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IL-33 Exacerbates Autoantibody-Induced Arthritis

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Rheumatoid arthritis pathogenesis comprises dysregulation in both innate and adaptive immunity. There is therefore intense interest in the factors that integrate these immunologic pathways in rheumatoid arthritis. In this paper, we report that IL-33, a novel member of the IL-1 family, can exacerbate anti–glucose-6-phosphate isomerase autoantibody-induced arthritis (AIA). Mice lacking ST2 (ST2<sup>−/−</sup>), the IL-33 receptor α-chain, developed attenuated AIA and reduced expression of articular proinflammatory cytokines. Conversely, treatment of wild-type mice with rIL-33 significantly exacerbated AIA and markedly enhanced proinflammatory cytokine production. However, IL-33 failed to increase the severity of the disease in mast cell-deficient or ST2<sup>−/−</sup> mice. Furthermore, mast cells from wild-type, but not ST2<sup>−/−</sup>, mice restored the ability of ST2<sup>−/−</sup> recipients to mount an IL-33–mediated exacerbation of AIA. IL-33 also enhanced autoantibody-mediated mast cell degranulation in vitro and in synovial tissue in vivo. Together these results demonstrate that IL-33 can enhance autoantibody-mediated articular inflammation via promoting mast cell degranulation and proinflammatory cytokine production. Because IL-33 is derived predominantly from synovial fibroblasts, this finding provides a novel mechanism whereby a host tissue-derived cytokine can regulate effector adaptive immune response via enhancing innate cellular activation in inflammatory arthritis.

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Rheumatoid arthritis (RA) is characterized by chronic inflammatory infiltration of the synovium, leading to cartilage and bone destruction (1–3). Although the etiology of RA remains obscure, the advent of effective treatment with B cell-depleting agents implicates a critical role for humoral immune responses in disease perpetuation (4, 5). The mechanisms whereby B cells mediate such processes are less clear. Ag presentation and immune regulatory cytokine production have been proposed as potential contributory pathways. Autoantibodies, including rheumatoid factor, anti-cyclic citrullinated peptide, and anti-type II collagen Abs, have been implicated as key effectors in RA (6–8). Transfer of anti-collagen or anti–glucose-6-phosphate isomerase Abs provokes articular inflammation and damage in recipient mice (9, 10). Autoantibodies in turn likely promote inflammatory synovitis by triggering the activation of a variety of innate effector cells primarily via FcR-dependent interactions (11, 12). Recently, there has been particular interest in the role of mast cells in this process (13, 14). Mast cells are present in abundance in RA synovium where their degranulation and proinflammatory cytokine production correlate with RA disease severity (15–18). The importance of mast cells in autoantibody-mediated synovial pathology was demonstrated using the K/BxN model (7). However, relatively little is known about the cytokine/autoantibody interactions that promote this synovial inflammatory pathway. We report here that IL-33 plays an important role in exacerbating K/BxN serum-mediated arthritis via induction of mast cell degranulation.

IL-33 is a recently identified cytokine of the IL-1 family (19, 20). IL-33 protein contains a DNA-binding domain that may mediate transcriptional repressor function (20, 21). IL-33 also can be released when cells undergo necrosis (22, 23) and thereafter trigger inflammation in an autocrine or paracrine fashion. IL-33 signals via a heteromeric receptor consisting of ST2 and IL-1R accessory protein (24, 25). IL-33 could contribute to the inflammatory process via enhanced type 1 or type 2 immune responses (19, 26–33). We have shown recently that IL-33 and ST2 are present in RA synovium and IL-33 and ST2 are associated closely with the pathogenesis of collagen-induced arthritis (33). This observation has been confirmed further by others (34). However, the immune mechanism underlying IL-33/ST2-mediated arthritogenesis remains unclear. We found previously that IL-33 signals markedly enhanced autoantibody production in the collagen-induced arthritis context (33).

We now show that IL-33 can exacerbate autoantibody-induced arthritis (AIA). ST2<sup>−/−</sup> mice developed attenuated AIA, whereas wild-type (WT) mice injected with IL-33 developed a more severe disease. Furthermore, IL-33 failed to exacerbate AIA in mast cell-deficient or ST2<sup>−/−</sup> mice. Moreover, ST2<sup>−/−</sup> mice reconstituted with WT, but not ST2<sup>−/−</sup>, mast cells restored their susceptibility to IL-33–enhanced AIA. Finally, IL-33 enhanced autoantibody-triggered mast cell degranulation in the inflamed joints. Thus, our results demonstrate that IL-33 is a key factor for humoral inflammatory arthritis, most probably via the induction of mast cell degranulation.

Materials and Methods

Mice

BALB/c mice were obtained from Harlan UK (Bicester, U.K.). ST2<sup>−/−</sup> or BALB/c mice were generated as described previously (35). Mast cell-deficient Kit<sup>Wsh</sup> mice in a C57B/6 background were from The Jackson Laboratory, and stained negative for both FcγRIII and FcγRII. 8TA, U.K. E-mail addresses: d.xu@clinmed.gla.ac.uk and f.y.liew@clinmed.gla.ac.uk

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Abbreviations used in this paper: AIA, autoantibody-induced arthritis; BMMC, bone marrow-derived mast cell; DLN, draining lymph node; RA, rheumatoid arthritis; WT, wild-type.

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Laboratory (Bar Harbor, ME). All of the mice were used at 8- to 10-wk-old and maintained at the Biological Services Unit, University of Glasgow. All of the animal experiments were carried out in accordance with the Home Office, U.K., guidelines.

rIL-33

Mouse IL-33 was produced as described previously (33). Endotoxin levels were <0.1 EU/μg of protein according to the Limulus amoebocyte lysate QCL-1000 pyrogen test (Lonza, Basel, Switzerland).

Generation of mouse bone marrow-derived mast cells

Bone marrow-derived mast cells (BMMCs) were derived from female BALB/c mice and maintained as described previously (33, 36). Briefly, bone marrow cells from femurs of mice were cultured with 10 ng/ml IL-3 and 20 ng/ml stem cell factor in complete RPMI medium (RPMI 1640 supplemented with 10% FCS, 2 mM l-glutamine, and 100 U/ml of penicillin and streptomycin) in a 5% CO₂ atmosphere. After 6 wk, BMMCs were collected and tested for c-Kit, Fcer1I, and ST2 expression by flow cytometry using anti-c-Kit (BD Biosciences, San Jose, CA), anti-Fcer1I (eBioscience, Hatfield, U.K.), and anti-ST2 (R&D Systems, Minneapolis, MN) Abs. For in vitro activation, BMMCs (2 × 10⁶ cells/ml) were preincubated with K/BxN or normal mouse serum overnight then stimulated with or without IL-33 for different times. β-Hexosaminidase released (45 min) in the culture supernatant was measured by the method described previously and represented as the percentage of the total enzyme (37). The levels of TNF-α, IL-1β, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IFN-γ, MCP1, MCP3, and MIP1α in the supernatant (48 h) were determined by Luminex (Cytokine 20-Plex; BioSource, London, U.K.) following the manufacturer’s instructions. For in vivo engraftment, ST2⁻/⁻ mice were injected i.v with BMMCs (1 × 10⁶ cells per mouse, the BMMCs contained 90% c-Kit⁺FcεRI⁺ mast cells) as described previously (33). Four weeks later, AIA was induced in the mice.

Induction of AIA and assessment of arthritis

AIA was induced in mice by the injection of anti–glucose-6-phosphate isomerase Ab (IgG1) containing sera from K/BxN transgenic mice as described previously (7). Briefly, 6- to 8-wk-old WT or ST2⁻/⁻ mice (six mice per group) were injected i.p. with 50 or 100 μg K/BxN serum to test the effect of IL-33 in AIA, mice received daily i.p. injections of IL-33 (0.1 μg per mouse) from days 0 to 4. Control mice received the same volume (100 μl) of PBS. All of the mice were monitored daily for signs of arthritis (7). Ankle and paw thickness were measured with a dial caliper (Kroepelin, Munich, Germany). Scores were assigned based on erythema, swelling or loss of function present in each paw on a scale of 0–3, giving a maximum score of 12 per mouse (32, 33).

Quantitative PCR

This was carried out as described previously (38, 39). Briefly, total RNA was extracted from joint tissue or draining lymph nodes (DLNs) using the TRIzol method (Invitrogen, Carlsbad, CA) and purified further with RNeasy columns (Qiagen, Valencia, CA). Real-time PCR was performed using the specific probe and primers from Applied Biosystems (Foster City, CA) (28).

Measurement of cytokines and serum Ab levels

All of the cytokine concentrations in serum and culture supernatant of DLN cells or spleen cells were detected by Luminex following the manufacturer’s instructions. The Ab titers of individual sera were determined by ELISA (BD Pharmingen, San Diego, CA).

Histological examination

For histological assessment, AIA and control mice were sacrificed at the end of the experiments, and the hind limbs were removed, fixed in 10% neutral-buffered formalin, and decalciﬁed. Sections (4 μm) were stained with H&E. The joint pathology was examined and scored as described previously (32, 33). The sections were stained with toluidine blue for mast cells and examined with a microscope (BX 51; Olympus, Melville, NY) using ×10, ×40, and ×100 UPlanApo lenses. Histological examination of the joints was performed using anti–IL-33 and control Abs (R&D Systems) as described previously (30, 33).

Statistical analysis

Clinical scores were analyzed using the nonparametric Mann-Whitney U test, and histological scores were analyzed by one-way ANOVA test. Differences between cumulative incidences at a given time point were analyzed by the χ² contingency analysis. Cytokine and IgG levels were compared using the Student t test.

Results

ST2⁻/⁻ mice develop impaired AIA

To examine the role of ST2 in AIA, groups of sex- and age-matched WT BALB/c and ST2⁻/⁻ BALB/c mice were injected i.p. with anti–glucose-6-phosphate isomerase Ab containing K/BxN serum, and arthritis development was monitored daily for 3 d. At the end of the experiment, mice were sacrificed, and serum, DLNs, and joint tissues were harvested. DLN cells were cultured with anti-CD3 Ab for 3 d, and the culture supernatant was analyzed for cytokines by ELISA. Cytokine mRNA expression in the joints was measured by quantitative PCR. Cytokine and IgG concentrations in the serum were determined by ELISA. WT mice developed AIA within 24 h after K/BxN serum injection. ST2⁻/⁻ mice showed significantly reduced AIA in all of the clinical parameters measured compared with those of the WT mice (Fig. 1A). The levels of TNF-α, IL-1, IL-17, and IFN-γ message in the joints were reduced significantly in ST2⁻/⁻ mice compared with those in WT AIA mice (Fig. 1B). The IFN-γ and IL-17 levels remained lower in the ST2⁻/⁻ group compared with those in WT AIA mice, indicative of impaired AIA. These results were consistent with what have been shown in the literature. The concentrations of serum Abs of individual mice (n = 10) were determined by ELISA. Data are mean ± SEM. *p < 0.05, WT versus ST2⁻/⁻ group by Mann-Whitney U test (n = 6). Data are representative of three experiments.
unchanged (Fig. 1B and data not shown). No significant differences in the levels of the inflammatory cytokines IL-1β, TNF-α, and IFN-γ in the serum and DLNs between WT and ST2−/− mice were detected (data not shown). ST2−/− AIA mice showed significantly reduced levels of serum IgG2a and IgG1 compared with those of WT mice (Fig. 1C). These results show that IL-33/ST2 signaling is associated closely with the disease development in AIA.

IL-33 exacerbates AIA

We next directly tested the arthritic role of IL-33 in AIA. To see a maximal disease-exacerbating effect of injected IL-33, AIA was induced in WT and ST2−/− mice on day 0 with a suboptimal dose of K/BxN serum (50 μl), and then mice were injected with IL-33 (0.1 μg per mouse) or PBS i.p. daily for 5 d. IL-33–treated mice developed significantly exacerbated AIA compared with that of the control PBS-treated mice (Fig. 2A). IL-33 injection did not have a significant effect on the severity of AIA in ST2−/− mice (Fig. 2B). IL-33 treatment alone without K/BxN serum did not induce inflammatory arthritis (data not shown). IL-33 administration markedly increased the mRNA levels of the proinflammatory cytokines IL-1β and TNF-α, but not IFN-γ and IL-17, in the joints of WT mice (Fig. 2C and data not shown). IL-33 administration also induced higher concentrations of serum IgG1 and IgG2a than those in PBS control mice (Fig. 2D). Histological analysis shows that the IL-33 treatment markedly increased mononuclear and polymorphonuclear cell infiltration into the joints, with significant synovial hyperplasia and adjacent cartilage and bone erosion in the AIA mice (Fig. 2E). Furthermore, we determined IL-33 expression in the joint tissues of normal and AIA mice by immunohistochemical staining. We found that IL-33 proteins were detected specifically in the inflamed joints of AIA mice but not in normal joints (Fig. 2F). The levels of IL-33 were enhanced markedly in the joints of mice treated with the K/BxN serum together with IL-33 (Fig. 2F). Moreover, mast cells were also clearly present in the joint tissue, and the mast cells were located among the IL-33–positive cells in the inflamed joints.

Together these data clearly indicate that IL-33 potentiates autoantibody-elicited clinical severity of AIA, local proinflammatory cytokine production, and articular damage.

IL-33 exacerbates AIA mainly via mast cells

We next identified the cellular target of IL-33 in the context of AIA. Because mast cells express membrane ST2 at a high density and are able to directly respond to IL-33 (33, 40–43) and mast cell-deficient KitW−/− mice are completely resistant to AIA (7), we sought to investigate the role of mast cells in IL-33–enhanced AIA. We first...
determined the arthritogenic role of IL-33 in AIA in the mast cell-deficient Kit\(^{W-sh}\) mice. AIA was induced in WT and mast cell-deficient mice, and mice were inoculated with PBS or IL-33 as previously. As expected, WT mice developed AIA, which was exacerbated further by IL-33 treatment (Fig. 3A). Unexpectedly, and in contrast to the Kit\(^{W-s}\) mice, Kit\(^{W-sh}\) mice also developed detectable arthritis when injected with the K/BxN serum, although the disease was significantly milder than that in the WT mice (Fig. 3A). However, IL-33 failed to increase AIA in the Kit\(^{W-sh}\) mice (Fig. 3A). These results therefore show that the IL-33-mediated exacerbation of AIA is associated with mast cell activities.

We further confirmed the importance of mast cells in IL-33-enhanced AIA by a mast cell reconstitution experiment. BMMCs were generated in vitro as reported previously (33, 36). Groups of mice were engrafted i.v. with BMMCs from ST2\(^{-/-}\) or WT mice of the same BALB/c background. Four weeks later, AIA was induced in the mice, and IL-33 was administered as described in Fig. 2A. A group of WT positive control mice also was included and induced for AIA and injected with IL-33 as the ST2\(^{-/-}\) recipients. WT mice treated with IL-33 developed significant AIA, whereas ST2\(^{-/-}\) mice engrafted with ST2\(^{-/-}\) BMMCs and treated with IL-33 developed minimal AIA (Fig. 3B). Importantly, WT BMMCs partially, but significantly, restored the disease severity of AIA in ST2\(^{-/-}\) mice following IL-33 administration (Fig. 3B). Because the only cells in the ST2\(^{-/-}\) recipients capable of responding to IL-33 were the engrafted WT BMMCs, these data therefore confirm that mast cells are associated with the increased severity mediated by IL-33 in AIA.

**IL-33 promotes mast cell degranulation in the joints of AIA mice**

We then determined whether IL-33 exacerbates AIA via its ability to enhance autoantibody-mediated mast cell degranulation. AIA was induced in ST2\(^{-/-}\) and WT mice, and mice were injected with IL-33 or PBS as previously. The inflamed ankle joints were removed at day 4, fixed, and sectioned. The mast cells in the joint tissues were identified by toluidine blue staining, and the intact and degranulated mast cells were quantified as described previously (36). Consistent with a previous report (7), K/BxN serum injection caused ∼37.5% of mast cells to degranulate in the joints of WT mice (Fig. 4A, 4E). The number of degranulated mast cells in the ST2\(^{-/-}\) mice injected with...
K/BxN serum was significantly lower (22.5%, \( p < 0.05 \)) (Fig. 4B, 4E). IL-33 injection resulted in 55% of mast cell degranulation in WT mice (Fig. 4C, 4E), whereas IL-33 treatment did not enhance mast cell degranulation in ST2\(^{-/-}\) mice (Fig. 4D, 4E). IL-33 alone failed to trigger mast cell degranulation in naive mice without autoantibody injection (data not shown). Thus, IL-33 enhances Ab-mediated mast cell degranulation in AIA, which also may contribute to the pathogenesis of AIA.

To demonstrate the synergistic effect of IL-33 and autoantibodies in the activation of mast cell degranulation, we preactivated BMMCs with K/BxN or normal mouse serum overnight and then stimulated the cells with or without IL-33 for different times. The levels of \( \beta \)-hexosaminidase and cytokines or chemokines in the culture supernatants were determined. Although IL-33 alone activated modest levels of \( \beta \)-hexosaminidase and cytokine or chemokine production by mast cells, these levels were markedly enhanced by the presence of the K/BxN serum but not by normal mouse serum (Fig. 5). Our result therefore demonstrated that IL-33 is involved directly in mast cell activities and both IL-33 and autoantibodies are necessary for a maximal induction of mast cell degranulation and proinflammatory mediator production.

**Discussion**

The mechanisms promoting the induction and function of autoantibodies in autoimmune diseases such as RA remain unclear. Data presented here demonstrate for the first time that IL-33 is required for the full induction and exacerbation of the clinical onset of AIA. Because AIA is an autoantibody-triggered, innate cell-mediated arthritic condition, our results provide a novel arthritogenic pathway for IL-33 in inflammatory arthritis.

Our result from Kit\(^{W/wsh}\) mice suggests that mast cells are important but not essential for the development of AIA (Fig. 3A). This is distinct from a previous report showing that mast cell-deficient Kit\(^{W/w}\) mice are completely resistant to AIA induction (7). The reason for this discrepancy is unclear but may be due to the different abnormalities of other cell lineages, in particular the neutrophils, in these mice. It is known that mast cell-deficient Kit\(^{W/w}\) mice also lack neutrophils (44, 45). In contrast, the Kit\(^{W/wsh}\) strain has normal neutrophil numbers and phenotype (44, 45). Because neutrophils also are involved critically in the pathogenesis of AIA (45–47), the complete AIA resistance in Kit\(^{W/w}\) mice may be due to their deficiency in both mast cells and neutrophils. Zhou et al. (45) reported that Kit\(^{W/wsh}\) mice developed arthritis indistinguishable from that in the WT mice when the mice were injected with anti-collagen Ab together with LPS. Because LPS is a strong neutrophil activator, these results are consistent with the notion that both mast cells and neutrophils are important for the manifestation of Ab-mediated arthritis. Nevertheless, the clinical parameters of AIA in Kit\(^{W/wsh}\) mice could not be further enhanced by IL-33, and IL-33 specifically exacerbated AIA in ST2\(^{-/-}\) mice repopulated with WT but not ST2\(^{-/-}\) mast cells. These results therefore demonstrated that mast cells are the main cellular target for IL-33 in enhancing AIA.

Our results suggest that IL-33 may amplify AIA by at least three mechanisms: First, IL-33 is involved critically in the maturation and function of mast cells (38, 39). Therefore, IL-33 likely worsens AIA by accelerating mast cell maturation and activation. Second, IgG-triggered mast cell degranulation and associated inflammatory mediator release are correlated with the severity of inflammatory arthritis (7). Thus, IL-33 also may exacerbate AIA by promoting mast cell degranulation in the joints. Because IL-33 alone fails to induce AIA or mast cell degranulation in vivo, IL-33 may do so by potentiating autoantibody-mediated mast cell activation. This is in agreement with early reports that IL-33 is unable to directly trigger mast cell degranulation in vitro (41–43). Third, we found that IL-33 signals drive the expression of articular proinflammatory cytokines (IL-1\( \beta \) and TNF-\( \alpha \)), which also are required for the initiation of AIA (38, 39). IL-33 also can enhance proinflammatory cytokine production by IgG-primed mast cells.

![Figure 5](http://www.jimmunol.org/pdf/2017/62424A.png)  
**Figure 5.** IL-33 directly promotes autoantibody-initiated mast cell degranulation and proinflammatory mediator production in vitro. Mast cells were precultured overnight with K/BxN serum or normal mouse serum and then stimulated with or without IL-33 (2 ng/ml). A, \( \beta \)-Hexosaminidase release was detected 45 min after the culture. B, For cytokine and chemokine production, the cells were cultured for 48 h, and cytokine and chemokine concentrations in the supernatant were determined by Luminex. Data are mean ± SD. \( * p < 0.05 \) and \( ** p < 0.01 \), compared with controls by Student’s \( t \)-test. Data are representative of two experiments.
We have reported recently that IL-33 is also capable of promoting IgE-mediated mast cell degranulation and enhancing anaphylaxis by activating the phospholipase D1 and sphingosine kinase 1 pathway (36). It will be intriguing to know whether IL-33 uses the same or different mechanisms to promote IgG-mediated mast cell activation and degranulation in AIA. We observed that IL-33 signals also are able to promote IgG1 and IgG2a production in AIA. This is consistent with our previous report that IL-33 signals drive Ag-specific IgG2a and IgG1 in collagen-induced arthritis (33). Although the Ag specificity and the precise role of these Abs in AIA is still unknown, our results nevertheless demonstrated that IL-33 is a powerful Ab inducer leading to arthritogenic pathology. Our results also suggest that IL-33 may promote humoral arthritis by inducing autoantibody production and subsequently potentiating Ab-mediated arthritis reactions.

Together, our data demonstrate that IL-33 is an innate cell-derived arthritogenic factor that is able to drive both cellular and humoral arthritis responses. This finding provides an intriguing mechanism whereby host tissue articular cells of the fibroblast lineage can regulate and promote humoral and innate immune activation to the detriment of joint structure and homeostasis. As such, it offers novel therapeutic potential.

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Disclosures
The authors have no financial conflicts of interest.

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