IL-33 promotes ST2-dependent lung fibrosis by the induction of alternatively activated macrophages and innate lymphoid cells in mice

Dong Li, PhD,[a]* Rodrigo Guabiraba, PhD,[a,b]* Anne-Gaëlle Besnard, PhD,[a] Mousa Komai-Koma, PhD,[c] Majid S. Jabir, MSc,[a,d] Li Zhang, BSc,[e] Gerard J. Graham, PhD,[a] Mariola Kurowska-Stolarska, PhD,[a] Foo Y. Liew, PhD,[a,f] Charles McSharry, PhD,[a] and Damo Xu, PhD[a,e] *Glasgow, United Kingdom, Nouzilly, France, Mecca and Jeddah, Saudi Arabia, Baghdad, Iraq, and Beijing, China*

Background: The initiation and regulation of pulmonary fibrosis are not well understood. IL-33, an important cytokine for respiratory diseases, is overexpressed in the lungs of patients with idiopathic pulmonary fibrosis.
Objectives: We aimed to determine the effects and mechanism of IL-33 on the development and severity of pulmonary fibrosis in murine bleomycin-induced fibrosis.
Methods: Lung fibrosis was induced by bleomycin in wild-type or *Il33r* (*St2*)$^{-/-}$ C57BL/6 mice treated with the recombinant mature form of IL-33 or anti–IL-33 antibody or transferred with type 2 innate lymphoid cells (ILC2s). The development and severity of fibrosis was evaluated based on lung histology, collagen levels, and lavage cytology. Cytokine and chemokine levels were quantified by using quantitative PCR, ELISA, and cytometry.
Results: IL-33 is constitutively expressed in lung epithelial cells but is induced in macrophages by bleomycin. Bleomycin enhanced the production of the mature but reduced full-length form of IL-33 in lung tissue. ST2 deficiency, anti–IL-33 antibody treatment, or alveolar macrophage depletion attenuated and exogenous IL-33 or adoptive transfer of ILC2s enhanced bleomycin-induced lung inflammation and fibrosis. These pathologic changes were accompanied, respectively, by reduced or increased IL-33, IL-13, TGF-β1, and inflammatory chemokine production in the lung. Furthermore, IL-33 polarized M2 macrophages to produce IL-13 and TGF-β1 and induced the expansion of ILC2s to produce IL-13 *in vitro* and *in vivo*.
Conclusions: IL-33 is a novel profibrogenic cytokine that signals through ST2 to promote the initiation and progression of pulmonary fibrosis by recruiting and directing inflammatory cell function and enhancing profibrogenic cytokine production in an ST2- and macrophage-dependent manner. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *IL-33, lung fibrosis, alternatively activated macrophages, type 2 innate lymphoid cells*

Bleomycin is an important cancer chemotherapeutic agent. However, its cytotoxic activity associated with DNA strand cissation and reactive oxygen species induction can cause severe side effects, including pulmonary fibrosis. This can be recapitulated in experimental models designed to investigate the pathogenesis of pulmonary fibrosis and some aspects of idiopathic pulmonary fibrosis (IPF),[1-3] a devastating treatment-refractory interstitial lung disease of unknown origin.[4,5] A better understanding of the fibrotic process might lead to novel therapeutic approaches for this unmet clinical need. Bleomycin-induced fibrosis in susceptible C57BL/6 mice provides a reliable model to study the underlying mechanisms of fibrosis.[1]

Although the pathogenic mechanisms of bleomycin-induced fibrosis and IPF are not fully understood, both conditions are characterized by alveolar epithelial injury, accumulation of fibroblasts and myofibroblasts, and deposition of collagenous extracellular matrix in the lung, which together compromise functional gas exchange.[1,2,4,5] Lung histology and bronchoalveolar lavage (BAL) show inflammatory cytology, including neutrophils, lymphocytes, and macrophages, which are thought to contribute to fibrogenesis.[1,2,4,5] Macrophages can be polarized into 2 phenotypes: classically activated macrophages (M1 macrophages), which are activated by IFN-γ and LPS, or alternatively activated macrophages (M2 macrophages), which are activated by IL-4 and IL-13.[6,7] M1 macrophages express inducible nitric oxide synthase and proinflammatory cytokines and protect against infection, whereas M2 macrophages express arginase 1 and TGF-β1 and are critically involved in tissue repair and fibrosis.[6,7]

The profibrogenic cytokines TGF-β1 and IL-13 are essential for the development of lung fibrosis by promoting myofibroblast differentiation and stimulating production of extracellular matrix proteins, primarily collagen,[4,5] and thus are important potential therapeutic targets in fibrosis. Similar strategies can be applied to other mediators, including cytokines of the IL-1 family, among which IL-1 and IL-18 have a role in clinical and experimental

From [a]the Institute of Infection, Immunity and Inflammation, University of Glasgow; [b]INRA, Infectiologie et Santé Publique, Nouzilly; [c]the Department of Haematology & Immunology, Faculty of Medicine, Umm Al-Qura University, Mecca; [d]the Department of Biotechnology, University of Technology, Baghdad; [e]the Institute of Laboratory Animal Science, Peking Union Medical College, Beijing; and [f]CEGMR, King Abdul-Aziz University, Jeddah.

*These authors contributed equally to this work.

Supported by Arthritis Research UK (AR UK 18912 to D.X.), the Medical Research Council UK, and the Wellcome Trust (to F.Y.L.).

Disclosure of potential conflict of interest: G. J. Graham has received research support from and travel support from the Medical Research Council (MRC), has received participation fees from and is a board member for the Wellcome Trust, and is employed by the University of Glasgow. F. Y. Liew has received research support from MRC. C. McSharry is employed by the UK National Health Service. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication September 9, 2013; revised April 22, 2014; accepted for publication May 1, 2014.

Corresponding author: Damo Xu, PhD or Charles McSharry, PhD, Institute of Infection, Immunity and Inflammation, 120 University Place, University of Glasgow, Glasgow G12 8TA, United Kingdom. E-mail: Damo.Xu@glasgow.ac.uk. Or: Charles.McSharry@glasgow.ac.uk.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2014.05.011

Abbreviations used
APC: Allophycocyanin
Arg1: Arginase 1
BAL: Bronchoalveolar lavage
BMDM: Bone marrow–derived macrophage
CT: Computed tomography
FITC: Fluorescein isothiocyanate
flIL-33: Full-length IL-33
ICOS: Inducible costimulator
ILC2: Type 2 innate lymphoid cell
IPF: Idiopathic pulmonary fibrosis
M1 macrophage: Classically activated macrophage
M2 macrophage: Alternatively activated macrophage
mIL-33: Mature IL-33
Nos2: Inducible nitric oxide synthase 2 gene
PE: Phycoerythrin
qPCR: Quantitative PCR
WT: Wild-type

lung fibrosis.[8] IL-33 is a new member of the IL-1 family and is overexpressed in the lungs of patients with IPF.[9]

IL-33 is a dual-function cytokine: the full-length IL-33 (flIL-33) form serves as an intracellular gene regulator in the nucleus, and the mature IL-33 (mIL-33) form serves as an extracellular cytokine after release when cells sense inflammatory signals or undergo necrosis.[10-15] Once released, flIL-33 can be processed by neutrophil-derived proteases into mIL-33.[13] Although both flIL-33 and mIL-33 are able to bind to and signal through their receptor, ST2, mIL-33 has a 10-fold higher affinity and bioactivity than flIL-33.[13] ST2 is expressed on most innate cells, including macrophages and the newly identified type 2 innate lymphoid cells (ILC2s), and IL-33 plays a direct role in the function of these cells.[16-19] mIL-33 mainly elicits a type 2 immune response and is closely associated with allergic and parasitic diseases.[11,18-22] It has recently been reported that nuclear flIL-33 potentiates bleomycin-induced lung injury in an undefined but ST2-independent manner.[9] The expression of IL-33 mRNA is increased in IPF lung tissue[9]; however, the role of mIL-33 as a cytokine in the fibrotic process is unknown.

We have investigated the effect and mechanism of mIL-33 in the initiation and exacerbation of bleomycin-induced fibrosis in mice. We report here that mIL-33, through ST2, strongly enhances bleomycin-induced pulmonary fibrosis, mainly by promoting inflammatory cell infiltration and function, including polarization of M2 macrophages and ILC2s, and enhancing their IL-13 and TGF-β1 production.

METHODS

Experimental details are provided in the Methods section in this article's Online Repository at www.jacionline.org.

RESULTS

Bleomycin-induced fibrosis is impaired in *St2*$^{-/-}$ mice

Groups of wild-type (WT) and *St2*$^{-/-}$ C57BL/6 mice were given bleomycin or PBS intranasally on day 0. The mice were killed on day 7 or 14 to investigate the role of the cytokine IL-33 in the development of bleomycin-induced fibrosis. WT mice that received bleomycin had progressive lung inflammation (Fig 1, *A*) and fibrosis (Fig 1, *B*) from day 7 compared with PBS control mice. This bleomycin-induced inflammatory and fibrotic response was demonstrated by enhanced inflammatory cell infiltration and collagen deposition in the lung and quantified by using histologic inflammatory and fibrosis scores (Fig 1, *C*). The pathologic changes observed in WT mice given bleomycin were significantly reduced in *St2*$^{-/-}$ mice given bleomycin (Fig 1, *A-C*).

Compared with bleomycin-treated WT mice, bleomycin-treated *St2*$^{-/-}$ mice also had significantly reduced infiltration of neutrophils on day 7 and total leukocytes, including macrophages, neutrophils, and lymphocytes, on day 14 in BAL fluid (Fig 1, *D*). Furthermore, bleomycin-enhanced concentrations of soluble collagen and the expression of collagen 3, which is associated with early-repair fibrosis, were reduced in *St2*$^{-/-}$ compared with WT mice (Fig 1, *E* and *F*), whereas the expression of collagen 1 remained unchanged (data not shown). Moreover, bleomycin-treated *St2*$^{-/-}$ mice have reduced concentrations of IL-33, IL-1, and chemokines (CXCL1, CXCL2 and CCL2) in lung tissue extracts compared with concentrations seen in bleomycin-treated WT mice (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org).

Neutralizing anti–IL-33 antibody attenuates bleomycin-induced fibrosis

We next assessed the role of endogenous IL-33 in the development of bleomycin-induced fibrosis by treating WT mice with anti–IL-33 antibody. C57BL/6 mice were injected intraperitoneally with anti–IL-33 every fifth day from day 0 of bleomycin administration and killed on day 14. Anti–IL-33 antibody reduced IL-33 and IL-1 levels in the lung tissue of bleomycin-treated mice compared with that seen in control IgG-treated mice (see Fig E1, *B*). The antibody treatment also markedly reduced bleomycin-induced airway inflammation and lung fibrosis (Fig 2, *A-C*) and the number of macrophages, neutrophils, and lymphocytes in BAL fluid on day 14 compared with IgG control values (Fig 2, *D*). Furthermore, the antibody treatment significantly reduced lung tissue soluble collagen (Fig 2, *E*) and collagen 3 mRNA expression (Fig 2, *F*).

Recombinant mIL-33 exacerbates bleomycin-induced fibrosis in mice

Mice were administered intranasal mIL-33 together with bleomycin on day 0 and lung tissues were analyzed on day 7 to directly assess the role of the cytokine IL-33. Control mice were given either PBS, mIL-33, or bleomycin alone. One administration of exogenous mIL-33 significantly enhanced bleomycin-induced lung inflammation (Fig 3, *A*), collagen deposition (Fig 3, *B*), and pathology score (Fig 3, *C*), compared with controls. The IL-33–enhanced histologic changes were accompanied by significantly increased total numbers of cells in BAL fluid, mainly neutrophils and lymphocytes, compared with control values (Fig 3, *D*). The coadministration of IL-33 did not change the macrophage numbers in BAL fluid at this time point (7 days) compared with bleomycin alone. IL-33 further increased the levels of bleomycin-induced collagen production (Fig 3, *E*)