CD23 Mediates Innate Immunity Against *Aspergillus Fumigatus* Infection in Human Alveolar Macrophages Through Activation by PU.1

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Research Article

Keywords: *Aspergillus fumigatus*, innate immunity, alveolar macrophages, CD23, PU.1

Posted Date: November 17th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1053234/v1>

Abstract

Aspergillosis is a common cause of morbidity and mortality in immunocompromised populations. CD23 is a novel C-type lectin receptors (CLR) recognizing α -mannan and β -glucan in the cell wall of *Aspergillus fumigatus* (AF) that exerts a host innate immune response. However, the molecular mechanism underlying CD23 mediating immunity against AF infection in human alveolar macrophages is still unclear. In this study, we detected the expression of CD23 and PU.1 and the inflammatory markers IL-1 β , IL-6, TNF- α and IL-10 by qRT-PCR, Western blotting and enzyme linked immunosorbent assay (ELISA) analysis in human alveolar macrophages (AMs) with AF infection. Phagocytosis of macrophages with altered CD23 expression and histological changes in lung tissues transfected with CD23-expressing adenoviruses in AF infection were investigated. Dual luciferase, chromatin immunoprecipitation assay (ChIP) and electrophoretic mobility shift assay (EMSA) was performed to detect the interaction of PU.1 and CD23. The results showed that the expression of CD23, PU.1 and these inflammatory markers increased significantly with the time of AF infection. Increasing CD23 expression strengthened the phagocytosis of AMs, and exogenous CD23 attenuated pathological defects in immunodeficient mouse lung tissues with AF infection. Moreover, CD23 was directly activated by PU.1. PU.1 siRNA resulted in downregulation of inflammatory marker expression, but overexpression of CD23 significantly increased the expression of these markers. Our study concluded that CD23 mediates innate immunity against AF in human AMs through activation of PU.1. Therefore, PU.1/CD23 may be a new anti-aspergillosis therapeutic for the treatment of invasive aspergillosis with the deepening of gene therapy and its wide application in the clinic.

Key Messages

- The expressions of CD23, PU.1 and the inflammatory markers IL-1 β , IL-6, TNF- α and IL-10 increased with AF infection time.
- Increasing CD23 expression strengthened the phagocytosis of AMs.
- Exogenous CD23 attenuated pathological defects in immunodeficient mouse lung tissues with AF infection.
- PU.1 directly activated CD23.
- CD23 overexpression rescued the inflammatory marker expressions which decreased by PU.1 interference.

Introduction

Invasive aspergillosis (IA) is a disease that seriously threatens human health. According to WHO statistics, the incidence of IA is increasing annually, with approximately 200,000 new IA patients worldwide each year, and the fatality rate is as high as 50%-95% [1, 2]. Although the development of antifungal drugs has greatly improved the prognosis of IA patients, the mortality rate of IA remains high due to the lack of rapid and reliable early diagnosis methods, the emergence of antifungal drug

resistance and the limitation of treatment options [3]. The main pathogen of IA is AF, which accounts for approximately 90% of infections. AF conidia can enter the respiratory tract and alveoli through respiration. Recently, the role and mechanism of human innate immunity against fungal infections has received extensive attention, as a healthy body can remove inhaled conidia via the immune system; otherwise, they become sick [4, 5]. Therefore, it is of great significance to understand the pathogenesis of AF and guide clinical treatment to study immune recognition and the response to AF infection [6].

AMs are the first sentinel of defence against pathogenic microorganisms and eliminate invading AF through pattern-recognition receptor (PRR)-mediated endocytosis, release cytokines, recruit neutrophils and monocytes to the infection site, balance proinflammatory factors and anti-inflammatory factors, and coordinate the anti-*Aspergillus* response [7, 8]. There are four main types of PRRs: toll-like receptors (TLRs), CLRs, nod-like receptors (NLRs), and RIG-I like receptors (RLRs) [9]. CLRs account for the main PRRs recognizing fungal infections through extracellular C-type lectin domains (CRDs), which recognize important carbohydrate structures of most pathogenic fungi, such as β -glucan and mannan D [10, 11].

The differentiation antigen 23 (CD23) encoded by the Fc fragment of the IgE receptor II (FCER2) gene was recently considered a novel CLR [12, 13]. CD23 is a typical type II transmembrane glycoprotein that includes intracellular N-termini, transmembrane regions and extracellular C-termini and is mainly distributed in B cells, macrophages, eosinophils, intestinal epithelial cells, etc. [14, 15]. CD23 is a low-affinity IgE receptor and plays key roles in the IgE-mediated immune response, regulating cell differentiation and inflammation [12, 14, 16]. The expression of CD23 was increased in mouse macrophages stimulated by *Candida albicans*. CD23 recognizes the α -mannan and β -glucan of *Candida albicans*, thereby inducing macrophages to produce nitric oxide (NO) and exerting innate immune functions [13, 17]. Recent research found that CD23 induced NF- κ B activation by recognizing α -mannan and β -glucan on the surface of AF, and CD23 knockout mice infected with AF had higher mortality than wild-type mice [17]. Taken together, these results indicate that CD23 is an important component exerting antifungal immunity. However, the molecular mechanism underlying CD23 in response to AF infection is still unclear.

The haematopoietic transcription factor PU.1 belongs to the E26 transformation-specific (ETS) family and plays a broad range of roles in cell function, positively regulating gene expression in granulocytes, macrophages, B cells and dendritic cells [18–20]. Previous studies showed that PU.1 was critical for innate immunity against AF by regulating important CLR expression in human macrophages [21]. Moreover, it directly activated the dendritic cell-associated C-type lectin receptor (Dectin1), enhancing the role of Dectin-1 in the host's innate immune response against AF [22]. It is well known that PU.1 activates downstream targets by binding to typical (A/G) AGGAAGTG motifs [20, 23, 24].

In this study, we speculate that CD23 might play pivotal roles in innate defence against AF infection in human AMs through activation by PU.1 based on bioinformatics analysis in which the CD23 promoter regions contain two putative PU.1 binding motifs. The results of CD23, PU.1 and inflammatory factor expression with AF infection in human AMs, phagocytosis with altered CD23 expression *in vitro* and

histological changes in immunodeficient mouse lung tissues with adenovirus AD-CD23 under AF conditions indicated that CD23 was a critical mediator in anti-aspergillosis immunity. In addition, PU.1 activated CD23 expression by directly binding to promoter regions in lung tissues. All the findings verified our hypothesis. PU.1/CD23 may be a new anti-aspergillosis therapeutic for the future treatment of invasive aspergillosis.

Materials And Methods

Induced differentiation of THP-1 macrophages

We cultivated human acute monocytic leukaemia mononuclear THP-1 cells with the cell density adjusted to $1.0 \times 10^6/\text{ml}$. THP-1 cells were induced to differentiate into adherent THP-1 macrophages by phorbol-12-myristate-13-acetate (PMA, 100 ng/ml) stimulation for 24 h, mimicking human AMs.

Plasmid construction and cell transfection

siRNA-mediated CD23/PU.1 interference and pRK5-HA (Promega, US) were used to silence and overexpress CD23/PU.1 expression, respectively. The siRNA sequences against CD23 were sense 5'-GGAGGAACUUCGAGCUGAACA-3' and antisense 5'-UUCAGCUCGAAGUCCUCCAG-3'. The siRNA sequences against PU.1 were sense 5'-CAAGUAAAGUUAUUCUCAAUC-3' and antisense 5'-UUGAGAAUAACUUUACUUGUU-3'. THP-1-derived macrophages were transiently transfected with CD23/PU.1 siRNA and pRK5-CD23 with Lipofectamine 2000 transfection reagent (Thermo Fisher Scientific, US) according to the manufacturer's instructions controlled by a negative control.

Observation of phagocytic ability of the macrophages

THP-1 macrophages with CD23 silencing and overexpression were incubated with FITC-labelled AFA1 (AF strains used in this study, referred to as AF) conidial suspensions (multiplicity of infection (MOI) = 1) for 4 hours. The nuclei and cell membranes of the macrophages were stained with 4',6-diamidino-2-phenylindole (DAPI) (Solarbio, C0065) and 1,1'-dioctadecyl-3,3',3'-tetramethylindodicarbocyanine perchlorate (DiD) (Absin, abs47014947) fluorescent probes. A laser scanning confocal microscope (Lecia SP8, Germany) was used to observe the phagocytic ability of THP-1 macrophages to AF.

Establish of the AF infection model

THP-1 macrophages were incubated with AF conidial suspensions (MOI = 1). Macrophages were harvested at 0 h, 8 h, 16 h and 24 h after AF conidial stimulation. Quantitative real-time polymerase chain reaction (qRT-PCR), Western blotting analysis and enzyme-linked immunosorbent assay (ELISA) were performed to quantify CD23 and PU.1 expression and inflammatory factor levels.

qRT-PCR

Total RNA was extracted from THP-1-derived macrophages with TRIzol reagent. The mRNA was reverse transcribed to cDNA and subjected to qRT-PCR with the PrimeScript® RT Master Mix Perfect Real Time kit (TAKARA Bio Inc., Kusatsu, Japan) and SYBR Green Master Mix (Applied Biosystems, Foster City, CA, US). The reaction was conducted on a qPCR instrument (Applied Biosystems 7900HT Real-Time System, US). The relative gene expression levels were analysed by the $2^{-\Delta\Delta C_t}$ method and normalized to actin. The primer sequence information was forward primer 5'-GCACCTTCCAGTTCTCGTCCAAGC-3' and reverse primer 5'-CGCCGCTGAACTGGTAGGTGAGCT-3' for PU.1 and forward primer 5'-ACTGCGTGATGATGCGGGGCTCC-3' and reverse primer 5'-GTCAGGGTCTGGTCTTGAATCAG-3' for CD23.

Western blotting analysis

The collected macrophages were lysed with RIPA protein extraction reagent (Beyotime, Beijing, China) supplemented with protease inhibitor cocktail (Roche, Pleasanton, CA, US). The lysis products were subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) gels for electrophoresis, transferred to polyvinylidene fluoride (PVDF) membranes, blocked in 5% milk, and incubated with primary antibodies (1:1000, rabbit anti-CD23, rabbit anti-PU.1) (Abcam Inc., Cambridge, MA, US) and secondary antibodies (1:5000, goat anti-rabbit IgG) (Abcam Inc., Cambridge, MA, US). Autoradiograms were quantified by densitometry with GAPDH as a control.

ELISA

Intracellular inflammatory factor levels were detected using an ELISA kit (Solarbio, Beijing) according to the manufacturer's instructions.

CD23-expressing adenovirus

The recombinant adenovirus plasmid pAD-CD23-pIRES2-ERFP was obtained and transfected into human embryonic kidney 293 (HEK293) cells after linearization by Pac I. HEK293 cells amplified the recombinant adenovirus AD-CD23-ERFP (referred to as AD-CD23) in large quantities. The recombinant adenovirus AD-ERFP served as a control.

Animal experiment

To investigate the effect of CD23 in mouse lungs, we anaesthetized BALB/c mice with pentobarbital (70 mg/kg) and transfected 30 μ l CD23-expressing adenovirus AD-CD23 ($\sim 3 \times 10^8$ PFU) into the mice via trachea controlled by adenovirus expressing ERFP (Ad-ERFP). CD23 mRNA expression in lung tissues of each group was detected by qRT-PCR.

CD23-upregulated mice were obtained following the above method, and wild-type mice were injected intraperitoneally with cyclophosphamide (200 mg/kg) 1 d after adenovirus AD-CD23 administration. The immunodeficiency mouse model was established after 4 d of continuous cyclophosphamide administration. Immunodeficient mice were intratracheally injected with AF conidia (5×10^6 PFU) controlled by normal saline treatment. The mice were sacrificed with

pentobarbital (70 mg/kg) 7 d after AF infection. The diseased lung tissue in mice was separated, and haematoxylin and eosin (HE) staining and immunochemistry analysis were performed to detect the pathological changes in the mouse lung tissues.

Immunohistochemistry

Paraffin-embedded blocks were cut into 4- μ m thick sections. The dewaxed and hydrated tissue sections were incubated with anti-PU.1 (Abcam, ab88082) or anti-CD23 (Abcam, ab254162) antibody for 2 h at room temperature and subsequently incubated with a goat-anti-rabbit antibody for 40 min. The degree of staining was determined by development with diaminobenzidine (DAB) chromogen (Bio-Rad, Inc., CA, USA) and detection using a microscope (Olympus, Japan).

Luciferase assay

pRK5-PU.1 cells overexpressing PU.1 and pGL3-CD23 with the LUC reporter were constructed and transfected into HEK293T cells with Lipofectamine 2000 transfection reagent (Thermo Fisher Scientific, US). Twenty-four hours after transfection, the HEK293T cells were incubated with AF conidia for 12 h. The LUC signals were analysed by a dual luciferase reporter gene detection kit (Promega, US) and FlowJo V10 software (Ashland, USA).

ChIP

THP-1 macrophages and AF conidia were co-incubated (MOI = 1) for 8 h. Then, formaldehyde (1%) was applied to crosslink the proteins and chromatin for 10 minutes at room temperature. After that, the macrophages were lysed with an Ultrasonic Breaker (Bioruptor, Belgium). Immunoprecipitation was performed with an IP-level PU.1 antibody (Abcam, US) and an EZ ChIP kit (Millipore, Germany). qPCR was performed to detect the binding activity of PU.1 to the promoter of CD23 after the immune precipitate was washed.

EMSA

According to the ChIP results, biotin-labelled probes for different sites were designed. The nonbiotin-labelled probes were applied as competitive controls with 25-fold, 50-fold and 100-fold competition concentrations. Total proteins were extracted with a cytoplasm-nucleus protein extraction kit (KeyGEN BioTECH, Nanjing, China) following the instructions. The nucleoprotein and nucleic acid probes were combined and reacted. After electrophoresis, membrane transformation and ultraviolet crosslinking, chemiluminescence was used to detect the binding activity of PU.1 to CD23.

Statistical analysis

SPSS 20.0 and SigmaPlot 12.0 were applied for statistical analysis. All data were represented as means $\pm$ SD. Independent groups tests were performed using Student's *t*-test and one-way ANOVA test. $P < 0.05$ was considered statistically significant.

Results

AF infection increased the expression of PU.1, CD23 and inflammatory factors in human AMs.

To investigate the response of human AMs to AF infection, we induced human acute monocytic leukaemia mononuclear THP-1 cells to differentiate into macrophages and infected them with AF conidia. Western blotting analysis showed that the protein levels of PU.1 and CD23 were increased significantly after 16/24 h of infection compared to the expression at 0 h (** $P < 0.01$) (Fig. 1A-1C). The qRT-PCR experiment revealed the following similar results: the mRNA expression of PU.1 and CD23 increased significantly with AF infection over time (Fig. 1D and 1E).

Macrophages play key roles in inflammation. Thus, we detected the expression of the inflammatory factors IL-1 β , IL-6, TNF- α and IL-10 in AMs with AF infection. The ELISA results showed that the expression of these inflammatory factors increased significantly with AF infection compared to the negative control group (* $P < 0.05$, ** $P < 0.01$) (Fig. 2). The expression of inflammatory factors continued to increase within 24 h of infection (Fig. 2).

Overexpression of CD23 strengthened the phagocytosis of AMs against AF conidia.

To clarify the immune function of macrophages in response to AF infection, we established CD23-overexpressing and CD23-silenced TPH-1 macrophages. After incubation of the CD23-OE/siRNA macrophages with FITC-labelled AF conidia, laser scanning confocal microscopy showed that CD23-OE macrophages exhibited stronger phagocytosis against AF conidia than the control group (Fig. 3). In contrast, CD23-siRNA macrophages revealed very weak phagocytic ability against AF conidia (Fig. 3).

Exogenous CD23 attenuates pathological defects in immunodeficient mouse lung tissues with AF infection.

To further clarify the function of CD23 in the immune response, we established CD23-high-expressing immunodeficient mice via intratracheal adenovirus transfection. As shown in Supplemental Fig. 1, ERFP signals were obvious in lung tissues (Supplemental Fig. 1A-1C). The relative levels of CD23 were significantly increased following Ad-CD23-ERFP adenovirus administration compared to the Ad-ERFP and PBS groups (Supplemental Fig. 1A-1D) (** $P < 0.01$). The immunodeficient mice treated with PBS recruited massive leukocytes into the lungs from histopathological analysis after AF infection compared to the normal saline group (Fig. 4A). The alveoli and bronchioles were filled with macrophages, neutrophils, and lymphocytes, exhibiting symptoms of inflammation (Fig. 4A). However, the immunodeficient mice with high CD23 expression revealed obviously milder inflammatory lesions in lung tissues (Fig. 4A). Immunohistochemistry revealed obviously higher CD23 expression in AD-CD23 immunodeficient mice than in PBS-treated mice under normal saline or AF infection conditions (Fig. 4B). CD23 expression in AD-CD23 immunodeficient mice with AF infection was increased compared to that in mice under normal saline conditions (Fig. 4B).

Exogenous PU.1 protected against pathological defects in immunodeficient mouse lung tissues with AF infection.

The expression of both PU.1 and CD23 was upregulated in TPH-1 macrophages during AF infection (Fig. 1). Thus, we performed histological observation of the immune function of PU.1 in immunodeficient mouse lung tissues. The HE staining results showed that exogenous PU.1 upregulation in Ad-PU.1 immunodeficient mouse lung tissues obviously protected against pathological defects in AF infection (Fig. 5A). The histological results from AD-PU.1- and AD-CD23-immunodeficient mice were similar (Fig. 4A, Fig. 5A). The PU.1 expression in mouse lung tissues from immunochemistry showed an obvious increase in Ad-PU.1 mice compared to the PBS group under normal saline, and it was stronger when the Ad-PU.1 mice were treated with AF conidia (Fig. 5B)

PU.1 directly activated the expression of CD23 in the mouse lung.

To characterize the molecular mechanism regulating CD23 expression in lung tissues, we carried out bioinformatics analysis and found that the CD23 promoter region contained two putative motifs for PU.1 binding starting at -659 and -326 (Fig. 6A and 6E). The relative luciferase activity from the dual luciferase reporter assay showed that the PU.1 protein could activate the LUC reporter efficiently in the construct containing CD23 promoter sequences from 2500 bp upstream of ATG to 50 bp downstream of ATP (pGL3-CD23, -2500/+50) compared to the basic plasmid (Fig. 6B). The deleted CD23 promoter containing -300/+50 sequences revealed significantly weak relative luciferase activity ($**P < 0.01$) (Fig. 6B). Moreover, we mutated the two putative PU.1 binding motifs in turn to detect the contributions of the two motifs to the activation of the LUC reporter (Fig. 6C). The results showed that mut-1, mut-2 and mut-3 revealed significantly decreased relative luciferase activity compared to pGL3-CD23 ($**P < 0.01$) (Fig. 6D). Mut-3 with simultaneous mutation of two putative motifs led to a sharp decrease in the activation of PU.1 ($**P < 0.01$) (Fig. 6D). ChIP assays revealed that the PU.1 antibody significantly immunoprecipitated the two putative motifs in the CD23 promoter compared to the Ig control group ($**P < 0.01$) (Fig. 6E and 6F). The relative DNA levels containing the -326/-320 motif were significantly higher than the level containing the -659/-651 motif, which was consistent with the results from the dual luciferase reporter assay and percentage relative to input DNA ($*P < 0.05$, $**P < 0.01$) (Fig. 6D, 6F and 6G). Furthermore, the EMSA verified the above results that the PU.1 protein could bind to the CD23 promoter region, which revealed an obvious mobility shift in electrophoresis via biotin labelling (Fig. 6H). The non-biotin-labelled probes contributed obvious competition activity as the amount increased (Fig. 6H).

Overexpression of CD23 partially rescued cell function after PU.1 interference with AF infection.

To further identify the relationship between PU.1 and CD23, we overexpressed CD23 in PU.1-silenced TPH-1 macrophages. Western blotting analysis showed that CD23 expression with PU.1 silencing decreased significantly under AF infection conditions ($**P < 0.01$) (Fig. 7A and 7B). However, its expression increased significantly after overexpressing CD23 in PU.1 siRNA macrophage ($**P < 0.01$) (Fig. 7A and 7B). Without AF infection, the expression of the inflammatory factors IL-1 β , IL-6, TNF- α , and IL-10 revealed no significant changes with PU.1 silencing or overexpression of CD23 (Fig. 7C-7F).

However, AF infections significantly decreased these expression levels in PU.1 siRNA macrophage compared to the control group ($*P < 0.05$, $**P < 0.01$) (Fig. 7C-7F). Overexpression of CD23 in PU.1 siRNA macrophage significantly increased the expression of these inflammatory factors in AF infection ($*P < 0.05$, $**P < 0.01$) (Fig. 7C-7F).

Discussion

AF as an opportunistic pathogen increases mortality in immunocompromised individuals, accounting for 90% of the IA incidence and 50-95% mortality rate [25–27]. The immune response is oriented by the interaction of pathogen-associated molecular patterns (PAMPs) in the cell wall and different PRRs from the host, leading to disease progression [28]. CD23 was suggested to be a fungal PRR sensing α -mannan and β -glucan in the AF cell wall [17]. In this study, the expression of CD23 was upregulated in TPH-1-derived macrophages with AF infection. In addition, as the infection time increased, the CD23 levels were enhanced significantly. These results suggested that CD23 positively responded to AF infection in human AMs, which confirmed the previous finding that CD23 functions as a fungal PRR in antifungal immunity.

AMs are highly abundant in the lungs, contributing to homeostasis and immunity, and their functions are closely related to lung infections and chronic inflammatory disease [29]. According to the results in this study, CD23-overexpressing TPH-1-derived macrophages improved phagocytosis against AF conidia. Phagocytosis is a fundamental process in immunity in which AMs recognize and remove pathogens and particulates entering the airway with respiration through phagocytosis to defend against pathogenic microbial infections and maintain the balance and stability of the body's environment [30, 31]. Thus, CD23 upregulation is beneficial to protect against AF infections by AMs. In addition, the histological results from HE staining proved that exogenous CD23 by adenovirus transfection attenuates pathological defects in immunodeficient mouse lung tissues with AF infection. The mouse kidney from the control group exhibited classical symptoms of inflammation with AF treatment, recruiting massive macrophages, neutrophils, and lymphocytes to the lesions in the kidney. However, increasing CD23 expression obviously alleviated inflammatory lesions in immunodeficient mice. Previous studies have indicated that CD23 expression is increased in AF keratitis to regulate antifungal immunity [32]. Taken together, we concluded that CD23 might be an irreplaceable component exerting critical roles in human innate immunity dampening *Aspergillus*-triggered immunopathology.

Considering the importance of CD23 in immunity, the regulatory mechanism mediating CD23 activity is under urgent demand. In this study, we suggested that CD23 was directly activated by PU.1 based on dual luciferase, ChIP and EMSAs. In PU.1 siRNA macrophage, the expression levels of the inflammatory factors IL-1 β , IL-6, TNF- α and IL-10 were significantly decreased with AF infection. However, the overexpression of CD23 in the PU.1 siRNA groups significantly increased the expression of these inflammatory factors. These results were consistent with the findings that the expression of CD23 and PU.1 and these inflammatory factors was elevated in macrophages with AF infection. This result indicated that the PU.1-CD23 regulatory pathway was a key mediator of the activity of the inflammatory factors IL-1 β , IL-6, TNF- α and IL-10. It is widely regarded that crucial inflammatory factors released by

immune cells play important roles in host antifungal immunity [33]. Deficiencies in immunity increase inflammatory markers and promote inflammation [34]. Inflammations involving immunity are normal responses to infection. However, unabated inflammation may cause autoimmune or autoinflammatory disorders, even cancer in severe cases [35]. In conclusion, this study elucidated that CD23 directly activated by PU.1 mediated innate immunity against AF infection in human AMs by regulating the expression of the inflammatory factors IL-1 β , IL-6, TNF- α and IL-10 to exert anti-aspergillosis activity. Although more investigations are needed in future studies, with the deepening of gene therapy and its wide clinical application, PU.1/CD23 may become a new anti-aspergillosis therapeutic for the prevention and treatment of invasive aspergillosis.

Declarations

Acknowledgements

This study was supported by grants from Henan Provincial Program for Science and Technology Research (202102310047) and Henan Provincial Program for Medical Science and Technology Research (LHGJ20200569).

Conflicts of interest

The authors declare that there are no relevant conflicts of interest.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of The First Affiliated Hospital, and College of Clinical Medicine, Henan University of Science and Technology. All animal experiments were carried out in accordance with the Chinese governing law on the use of medical laboratory animal.

Availability of data and materials

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

Author contributions

YMZ and YMM conceived and designed the study. MZ and JYQ analysed the data. CYL and YL conducted the experiments. MW designed and wrote the manuscript. The final manuscript was read and approved by all the authors.

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Figures

Figure 1

Expression of PU.1 and CD23 in human AMs with AF infection. (A) Western blotting analysis showing the expression of PU.1 and CD23 in human AMs with AF infection normalized to GAPDH. (B and C) Quantitative analysis of the expression of PU.1 and CD23 in A. (D and E) qRT-PCR results showing the expression of PU.1 and CD23 in human AMs with AF infection. The X axis represents the infection time. All data are presented as the mean $\pm$ SD, $N \geq 3$, * $P < 0.05$, ** $P < 0.01$.

Figure 2

Expression of the inflammatory factors IL-1 β (A), IL-6 (B), TNF- α (C) and IL-10 (D) in human AMs with AF infection. All data are presented as the mean $\pm$ SD, $N \geq 3$, * $P < 0.05$, ** $P < 0.01$.

Figure 3

Observation by confocal microscopy to detect the phagocytosis of AMs against AF conidia.

Figure 4

HE staining (A) and immunochemistry (B) results from mouse lung tissues injected with CD23-expressing adenovirus in AF infection. Normal saline conditions and PBS-treated mice served as controls. Bars = 200 μ m.

Figure 5

HE staining (A) and immunochemistry (B) results from mouse lung tissues injected with PU.1-expressing adenovirus in AF infection. Normal saline conditions and PBS-treated mice served as controls. Bars = 200 μ m.

Figure 6

PU.1 directly activated the expression of CD23. (A) Luciferase reporter plasmid containing two putative PU.1 binding sites in the CD23 promoter (2500 bp upstream of the ATG) compared to the full-length promoter deletion (300 bp upstream of the ATG). (B) Dual luciferase reporter assay showed that PU.1 could activate the LUC signal in the construct containing sequences from 2500 bp upstream of the ATG to 50 bp downstream of ATP. The luciferase activities were normalized to the β -galactosidase levels of the control. (C) Three artificial mutations in the PU.1 binding sites of the CD23 promoter. (D) Relative LUC activity showed the activating efficiency of PU.1 to the three CD23 promoters with artificial mutations. (E)

CD23 promoter revealed two putative PU.1 binding sites starting at -695 and -326. (F) Relative DNA levels of the CD23 promoter region containing two PU.1 binding sites were detected by PCR. (G) Percentage relative to input DNA for PU.1 ChIP was quantified. (H) EMSA showed that the PU.1 protein could bind to the CD23 promoter region in vitro. All data are presented as the mean $\pm$ SD, $N \geq 3$, * $P < 0.05$, ** $P < 0.01$.

Figure 7

CD23 overexpression partially rescued cell function after PU.1 interference. (A) Western blotting analysis showing the expression of CD23 and in THP-1 macrophages with AF infection after PU.1 silencing and PU.1 silencing plus CD23 overexpression, normalized to GAPDH. (B) Quantitative analysis of the relative protein level of CD23 in A. (C-F) Expression of the inflammatory factors IL-1β (C), IL-6 (D), TNF-α (E) and IL-10 (F) in THP-1 macrophages with AF infection after PU.1 silencing and PU.1 silencing plus CD23 overexpression. All data are presented as the mean $\pm$ SD, $N \geq 3$, * $P < 0.05$, ** $P < 0.01$.

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