Original Article

Effect of *Aspergillus fumigatus* on infection in immunosuppressed rats

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Background: Immunosuppression is believed to increase the risk of invasive pulmonary aspergillosis (IPA), but information on the mechanism is limited. Therefore, we analyze the effect and mechanism of the pathogenesis and disease progression of IPA in a combined immunosuppressed rat model.

Methods: The immunosuppressed rat model was established by intraperitoneal injection of cyclophosphamide (CTX) and dexamethasone (DXM). IPA was established by nasal inoculation of *Aspergillus fumigatus* spore suspension. Pathological sections and tissue homogenate culture were used to evaluate the lung tissue. Routine blood and inflammatory indexes were dynamically observed. The expressions of NLRP3/caspase-1/GSDMD protein and gene were determined using western blot and quantitative polymerase chain reaction (q-PCR) respectively. *T*-test or one-way repeated measures analysis were used to do statistical analysis on the groups.

Results: Following intraperitoneal of CTX and DXM injections, the rats showed depression, weight loss, and significant decreases in the numbers of leukocytes and classified cells. Pathological sections revealed more severe lung lesions in the immunosuppressed rats infected with *Aspergillus fumigatus*. The expression of NLRP3/caspase-1/GSDMD protein increased significantly in both the aspergillosis and immunosuppressed plus aspergillosis groups.

Conclusions: The pathological development of IPA in the immunosuppressed rats had the most serious effects, and the findings strongly implicated NLRP3/caspase-1/GSDMD pathway involvement.

Keywords: *Aspergillus fumigatus*; cyclophosphamide (CTX); dexamethasone (DXM); immunosuppression; pulmonary aspergillosis

Submitted Nov 17, 2022. Accepted for publication Jan 10, 2023. Published online Jan 31, 2023.
doi: 10.21037/atm-22-6600
View this article at: https://dx.doi.org/10.21037/atm-22-6600

Introduction

Invasive pulmonary aspergillosis (IPA) is a serious life-threatening lung infection occurring primarily in patients with severe immunodeficiency. When the host is immunocompromised or the number of inhaled spores exceeds the clearance ability of airway defense barrier, *Aspergillus fumigatus* spores can invade the body and lead to systemic infection. According to the latest epidemiological survey of invasive fungal diseases in China, *Aspergillus* infection accounted for 37.9% of all confirmed cases of pulmonary fungal infection, and was the most common

pathogenic fungus (1,2).

In recent years, due to the widespread use of broad-spectrum antibiotics, anticancer drugs and immunosuppressive agents, invasive diagnosis and treatment technologies, increased organ transplantation, increasing numbers of AIDS patients, aging populations and various other reasons, the incidence rate of IPA has increased dramatically (3-5), and the mortality rate is high at 80–90% (6).

Aspergillus fumigatus is a ubiquitous, opportunistic saprophytic fungal pathogen that frequently invades the upper and lower respiratory tracts of immunocompromised individuals (7-9), resulting in severe pulmonary disease (10,11). About 95.2% of the patients received medical treatment and 4.8% received surgical treatment, the patients who underwent operation were mainly lung cancer, organ transplantation, liver disease, human immunodeficiency virus (HIV) infection, blood disease, chronic lung disease and so on. The mortality of these patients was higher (12). Both cyclophosphamide (CTX) and dexamethasone (DXM) are crucial immunosuppressive agents widely used in clinical settings to mitigate complications and death (13). The combination of DXM, CTX, etoposide, and cisplatin is less toxic and has been shown to be more effective than high-dose CTX for peripheral stem cell mobilization in multiple myeloma (14).

Pyroptosis differs from apoptosis and other cell death modes as it is an inflammatory form of cell death, which can lead to sepsis if overactivated (15,16). A growing body of evidence suggests the factors involved in the classic pathway NLRP3/caspase-1/GSDMD process of focal death are highly expressed in people and animals with sepsis, and the use of related blockers of factors in this pathway or the knockout of genes of these factors can reduce the systemic inflammatory response, and alleviate organ damage to some extent (17-19).

To further elucidate the role of the pyroptosis pathway, NLRP3-caspase-1-GSDMD, we established rat models of immunosuppression and IPA to provide a new treatment for patients with IPA and immunosuppressive patients. We present the following article in accordance with the ARRIVE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6600/rc).

Highlight box

Key findings

- The increased expression of the NLRP3/caspase-1/GSDMD pathway was associated with susceptibility to invasive *Aspergillus fumigatus* infection in severely immunosuppressed rats.

What is known and what is new?

- The classical pathway of cell death, NLRP3/caspase-1/GSDMD, is highly expressed in sepsis patients and animals, and the use of related blockers of this pathway or the knockout of the genes of these factors can reduce the systemic inflammatory response, and alleviate organ damage to some extent.
- IPA is a serious, life-threatening lung infection occurring primarily in patients with severe immunodeficiency. Our findings highlight the role of the NLRP3/caspase -1/GSDMD pyrogenic pathway in IPA.

What is the implication, and what should change now?

- NLRP3/caspase-1/GSDMD involved in the pathogenesis of pulmonary aspergillosis has been identified, and it is necessary to continue to explore the application of pathway blockers as disease prevention.

Methods

Experimental animals

A total of 85 male pathogen-free Sprague-Dawley (SD) rats aged 8 weeks old weighing (200±20 g) were provided by Zhejiang Wei Tong Li Hua Experimental Animal Technology Co., Ltd. (animal license number: scxk; Zhejiang; 2019-0001).

The animals were housed under standard conditions and allowed access to food and water *ad libitum*. Animal experiments procedures were approved by the Animal Research Ethics Committee of Guangxi Zhuoqiang Technology Co. Ltd (No. ZQIA-2022-017), in compliance with Animal Research Ethics Committee of Guangxi Zhuoqiang Technology Co. Ltd guidelines for the care and use of animals. A protocol was prepared before the study without registration.

Experimental strains and drugs

Aspergillus fumigatus strain (CGMCC No. 3.15720), DXM (Merck, D022, 0.6 mg/mL, intramuscular (*i.m.*) injection, 0.6 mg/kg), CTX (Sigma, lrac0295, 15 mg/mL, intraperitoneal (*i.p.*) injection, 37.5 mg/kg), normal saline and glucose (Biologics, g708ba0050), peptone (Biologics, G 921ba0010), yeast extract (Biologics, G 916ba0009), and agar (Biologics, g828ba0005).

Equipment

XISEN Meikang automatic biochemical analyzer (XT-

2000i automatic five classification blood cell analyzer (Sysmex, XISEN Meikang Co., Ltd., Japan); flow cytometer (Beckman Coulter, USA, cytoflex s); real-time polymerase chain reaction (RT-PCR) instrument (Applied Biosystems 7500, America); Ultra Micro Nucleic Acid Analyzer (Nano-400, Aosen, Hangzhou, China).

Strain culture

Culture medium composition: 1 g glucose, 1 g peptone and 0.5 g yeast extract were added to 100 mL distilled water, mixed evenly, sub packed into 15 mL glass test tubes, sealed with a cotton stopper of appropriate size, and wrapped with kraft paper for sterilization.

Resurrected strains: 0.3 mL of liquid culture medium was added to the purchased strain ampoules, and the bacterial solution was then placed in two test tubes containing culture medium, and incubated in a constant temperature incubator at 25 ℃ for 5 days.

Strain amplification and colony count: 30 μL of the resurrected strain was placed in a test tube containing liquid culture medium and 25 ℃ cultured for 5 days. Next, 10 μL of bacterial solution was diluted 10^3-fold in a 10^6-fold gradient, after which 10 μL of bacterial solution was inoculated into sabouraud medium. The colony count was calculated after culturing for 2–3 days. The remaining bacterial solution was maintained at 4 ℃ as standby.

Preparation of colony suspension: cultured colonies were centrifuged at 1,006 ×g for 15 min, and the precipitation was diluted to 1.0×10^7/mL according to the count concentration.

Experimental groups

The 85 male SD rats were randomly divided into four groups after 1-week acclimation: (I) normal control group (n=15; NS) 3 mL 0.9% saline was administered per kilogramfor 3 days, and then 0.1ml 0.9% saline dripped through the nose on the 4th and 5th days; (II) Immunosuppressed group (n=20; DXM + CTX) 0.3 mg/kg DXM *i.m.* + 37.5 mg/kg CTX *i.p.* administered for 3 days, followed by 0.1 mL 0.9% saline nasal drip on the 4th and 5th days; (III) normal infection group (n=20; AF, *Aspergillus fumigatus*), 3 mL 0.9% saline was administered per kilogram continuously for 3 days, and 0.1 mL 1.0×10^7 colony-forming units (CFU)/mL spore suspension dripped through the nose on the 4th and 5th days; (IV) Immunosuppressed infection group: (n=30; DXM + CTX + AF) 0.3 mg/kg DXM *i.m.* + 37.5 mg/kg CTX *i.p.* administered for 3 days, followed by nasal drip with 0.1 mL 1.0×10^7 CFU/mL s pore suspension on the 4th and 5th days. Lung tissue and blood were sampled at five separate time points 10 days apart. All of the rats lost >25% of their original body weight and were humanely killed.

Blood biochemical indexes

Two mL venous blood was collected and injected into the collecting vessel containing ETDA anticoagulant. After collecting, the blood was mixed upside down for 3–5 times. The samples were mixed and detected white blood cells (WBC)/red blood cells (RBC)/platelets (PLT) were detected by whole blood classification in 2 hours through XT-2000i automatic five classification blood cell analyzer.

Flow cytometry

For FCM, 100 μL whole blood sample in anticoagulant was obtained, 1μg corresponding antibody solution was added, and the mixture incubated at room temperature in the dark for 30 min, following which erythrocyte lysate was added, the product was mixed well and left to stand in the dark for 10 min prior to centrifugation. The supernatant was discarded and 500 μL phosphate-buffered saline added to the remaining pellet, which was centrifuged again, the supernatant discarded again, and the washing procedure repeated twice. Following resuspension in 500 μL of flow loading buffer, the proportion of T lymphocyte (LYMPH) subsets $CD3^+$, $CD4^+$, and $CD8^+$ was analyzed (20).

Quantitative polymerase chain reaction (q-PCR)

Total RNA extraction: 50–80 mg lung tissue samples had 1 mL Trizol ice homogenate added, maintained at room temperature for 5 min before 0.2 mL of 1-bromo-3-chloropropane was added, then vortexed and mixed well, maintained at room temperature for 3 min, followed by centrifugation at 12,000 g at 4 ℃ for 15 min. Next, 500 μL of absorbed colorless supernatant was placed in a new centrifuge tube, 0.5 mL isopropanol was added and the tube gently inverted 10 times to mix before allowing precipitation at room temperature for 10 min and prior to a final 12,000 g chilled 4 ℃ centrifugation for 10 min, following which the supernatant was discarded. Next, 1 mL of 75% ethanol prepared with diethylpyrocarbonate (DEPC) water was added, vortexed and mixed well before 7,500 g chilled 4 ℃ centrifugation for 5 min, discarding of the supernatant

Table 1 RT-PCR primer sequences

Name	Sense/antisense (S/AS)	Sequence (5'-3')
GAPDH	S	GGCAAGTTCAACGGCACAG
	AS	CGCCAGTAGACTCCACGACAT
NLRP3	S	GACCAGCCAGAGTGGAATGATG
	AS	GCTGGGTGTAGCGTCTGTTGAG
Caspase-1	S	TCCAGGAGGGAATATGTGGG
	AS	TGATAACCTTGGGCTTGTCTTT
GSDMD	S	CATGACTTTAGTCTGCTTGCCGTAC
	AS	TCCTGTAAAATCCTCCCGATGTCT

RT-PCR, real-time polymerase chain reaction.

and washing twice. The RNA was allowed to dry slightly, then 20 μL DEPC dissolved in water was added. Nano 400 determines RNA 260/230 and 260/280, and RNA purity was detected. The cDNA obtained by reverse transcription was detected and analyzed by fluorescence quantitative PCR using ABI 7500 (*Table 1*) (21,22).

Western blotting

The protein content was evaluated using the bicinchoninic acid commercial protein assay kit (Beyotime Biotechnology, Jiangsu, China). The transferred membrane was blocked with 5% skimmed milk for 2 h at room temperature, then incubated with the primary antibody overnight at 4 ℃. After incubation with the horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature, the immunoblotting signals were visualized using a western luminescent detection kit (23,24).

Histopathological examination

Four rat lungs per group were randomly sampled and fixed in 4% paraformaldehyde, processed into paraffin blocks and a microtome was used to cut thin paraffin slices; xylene was used for dewaxing and an alcohol solution for hydration. Hematoxylin and eosin staining (Beyotime, Shanghai, China) was performed to discern the morphology of the lung tissue samples: the cell nucleus was stained with hematoxylin and the cytoplasm with eosin. The lung tissue samples were also evaluated under light microscopy. The pathological changes were assessed by a pathologist blinded to the allocation groups. Lesions (interstitial and alveolar edema, cellular infiltration, airway epithelial-cell damage, hemorrhage) were graded from 0 (normal), 1 (minimal), 2 (mild), 3 (moderate), 4 (severe), to 5 (very severe) (25).

ELISA

On day 10, rat serum samples were analyzed for inflammatory cytokines, including interleukin (IL)-1β, IL-8, and tumor necrosis factor (TNF)-α, using a commercially available sandwich enzyme-linked immunosorbent assay kit (ELISA Set, mlbio, Shanghai, China) specific for each cytokine processed according to the manufacturer's instructions.

Statistical analysis

Significance testing was performed using the two-tailed paired Student's *t*-test or one-way repeated measures analysis of variance with Dunnett's post-hoc test. All data are presented as mean ± standard deviation. Statistical significance was set at $P<0.05$. Data were analyzed and visualized using Microsoft Excel and GraphPad Prism 6.

Results

Pathological examination

The alveolar structure of the control group was complete, the alveolar septum was not widened, there was no obvious edema in the pulmonary stroma, and there were no inflammatory cells or fluid exudation in the alveolar cavities. Pathological changes including pulmonary edema, neutrophil infiltration and bleeding were observed in both the immunosuppressed and normal infection groups.

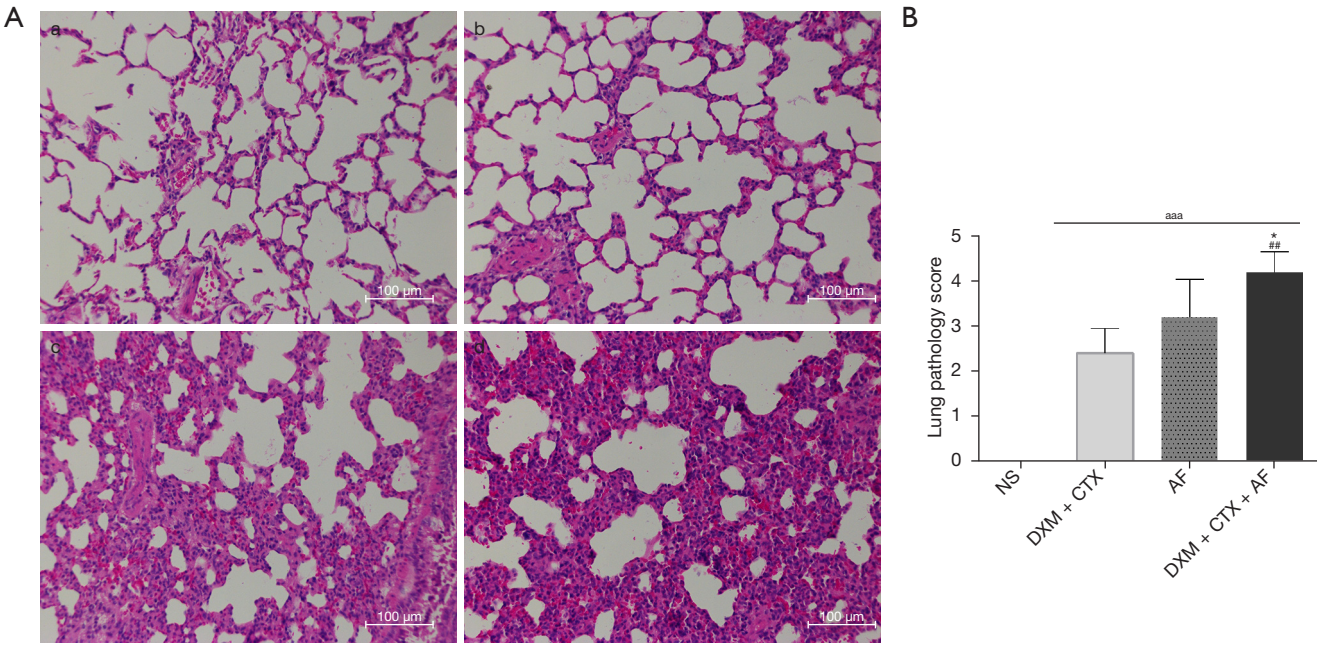

Figure 1 Pathological examination results. (A) Pathological sections of rat lung (HE; hematoxylin-eosin ×200): (a) control group (NS); (b) immunosuppressed group (DXM + CTX); (c) normal infection group (AF); (d) immunosuppressed infection group (DXM + CTX + AF). (B) Lung histopathological scores. compared with NS group. $^{aaa}P<0.001$; compared with DXM + CTX group, $^{\#\#}P<0.01$; compared with AF group, $^{*}P<0.05$, n=3. NS, normal saline; DXM, dexamethasone; CTX, cyclophosphamide; AF, *Aspergillus fumigatus*.

In the immunosuppressed infection group, the alveolar structure was seriously damaged, the alveolar and pulmonary interstitium were seriously edematous, the alveolar septum significantly thickened, and extensive inflammatory cell infiltration was observed, with pulmonary capillary blood flow and bleeding changes. The histopathological lung score showed the severity of injury in the immunosuppressed group, normal infection group and immunosuppressed infection group, which differed significantly from the normal group, and the injury in the immunosuppressed infection group was the most severe. The results are shown in *Figure 1*.

Blood biochemical indexes

The number of WBC in the DXM + CTX + AF and AF groups increased significantly compared with the NS group, and the number of WBC in the DXM + CTX + AF and DXM + CTX groups also increased significantly. The values for RBC, hemoglobin (Hb) decreased and PLT increased Compared with the NS group, platelets hematocrit (PCT), neutrophils (NEUT) and eosinophils (EO) in the DXM + CTX and DXM + CTX + AF groups were significantly increased. Cell types observed increased across the groups, with notable exceptions; the changes in LYMPH were consistent with the flow cytometry measurements. The results are shown in *Table 2*.

Determination of inflammatory factors

Compared with the NS group, IL-1β in the AF and DXM + CTX + AF groups was significantly higher; increased significantly in the AF and DXM + CTX + AF groups compared with the DXM +CTX group; the DXM + CTX + AF was significantly different from the AF group. IL-8 increased significantly in the DXM + CTX, AF and DXM + CTX + AF groups when compared with the NS group. Significant increases in TNF-α in the DXM + CTX, AF, and DXM + CTX + AF groups were observed when compared with the NS group, results are shown in *Figure 2*.

q-PCR

The results revealed significantly higher transcription of NLRP3, caspase-1 and Gasdermin D (GSDMD) in the AF

Table 2 Results of routine blood test of rats (mean ± standard deviation)

Group	WBC (10^9/L)	RBC (10^{12}/L)	Hb (g/L)	PLT (10^9/L)	PCT (%)	NEUT (%)	LYMPH (%)	MONO (%)	EO (%)
NS	8.28±2.90	7.20±0.59	146.00±11.58	982.80±67.43	0.86±0.08	19.02±4.00	80.36±4.20	0.30±0.16	0.42±0.30
DXM + CTX	6.67±1.23	6.21±0.53^{a}	130.40±9.35	2,348.40±383.91^{aa}	2.24±0.36^{aa}	59.72±1.60^{aa}	37.20±3.15^{aa}	0.20±0.12	2.70±1.90^{a}
AF	9.52±1.50^{a}	7.49±0.39$^{\#\#}$	149.40±6.62$^{\#}$	935.00±122.92$^{\#\#}$	0.85±0.11$^{\#\#}$	21.65±3.23$^{\#\#}$	65.00±21.85$^{\#}$	0.40±0.00	0.75±0.41
DXM + CTX + AF	16.85±5.53$^{a\#}$	6.47±0.28*	133.33±2.62*	2,502.00±333.72^{aa}**	2.28±0.42^{aa}**	59.60±6.96^{aa}**	37.03±7.81^{aa}*	0.30±0.17	2.07±1.45

Compared with the NS group, ^{a}P<0.05, ^{aa}P<0.01; compared with DXM + CTX group, $^{\#}$P<0.05, $^{\#\#}$P<0.01; compared with AF group, *P<0.05, **P<0.01, n=12. NS, control group; DXM + CTX, immunosuppressed group; AF, normal infection group; DXM + CTX + AF, immunosuppressed infection group. WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; PLT, platelets; PCT, platelets hematocrit; NEUT, neutrophils; LYMPH, lymphocyte; MONO, Monocytes; EO, eosinophils; NS, normal saline; DXM, dexamethasone; CTX, cyclophosphamide; AF, *Aspergillus fumigatus*.

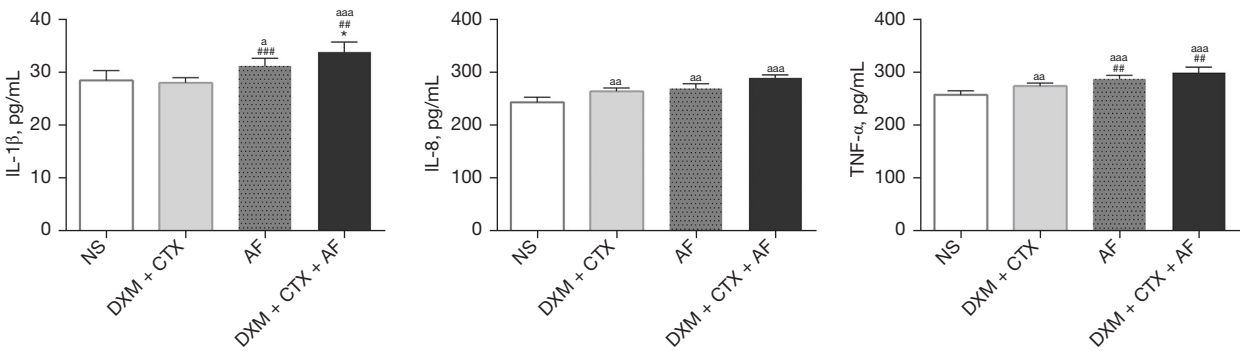

Figure 2 Changes in inflammatory factors. Levels of IL-1β, IL-8, and TNF-α. Compared with NS group, ^{a}P<0.05, ^{aa}P<0.01, ^{aaa}P<0.001; compared with DXM + CTX group, $^{\#\#\#}$P<0.001, $^{\#\#}$P<0.01, $^{\#}$P<0.05; compared with AF group, *P<0.05, n=12. NS, control group; DXM + CTX, immunosuppressed group; AF, normal infection group; DXM + CTX + AF, immunosuppressed infection group. IL, interleukin; NS, normal saline; DXM, dexamethasone; CTX, cyclophosphamide; AF, *Aspergillus fumigatus*; TNF, tumor necrosis factor.

and DXM + CTX + AF groups than in the NS and DXM + CTX groups. Additionally, a non-significant increase in the DXM + CTX group was observed. The transcription of NLRP3 and caspase-1 specifically was significantly higher in the DXM + CTX + AF group than in the AF group. The results are shown in *Figure 3*.

Western blotting

From the results, the expressions of NLRP3, caspase-1 and GSDMD in the AF and DXM + CTX + AF groups were significantly higher than in the NS and DXM + CTX groups. A non-significant increase was noted in the DXM + CTX group. Expressions of NLRP3 and caspase-1 in the DXM + CTX + AF group were significantly higher than in the AF group. The results are shown in *Figure 4*.

$CD3^+$, $CD4^+$, and $CD8^+$ content

There were four notable results. (I) $CD3^+$ content in DXM + CTX + AF group decreased significantly when compared with the NS group; similarly the content in the DXM + CTX + AF group decreased significantly when compared with the AF group. (II) $CD4^+$ content in the DXM + CTX + AF increased significantly when compared with the NS group. (III) $CD8^+$ content in the DXM + CTX + AF was markedly lower than that of the NS and DXM + CTX groups. (IV) $CD4^+/CD8^+$ ratio in the DXM + CTX + AF group was significantly higher than in the NS and DXM + CTX groups. The results are shown in *Figure 5*.

Discussion

Aspergillosis has emerged as one of the most common

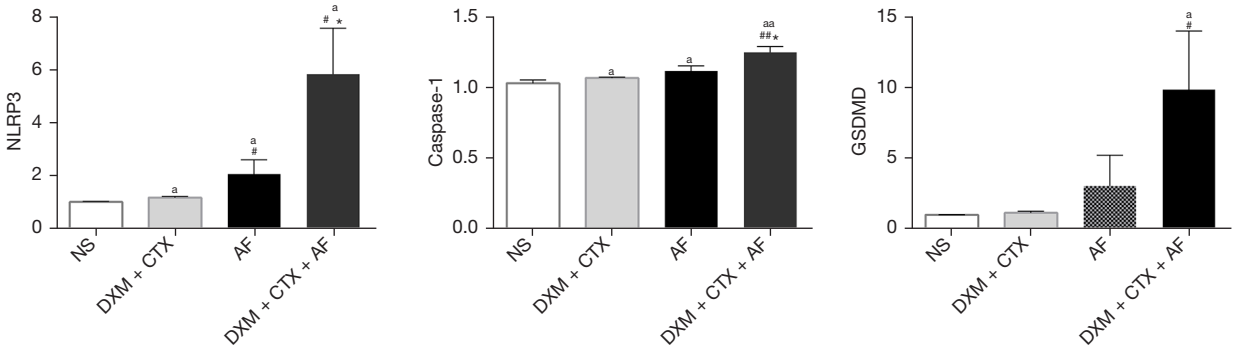

Figure 3 q-PCR results. Compared with NS group, [a]P<0.05, [aa]P<0.01, compared with DXM + CTX group, [##]P<0.01, [#]P<0.05; compared with AF group, *P<0.05, n=6. q-PCR, quantitative polymerase chain reaction; NS, control group; DXM + CTX, immunosuppressed group; AF, normal infection group; DXM + CTX + AF, immunosuppressed infection group. NS, normal saline; DXM, dexamethasone; CTX, cyclophosphamide; AF, *Aspergillus fumigatus*.

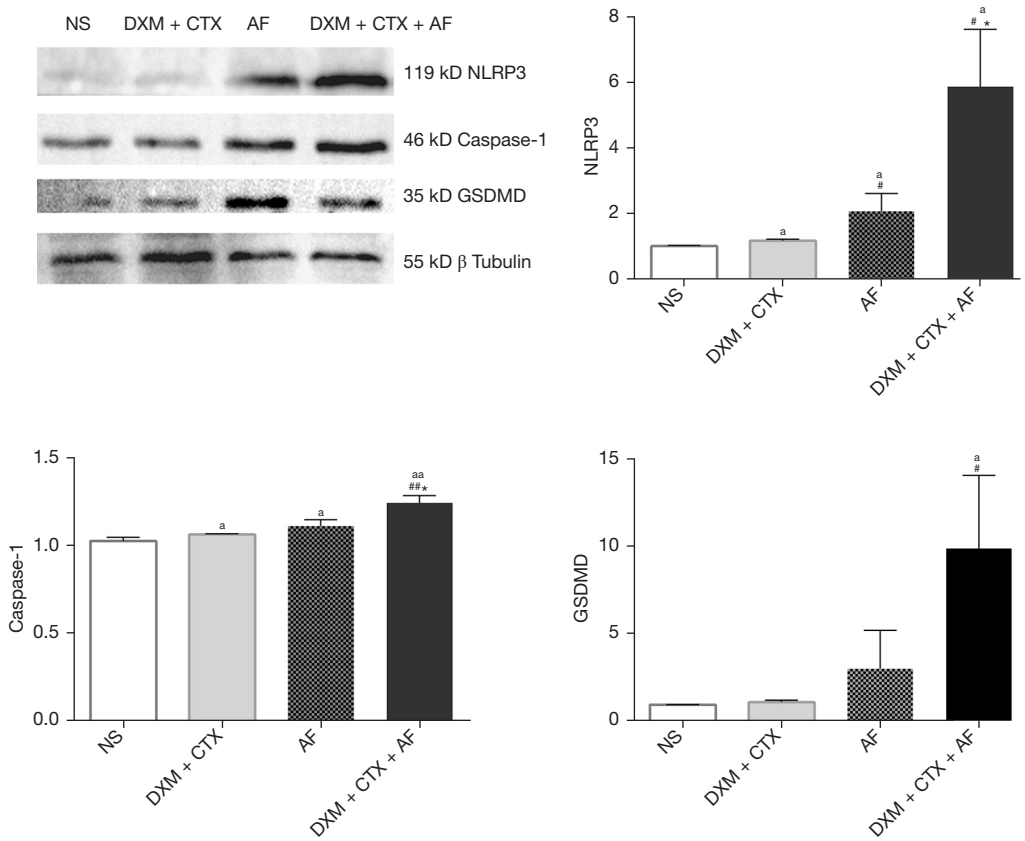

Figure 4 Expression of NLRP3, caspase-1 and GSDMD in lung tissue from rats. Compared with NS group, [a]P<0.05, [aa]P<0.01; compared with DXM + CTX group, [##]P<0.01, [#]P<0.05; compared with AF group, *P<0.05, n=3. NS, control group; DXM + CTX, immunosuppressed group; AF, normal infection group; DXM + CTX + AF, immunosuppressed infection group. NS, normal saline; DXM, dexamethasone; CTX, cyclophosphamide; AF, *Aspergillus fumigatus*.

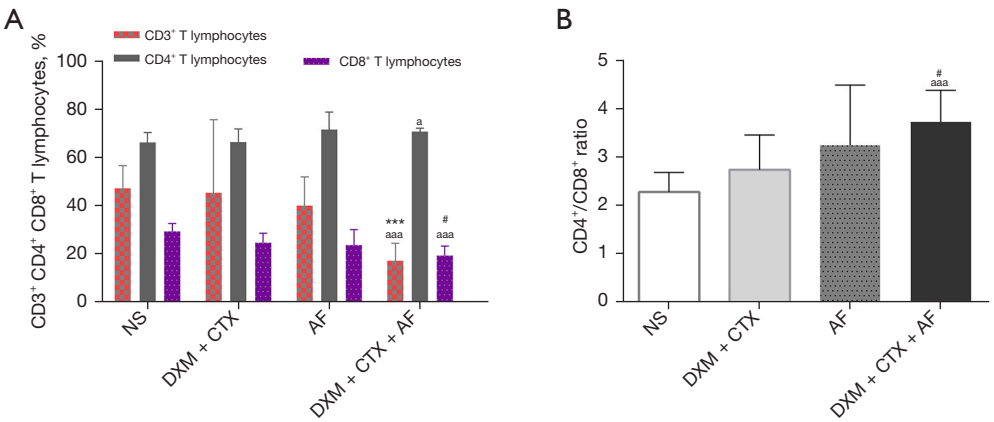

Figure 5 $CD3^+$, $CD4^+$, and $CD8^+$ content results. (A) Content of total lymphocytes, and of $CD3^+$, $CD4^+$, and $CD8^+$ cells in rat blood. (B) $CD4^+/CD8^+$ ratio in the rat groups. Compared with NS group, $^{a}P<0.05$, $^{aaa}P<0.001$; compared with DXM + CTX group, $^{\#}P<0.05$; compared with AF group, ***P<0.001, n=6. NS, control group; DXM + CTX, immunosuppressed group; AF, normal infection group; DXM + CTX + AF, immunosuppressed infection group. NS, normal saline; DXM, dexamethasone; CTX, cyclophosphamide; AF, *Aspergillus fumigatus*.

infectious causes of death in severely immunocompromised patients (26-29). Our study demonstrated that DXM + CTX + AF treatment increased the expression of proinflammatory cytokines and chemokines such as IL-1β, IL-8 and TNF-α, and these results were strongly associated with lung injury score results, and neutrophil infiltration, indicating that DXM + CTX + AF treatment can increase lung infection injury by inducing the expression of proinflammatory mediators.

Characteristic pathological changes in leukocytes, TNF-α, and inflammatory factors such as IL-8 are the most common response to diseases. In our experimental results, the pathological changes in the lung tissue samples from the DXM + CTX + AF rats were more severe than in the AF or DXM + CTX groups, highlighting the need for prevention of IPA in patients on immunosuppressive regimens.

The NLRP3 inflammasome is a widely studied macromolecular multiprotein complex mainly composed of NLRP3, and the apoptosis-related dot-like protein, caspase-1 (30). NLRP3 can mediate the host's immune response to microbial infection and potentially reduce cell damage. When pathogen- or cell damage-related molecules bind to the corresponding cell receptors, nuclear factor kappa B (NF-κB) or reactive oxygen species are activated, which in turn activate NLRP3 (31). Activation of NLRP3 activates caspase-1, followed by IL-1β and IL-8 activation, resulting in inflammation. Caspase is important in the regulation of the classical pathway of cellular pyrogens (32). The process of cell scorch death or pyroptosis, performed by the GSDMD protein (33,34), is a type of inflammatory programmed cell death. Pyroptosis includes activation of the classical and nonclassical pathways of caspase-dependent cell death (4,5,11). NLRP3 is activated by a myriad of molecules, bacteria and viruses during the classical activation pathway of pyroptosis (35). Recruitment of activated caspase precursors into active caspase cleaves the GSDMD protein and forms pore domains at the exposed ends (23,24). Peptides containing the amino terminal active domain of GSDMD induce cell rupture, lead to cell scorch death and release of IL-1β, IL-18 and other material that induce an inflammatory reaction. Conversely, activated caspase-1 has no effect on precursor IL-1β; IL-18 is excised to form active IL-1β and IL-18; after being released, the extracellular factors lead to inflammatory cell damage. In the present experiment, after rats were infected with *Aspergillus fumigatus*, the NLRP3/caspase-1/GSDMD pathway was activated, and inflammatory protein expression and immunosuppression increased significantly.

In conclusion, infection with *Aspergillus fumigatus* increased the pathological score of lung tissue damage, significantly changed routine blood indices, and also significantly progressed the deterioration in the health of immunosuppressed rats. This mechanism has a role in the upregulation of the focal cell death pathway of NLRP3/caspase-1/GSDMD expression. Further studies are necessary to elucidate the correlation between *Aspergillus fumigatus* and DXM + CTX therapy in future therapeutic recommendations.

Conclusions

In immunosuppressed rats, *Aspergillus fumigatus* induced inflammatory reaction. The most severe pathological changes were related to the enhanced expression of NLRP3/caspase-1/GSDMD pathway.

Acknowledgments

Funding: This research was supported by the Guangxi Zhuang Autonomous Region Nanning Science Research and technology development programme (No. 20183039-5) and the Nanning Talent Guangxi Zhuang Autonomous Region Programme – LRB (No. 2018014).

Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-6600/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-6600/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6600/coif). YY is an employee of Guangxi Zhuoqiang Technology Co., Ltd. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Animal experiments procedures were approved by the Animal Research Ethics Committee of Guangxi Zhuoqiang Technology Co., Ltd. (No. ZQIA-2022-017), in compliance with Animal Research Ethics Committee of Guangxi Zhuoqiang Technology Co., Ltd. guidelines for the care and use of animals.

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(English Language Editor: K. Brown)

Cite this article as: Huang W, Mo L, Yang Y, Chen S, Liu Z, Ma Q, Luo X. Effect of *Aspergillus fumigatus* on infection in immunosuppressed rats. Ann Transl Med 2023;11(2):88. doi: 10.21037/atm-22-6600