study of the zeta potential of Fe (O) OH colloids. *J Mater Sci.* (1995) 30:5473–8. doi: 10.1007/BF00351560
51. Tretina K, Park ES, Maminska A, MacMicking JD. Interferon-induced guanylate-binding proteins: guardians of host defense in health and disease. *J Exp Med.* (2019) 216:482–500. doi: 10.1084/jem.20182031
52. Wang Y, Zhong Y, Liao J, Wang G. PM2.5-related cell death patterns. *Int J Med Sci.* (2021) 18:1024–9. doi: 10.7150/ijms.46421
53. Bergsbaken T, Fink SL, Cookson BT. Pyroptosis: host cell death and inflammation. *Nat Rev Microbiol.* (2009) 7:99–109. doi: 10.1038/nrmicro2070
54. Zhang H, Du Y, Guo Y, Wang Z, Li H, Lv Z, et al. TLR4-NLRP3-GSDMD-mediated pyroptosis plays an important role in aggravated liver injury of CD38^{−/−} sepsis mice. *J Immunol Res.* (2021) 2021:1–15. doi: 10.1155/2021/6687555
55. Dickinson MS, Kutsch M, Sistemich L, Hernandez D, Piro AS, Needham D, et al. LPS-aggregating proteins GBP1 and GBP2 are each sufficient to enhance caspase-4 activation both in cellulo and in vitro. *Proc Natl Acad Sci USA.* (2023) 120:e2216028120. doi: 10.1073/pnas.2216028120
56. Ma J, Liu R, Wang X, Liu Q, Chen Y, Valle RP, et al. Crucial role of lateral size for graphene oxide in activating macrophages and stimulating pro-inflammatory responses in cells and animals. *ACS Nano.* (2015) 9:10498–515. doi: 10.1021/acs.nano.5b04751
57. Chen Z, Liu Y, Sun B, Li H, Dong J, Zhang L, et al. Polyhydroxylated metallofullerenols stimulate IL-1 β secretion of macrophage through TLRs/MyD88/NF- κ B pathway and NLRP3 inflammasome activation. *Small.* (2014) 10:2362–72. doi: 10.1002/smll.201302825