Effect of HMGB1 on monocyte immune function in respiratory syncytial virus bronchiolitis

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Abstract. Expression of high mobility group protein box 1 (HMGB1) in children with respiratory syncytial virus bronchiolitis and its effect on the inflammatory function of monocytes were investigated. A total of 30 cases of respiratory syncytial viral bronchitis and 30 cases of healthy persons from physical examination were collected from January 2017 to September 2019 in the pediatric department of Xuzhou Children's Hospital, Xuzhou Medical University. HMGB1 expression level in plasma was detected by ELISA. All participants in the study were followed up for 18 months. Human recombinant respiratory syncytial virus (RSV)-A2 virus was used to infect human bronchial epithelial cell line 16HBE, and cell culture supernatant was collected to detect HMGB1. Transwell plate was used to co-culture infected or no-infection groups of epithelial cells and monocytes THP-1. Western blot was used to detect the level of Toll-like receptor (TLR)4 and TLR7 in monocytes. HMGB1 expression level in peripheral blood of children with bronchiolitis was significantly increased compared with that in healthy controls ($P<0.0001$), and was significantly correlated with the severity of the children's condition ($P<0.01$). The expression level of HMGB1 was significantly correlated with the number of monocytes, lymphocytes and CRP expression level. HMGB1 was also significantly increased in cell culture supernatant compared with no-infection group ($P<0.0001$). TLR4 expression in monocytes could be activated by the virus infected cell lines. Follow-up results showed that children with bronchiolitis had a higher incidence of asthma within 18 months ($P<0.05$). The independent risk factors for children to develop asthma were age, number of monocytes and HMGB1 level. HMGB1 is highly expressed in peripheral blood of children with respiratory syncytial virus bronchitis, and RSV epithelial cells can activate TLR4 expression in monocytes, suggesting that HMGB1 plays an important role in monocyte mediated immune inflammation. HMGB1 expression level is related to the development of asthma in children, which is of great significance for understanding the pathogenesis of bronchiolitis and suggesting the prognosis of children.

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Introduction

Bronchiolitis is common in infants between 1 and 6 months. The characteristic lesion is the bronchioles of the lungs, which is a common acute lower respiratory tract infectious disease in children. The major clinical symptoms of the disease are wheezing, shortness of breath, and tri-concavity signs. The disease often occurs in winter, and the most common pathogen is respiratory syncytial virus (1). Researchers have shown that respiratory syncytial virus (RSV) bronchiolitis is one of the high risk factors for Bronchiolitis in infants and young children to develop bronchial asthma (2). RSV is an enveloped, non-segmented antisense RNA virus of the paramyxoviridae family. It is the most common respiratory pathogen in Bronchiolitis in infants and young children worldwide (3). Prospective epidemiological research suggests that RSV-associated bronchiolitis is significantly correlated with subsequent recurrent wheezing and asthma in childhood (4). This phenomenon has also been confirmed in animal models. RSV can be vertically transmitted from mother to the fetal lung respiratory tract, and the persistence of the virus after birth has a significant correlation with persistent airway hyperresponsiveness (5,6). After years of research, there is still a lack of reliable biomarkers, effective vaccines and treatment strategies for RSV bronchiolitis in infants and young children.

High mobility group protein box 1 (HMGB1) is a highly conserved nuclear DNA binding protein and a molecule of damage associated molecular pattern (DAMP). HMGB1 is the key mediator molecule that initiates the innate and adaptive immune response in inflammation (7). HMGB1 exists mainly in the nucleus and is involved in nucleosome stabilization, cell differentiation, DNA repair, and gene transcription. When cells are stimulated by 'danger signals', HMGB1, as the molecule of the alarmins family, can be rapidly activated and released to the outside of cells. It acts directly as inflammatory

cytokine to initiate and maintain the immune response caused by infectious or non-infectious diseases, and maintain the homeostasis balance in the damaged tissue (8). HMGB1 not only participates in the inflammatory response caused by a variety of cytokines and chemotactic proinflammatory cells, but also participates in asthmatic airway sensitization and induction of airway inflammation by directly affecting immune cells (9). In recent years, it is suggested that HMGB1 can be used as a biological marker. It plays an important role in the development of mouse RSV infection and the development of chronic airway dysfunction (6). However, there are still few reports on the expression of HMGB1 in clinical patients with bronchiolitis and its role in activating immune cells during the development of disease. Therefore, this experiment probes into the expression of HMGB1 in the clinical sample and *in vitro* cell model. The effect of HMGB1 on the function of macrophage inflammation activation was explored, aiming at providing a new train of thought for the diagnosis and treatment of RSV bronchiolitis.

Patients and methods

Research objects. The study was approved by the Ethics Committee of Xuzhou Children's Hospital, Xuzhou Medical University (Xuzhou, China). All the families of the child patients were informed of the experimental purpose and research plan before blood drawing, and signed written informed consent. Thirty patients with respiratory syncytial virus bronchiolitis were hospitalized in Department of Pediatrics, Xuzhou Children's Hospital, Xuzhou Medical University from January 2017 to September 2019. Their average age was (10.23±0.62) months in a ratio of male to female 18:12. Inclusion criteria: i) children aged up to 2.5 years with the disease for less than 3 days; ii) the serum-specific RSV-specific IgM was detected to be positive after admission, iii) children who met the diagnostic criteria for bronchiolitis in the 7th edition of Zhufutang Practical Pediatrics; iv) children diagnosed with bronchiolitis for the first time. Exclusion criteria: i) children with a family history of asthma-related diseases; ii) children with congenital heart and pulmonary insufficiency; iii) children with suspected immune deficiency and allergic diseases; iv) children on antiviral drugs for 4 weeks before treatment. The healthy control group comprised of 30 infants and young children who underwent a physical examination in the hospital during the same period. All of the infants were full-term babies, without recent suspicious infection status, and without previous history of diseases such as immunodeficiency, allergic diseases, and long-term medication history. Their age and gender match the patient group, respectively. The age was (10.31±0.63) months, which was comparable (P=0.077). According to the 2014 version of Bronchiolitis Diagnosis and Prevention, the severity of the included patients was graded into mild, moderate and severe according to the feeding amount (normal/normal to drop by half/drop by more than half), respiratory rate (normal/>60 times/min/>70 times/min), inspiratory trident sign on chest wall (mild/moderate/severe), nasal agitating (none/yes), blood oxygen saturation (>92%/88-92%/<88%) and mental condition (normal/minor irritability/irritable coma). All patients included in the research group were followed up for an 18-month outpatient visit to assess whether the children had symptoms such as chest tightness, dry cough, wheezing, and expiratory dyspnea, bronchodilation. Bronchodilation test was positive if FEV1 increased by ≥12% 15 min after inhaling available β2 receptor agonists. There was reversible airflow restriction if FEV1/FVC >0.8. The infants were checked to see if they had asthma in hospital during the follow-up period.

Analysis of peripheral blood. Two milliliters of peripheral venous blood (EDTA) was extracted from all the subjects and placed at 4˚C. The blood samples were sent to the laboratory for detection within 4 h. Then the blood cell analysis and CRP protein expression level of patients upon admission were collected according to the report results.

Enzyme linked immunosorbent assay (ELISA). HMGB1 kit (E-EL-H1554c; Ellerat) was used according to the instructions to detect the concentration of HMGB1 in the peripheral blood plasma of patients. A microplate reader (iMark; Bio-Rad) was used to read the absorbance at 450 nm.

Cytology experiments

Cell culture. Keratinocyte Complete Medium (zq-1303; Zhong Qiao Xinzhou) was used in this experiment to culture human bronchial epithelial cells 16HBE (ZQ0001; Zhong Qiao Xinzhou). The monocyte THP-1 cell line was purchased from (ZQ0086) Zhong Qiao Xinzhou. 90% 1640 (HyClone, Sv30087.01), 10% FBS, 100 μ/ml penicillin and 100 μ/ml streptomycin (Biyuntian, C0222) were used for culture. The cells were cultured in a constant temperature incubator containing 5% CO_2 at 37˚C, and it was used when its density increased to 90%.

Preparation of RSV and infection. Human recombinant RSV-A2 strain was purchased from ATCC in the United States. The RSV was purified by discontinuous sucrose gradient centrifugation at 5,000 x g for 10 min at 4˚C, as described (7). The titer of the virus was confirmed to be 10^9 PFU/ml by methyl cellulose empty spot method. The virus was repacked and stored in a freezer at -80˚C for use. Six hours before RSV virus infected 16HBE cells, the cell culture medium was replaced with basic culture medium. When the cell density reached 90%, the infection was carried out for 6 h according to the complex number of multiplication of infection (MOI)=1.0. The uninfected 16HBE cells were treated with the same amount of 30% sucrose solution as the infection control group. The cell culture supernatant and protein were extracted and stored at -80˚C.

Cell treatment grouping. After the cells in the infected RSV group and the infected control group grew to 30-50% after passage, the cells were divided into HMGB1 inhibition group and observation group. DMSO was added to the observation group, and 0.16 μM (glycyrrhizin, S2302; Selleck) was added to the HMGB1 inhibition group, until the cells grew to the appropriate concentration.

Co-culture of macrophages. Transwell plate (3452; Corning) was used for co-culture of monocytes and 16HBE. THP-1 monocytes with a density of $1x10^6$/ml (ZQ0086; Zhongqiao Xinzhou) was added to the lower wells of Transwell at

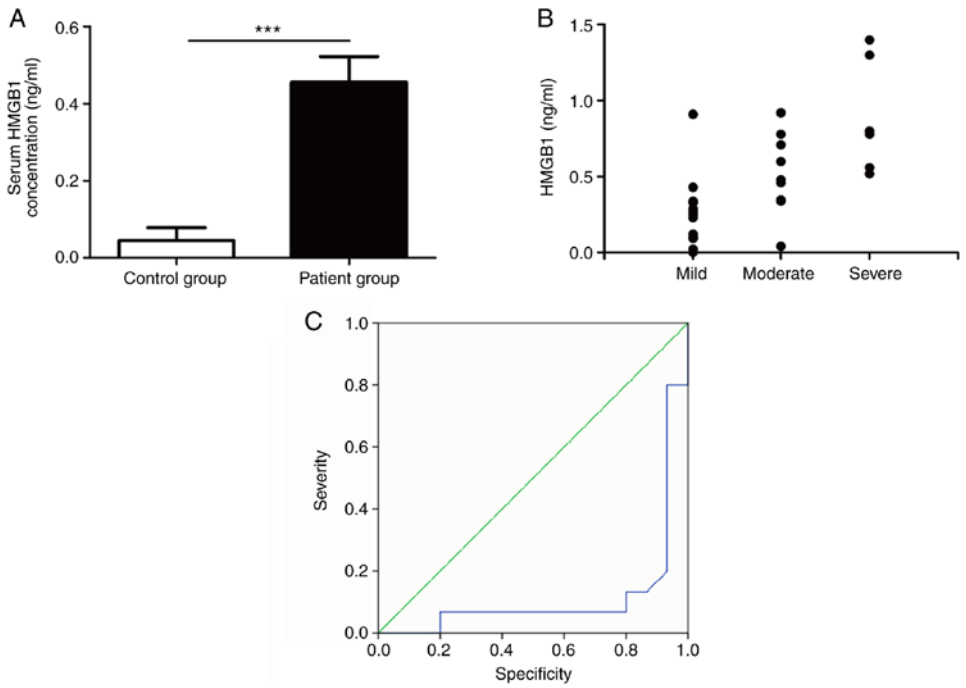

Figure 1. HMGB1 level in peripheral blood of children with RSV and control group and its correlation with severity of the disease. (A) HMGB1 expression level in peripheral blood. (B) Correlation between HMGB1 expression level and the severity of the disease by Spearman Correlation Analysis. (C) ROC curve analysis showed the specificity and sensitivity of HMGB1 in judging the condition of children with bronchiolitis. ***P<0.001. HMGB1, high mobility group protein box 1; RSV, respiratory syncytial virus.

5 ml/well, and 1x10^6 HMGB1 inhibition group infected with RSV virus and 16HBE cells in the observation group were added to the upper wells, respectively. After co-culture of 16HBE cells in the uninfected HMGB1 inhibition group and the observation group for 48 h, the total protein in the lower layer of the cells was extracted for use.

Quantitative real-time polymerase chain reaction (qRT-PCR). RNeasy Mini Kit (74104; Qiagen) was used to extract the total RNA of 16HBE cells infected with RSV and infected control cells. The RNA samples were quantified using a Nanodrop spectrophotometer. Reverse transcription was performed according to the specification of Taqman reagent. The forward and reverse primers of RSV-A2 virus infection are: 5'-ACT GCAATCAYACAAGATGCAACRA-3' and 5'-CAGATT GRAGAAGCTGATTCCA-3'. The expression level of gene change was ΔCt value-ACTB reference value, the expression was calculated based on the results of the $2^{-\Delta\Delta Ct}$ method.

Western blot protein detection. Cells were added with RIPA lysate (R0020; Solarbio) and protease inhibitor PMSF (P0100; Solarbio) with a final concentration of 1 mM, lysed and centrifuged at 15,000 x g for 10 min at 4˚C to extract the cell protein. The protein concentration was quantified by BCA method, and the final protein concentration was adjusted. The sample protein concentration was 5-10 μg/μl with 10 μl applied to each well, and the gel was electrophoresed using an 8% SDS-PAGE separation gel. After electrophoresis, the protein was transferred to the PVDF membrane by a semi-wet transfer method. 5% Skim milk powder was blocked for 1 h, and then Toll-like receptor (TLR)4 (1:2,000, 66350-1-Ig; Proteintech), TLR7 (1:500, 17232-1-AP; Proteintech), GAPDH (1:5,000, 60004-1-Ig; Proteintech) were added. The primary antibody dilution solution was left at 4˚C overnight, then it was washed three times with 0.1% PBST. The corresponding HRP-labeled goat anti-rabbit (mouse) immune secondary antibody (1:3,000, SA00001-1/2; Proteintech) was added, then incubated at room temperature for 1 h. Then washed 3 times with 0.1% PBST. ECL chemiluminescence solution (PE0010; Solarbio) was added, after that, it was developed, fixed and images were taken in a dark room (LAS 4000; ImageQuant) for recording. ImageJ software was used for image analysis.

Data statistics and analysis. All data were counted and analyzed by SPSS 23.0. The results of ELISA and qRT-PCR experiments were expressed as mean ± standard deviation (mean ± SD). The comparison between groups was performed using t-test of paired samples. The correlation between the number and the expression of HMGB1 was analyzed using the Pearson test. The comparison of cells between different treatment groups was performed using one-way ANOVA, Tamhane test was employed as the post hoc test. Unconditional logistic regression was used to analyze the independent risk factors of asthma symptoms in children with bronchiolitis infected with RSV. P<0.05 indicates that the difference was statistically significant.

Results

HMGB1 expression and immune cell levels in peripheral blood of children with RSV bronchiolitis are significantly increased, and correlate with the severity of the disease. ELISA was used to detect HMGB1 expression level in the peripheral blood plasma of the two groups of infants. The results

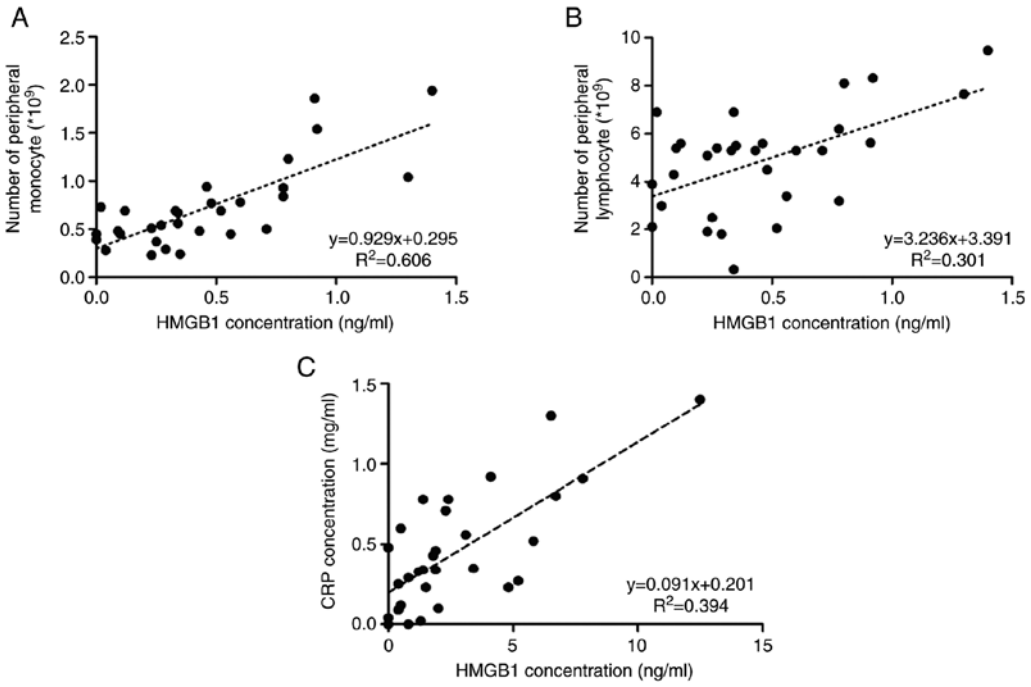

Figure 2. The significantly increased HMGB1 expression in peripheral blood of children with RSV bronchiolitis was significantly correlated with immune indicators. (A) Correlation between the number of monocytes in peripheral blood and the expression level of HMGB1. (B) Correlation between the number of monocytes in peripheral blood and the expression level of HMGB1. (C) Correlation between the expression level of CRP in peripheral blood and the expression level of HMGB1. HMGB1, high mobility group protein box 1; RSV, respiratory syncytial virus.

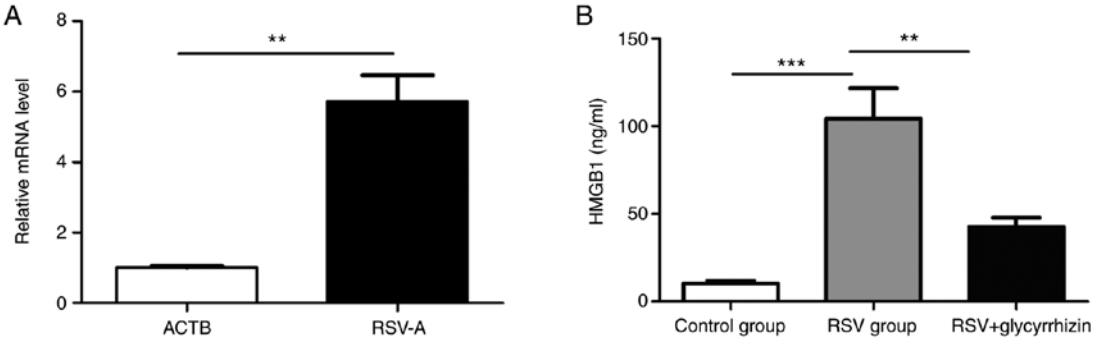

Figure 3. 16HBE cells can up-regulate HMGB1 expression after RSV infection. (A) qRT-PCR verification of RSV infection status, actin-β (ACTB); (B) HMGB1 expression level in the cell supernatant of the control group and RSV infection group. **P<0.01 and ***P<0.001. HMGB1, high mobility group protein box 1; RSV, respiratory syncytial virus.

showed that HMGB1 expression level in children with RSV bronchiolitis was significantly higher than that in the control group (0.45±0.06 vs. 0.04±0.03, t=5.835, P<0.0001 (Fig. 1A). Spearman Analysis was used for further analysis of HMGB1 expression level in peripheral blood and the severity of the disease. The results showed that the HMGB1 expression level was significantly correlated with the severity of the disease (P<0.01, Fig. 1B). The ROC curves showed that HMGB1 can be used as a marker to judge the condition of children with bronchiolitis (95% CI: 0-0.23, P<0.001) (Fig. 1C).

The significantly increased expression of HMGB1 in peripheral blood of children with RSV bronchiolitis is significantly correlated with immune indicators. The correlation of HMGB1 expression in peripheral blood and clinical indicators was analyzed. It was found that the expression of HMGB1 was correlated with monocytes and lymphocytes in the peripheral blood, as well as the CRP expression level upon admission. It suggested that the high expression of HMGB1 in children with RSV bronchiolitis may be correlated with the activation of inflammatory immunity *in vivo* (Fig. 2A-C).

Human bronchial epithelial cell lines can up-regulate HMGB1 after RSV infection. After the 16HBE cells infected with human recombinant RSV-A2 virus (Fig. 3A) were verified by qRT-PCR (1.00±0.05 vs. 5.73±0.73, t=6.455, P<0.01, respectively), it was found that the expression of HMGB1 in the culture medium of 16HBE cells increased significantly after infection (10.22±1.65 vs. 104.31±17.31, t=5.413, P<0.0001), which suggested that *in vitro* cell experiment simulates the RSV process of bronchial epithelial cells (Fig. 3B). In addition, the results of ELISA experiments also found that the addition of glycyrrhizic acid to

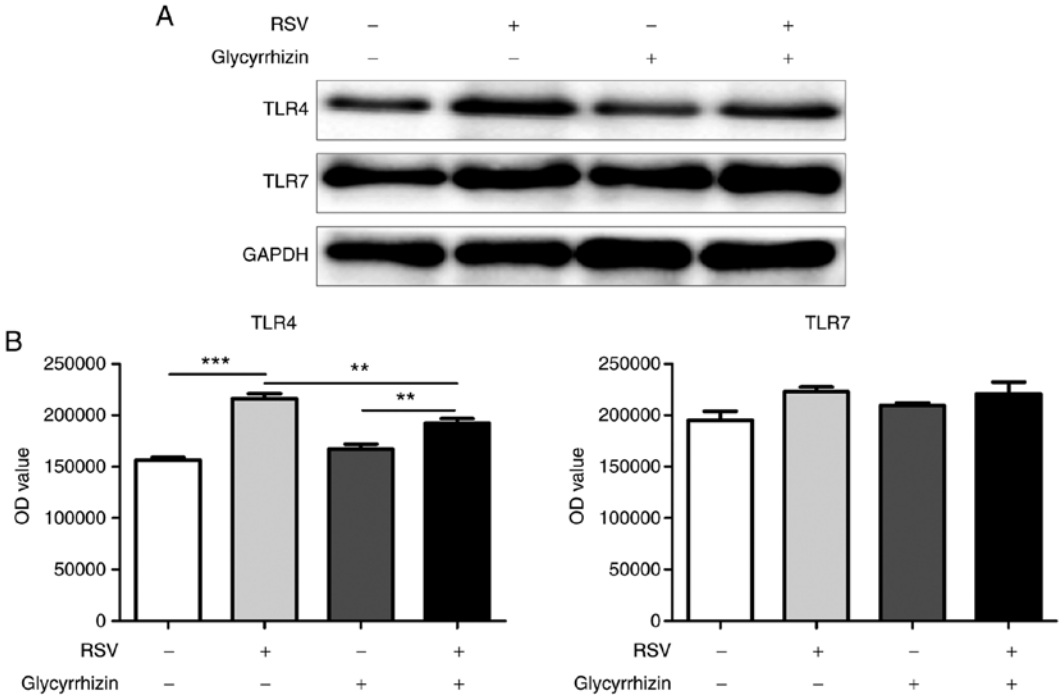

Figure 4. Toll-like receptor expression in monocytes in the treatment groups. (A) Western blot detection of TLR4 and TLR7 protein expression levels in monocytes after co-culture with bronchial epithelial cells infected with RSV and infection control groups, HMGB1 inhibition group and observation group, respectively. (B) TLR4, TLR7 expression statistics. **P<0.01 and ***P<0.001. HMGB1, high mobility group protein box 1; RSV, respiratory syncytial virus. TLR, Toll-like receptor.

Table I. Prognosis of children with RSV in infection group and control group.

Follow-up prognosis	Control group (n=30)	Children with RSV bronchiolitis (n=30)	P-value
Chest tightness, dry cough, wheezing, dyspnea and other symptoms	3	2	0.640
Bronchodilator test is positive	2	7	0.071
FEV_1/FVC >0.8	1	6	0.044[b]
Asthma[a]	2	6	0.128

[a]Asthma diagnosis is based on the Guidelines for Diagnosis and Prevention of Bronchial Asthma in Children (2016). [b]P<0.05. RSV, respiratory syncytial virus.

bronchial epithelial cells after RSV virus infection can significantly reduce the expression level of HMGB1 (Fig. 3B).

RSV-infected human bronchial epithelial cell line activates TLR4 expression in monocytes by up-regulating HMGB1. 16HBE cells infected with RSV virus were co-cultured with monocytes, which showed that the expression of TLR4 in monocytes significantly increased, while TLR7 had no significant change (Fig. 4A). The expression of TLR4 in the mononuclear cells can be significantly inhibited after the addition of glycyrrhizic acid to the culture medium to inhibit the activity of HMGB1 in the infected RSV cell lines (Fig. 4B). This result suggested that RSV may activate monocyte-related immune functions through HMGB1 after 16HBE infection.

Relationship between HMGB1 expression and prognosis in children with RSV infection. The patients with RSV bronchiolitis were followed up for chest distress, dry cough, wheezing, exhale dyspnea and other symptoms for 18 months. The bronchodilation test, the reversible airflow restriction (FEV1/FVC >0.8) test and asthma test were carried out during follow-up. The results showed that the rate of reversible airflow was higher in the patients with RSV (1/30, 6/30, P<0.05) (Table I). The related factors of asthma in children with bronchiolitis were analyzed by unconditional logistic regression. The variables of asthma-related factors included age (<12 months), gender (men), duration of disease (≤5 days), the number of monocytes in blood, and the expression of HMGB1 in peripheral blood plasma on admission for the first time. The results showed that the independent risk factors for asthma symptoms in children were age (OR=0.84, 95% CI: 3.64-12.58, P<0.05), number of monocytes (OR=2.13, 95% CI: 4.88-12.55, P<0.01) and HMGB1 levels upon admission (OR=1.47, 95% CI: 2.56-5.72, P<0.01).

Discussion

Bronchiolitis in infants and young children is also known as asthmatic pneumonia, which usually occurs in winter. Generally, the disease is relatively severe, which may cause local epidemic. Acute RSV infection may be complicated by secondary bacterial infections and respiratory failure. Mechanical ventilation is needed to prolong hospitalization time (10). In developed countries, most RSV infections are self-limiting. However, RSV is globally an important cause of death in children under 5 years of age. As a result, the extensive disease burden caused by the disease and the mortality rate indicate the urgent need for markers and related therapeutic targets in the active phase of RSV infection. This study collected peripheral blood serum from children with RSV capillary bronchitis and healthy children. The results showed that the expression of HMGB1 in children with bronchiolitis infected with RSV was significantly increased. It is consistent with previous results in the RSV capillary bronchitis animal model (7), which suggests that HMGB1 may be used as a biomarker of disease. In addition, the children with RSV bronchiolitis were followed up for 1-2 years. Combined with the prognosis of the children and HMGB1 expression level in peripheral blood at the first admission, the results of Logistic regression analysis showed that the possibility of asthma and allergic related diseases increased with the increase of HMGB1 expression level in the children. Due to the limitation of time and follow-up, this research could not reach the 3-year follow-up (2), and the sample size was relatively small. The expression level and prognosis of HMGB1 in the disease still need to be verified by larger data and high-quality clinical controlled trials.

In this study, human bronchial epithelial cell line 16HBE was used to infect human recombinant RSV-A2 strain virus. The results showed that HMGB1 expression level in epithelial cell culture supernatant could also significantly increase, suggesting that the model could mimic human bronchial epithelial cells infected with RSV. Previous research has shown that after cell infection or apoptosis, HMGB1 can be passively released by necrotic cells or actively produced by macrophages, dendritic cells and natural killer cells (11). In combination with the receptors of TLR2, 4 and RAGE (12), HMGB1 can promote the synthesis of inflammatory cytokines, induce the chemotaxis of inflammatory cells, and support the proliferation of interstitial fibroblasts, the chemotaxis and the synthesis of matrix metalloproteinases (13), leading to the occurrence of acute and chronic diseases (14). Research has shown that both RSV infection and advanced glycation end product receptor (RAGE) mutations are a risk factor for asthma. It has recently been reported that HMGB1 as a cytokine can play an important role in allergic airway sensitivity and airway inflammation in patients with asthma (6). In our study, co-culture of bronchial epithelial cell lines and monocytes after RSV infection was found to significantly increase the expression of TLR4 in monocytes, and there was no significant change in TLR7 in monocytes after RSV infection. However, the results of Qi *et al* (15) and Kim *et al* (16) were different from our research. There may be two reasons. One may be that previous reports have been performed using mouse *in vivo* models and cell lines. There may be some differences with human cells. The other reason may be that cytology experiments directly use RSV to infect immune cells without considering the potential impact of epithelial cells on monocytes.

Although HMGB1 has been reported to play a key role in multiple stages of several DNA (herpes simplex virus type 2) and RNA (western nile virus, dengue) virus infections, literature reports on its role in RSV infection remain limited (7). Hou *et al* (17) reported an increase in HMGB1 levels in lung tissue of RSV-infected mice. The results of this animal model are consistent with the results of previous research (17). Some other research (18) showed that purified HMGB1 was co-cultured with human airway epithelial cell line (AEC) and peripheral mononuclear cells (PBMC) and various immune cells isolated and induced from PBMC, including monocytes. The results showed that although HMGB1 treatment can significantly increase the phosphorylation of NF-κB and P38 MAPK in AECs, it failed to promote the release of cytokines or chemokines. The addition of HMGB1 to immune cells can simultaneously promote the significant induction of cytokine/chemokine release and activate the NF-κB and P38 MAPK pathways. These results suggest that HGMB1 can promote the inflammatory response involved in proinflammatory mediators secreted by immune cells and pathogenesis of RSV by promoting the mechanism. Thus, the results of this experiment further explored the effects of human bronchial epithelial cells on monocytes. It can be found that 16HBE cells infected with RSV can significantly activate the TLR4 signaling pathway in monocytes, thus inducing the generation of innate immune system activation *in vivo*, leading to the activation of inflammatory cytokines and chemokines in downstream pathways. Therefore, blocking the pro-inflammatory function of HMGB1 may be an effective way to develop new treatments. This research was further deepened on the basis of previous studies and focused on the immunomodulatory effects of HMGB1 on monocytes from the 16 HBE cell-derived sources.

Monocyte-macrophages are the key to coordinating the immune system response to infection and injury. We observed that HMGB1 can mediate a series of downstream cytokine/chemokine cascades by activating TLR4 in monocytes. It has important significance in RSV infection. Previous research has shown that HMGB1 cannot stimulate human bronchial epithelial cells, and its effect only shows a certain effect in the presence of RSV (7). Therefore, four groups of bronchial epithelial cells were set up (the control group, the infection group, the non-infection and inhibition group, and the infection and inhibition group) and the cells were co-cultured with monocytes. We found that there was activation induction of the HMGB1-dependent activation of monocytes in the presence of RSV. HMGB1 not only activates monocytes. Research has shown that HMGB1 may trigger more inflammatory reactions by activating other immune cells in the airway to release more pro-inflammatory cytokines, such as mediating neutrophils, proliferation and activation of acid granulocytes (19). Combined with the results of this study, it is suggested that HMGB1 itself or in combination with other proinflammatory cytokines may also cause chronic inflammation of airway epithelial cells. These observations support the idea that increased secretion of HMGB1 can trigger and maintain the pathological state of the disease. Next, the appropriate animal

model should be used to further confirm the pathological role of HMGB1 in bronchiolitis infected with RSV and the physiological process of HMGB1 in the process of RSV infection.

The transfer of HMGB1 from nucleus to cytoplasm or extracellular cells not only indicates that it plays an important role in apoptosis, necrosis and other cell processes, but also indicates that it can affect the host immunity to respiratory tract virus infection by regulating autophagy (20,21). HMGB1 promotes nutrient deficiency and oxidative stress-induced autophagia in mouse embryonic fibroblasts and cancer cells by directly interacting with the key regulatory factor Beclin1 of autophage and apoptosis. Research on the effect of RSV infection on autophagy has shown that RSV can enter the cytoplasm of host cells directly through membrane fusion (22). While DC activation is dependent on the production and immune response of autophagy-mediated TLR-dependent cytokines, autophagy can also promote the identification of intracellular pathogens, the maturation of dendritic cells, and the production of pro-inflammatory cytokines (23-25). HMGB1 mediated autophagy can also control the differentiation of acute promyelocytic leukemia cells by regulating the protein degradation of promyelocytic leukemia and the retinal acid receptor α (26). These studies can lay a solid foundation for further exploration of the role of HMGB1 in airway inflammation caused by RSV infection, and provide new insights into its occurrence mechanism, which is helpful to develop new treatment strategies to treat and prevent bronchiolitis in children caused by a virus.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MN and ZJ designed the study and drafted the manuscript. XX, JZ and JY were responsible for the collection and analysis of the experimental data. MD, GQ and BQ revised the manuscript critically for important intellectual content and were also involved in the conception of the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Xuzhou Children's Hospital, Xuzhou Medical University (Xuzhou, China). Signed informed consents were obtained from the guardians of the child patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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