

Cover Page



Universiteit Leiden




The handle <http://hdl.handle.net/1887/38587> holds various files of this Leiden University dissertation.

**Author:** Suurmond, Jolien

**Title:** Immune regulation by mast cells

**Issue Date:** 2016-03-22



ABILITY OF IL-33- AND IMMUNE COMPLEX-TRIGGERED  
ACTIVATION OF HUMAN MAST CELLS TO DOWN-  
REGULATE MONOCYTE-MEDIATED IMMUNE RESPONSES

Chapter 6

Felice Rivellese,<sup>1,2,3</sup> Jolien Suurmond,<sup>1</sup> Kim LL Habets,<sup>1</sup>  
Annemarie L Dorjée,<sup>1</sup> Nandhini Ramamoorthi,<sup>4</sup> Michael J Townsend,<sup>4</sup>  
Amato de Paulis,<sup>2</sup> Gianni Marone,<sup>2</sup> Tom WJ Huizinga,<sup>1</sup> Costantino Pitzalis,<sup>3</sup>  
René EM Toes<sup>1</sup>

<sup>1</sup>Leiden University Medical Center, Leiden, The Netherlands; <sup>2</sup>University of Naples Federico II, Naples, Italy; <sup>3</sup>William Harvey Research Institute and Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK; <sup>4</sup>Genentech Research and Early Development, South San Francisco, USA

*Arthritis Rheumatol.* 2015;67(9):2343-53

## ABSTRACT

**Objective** Mast cells have been implicated in the pathogenesis of rheumatoid arthritis (RA). In particular, their activation by interleukin-33 (IL-33) has been linked to the development of arthritis in animal models. The aim of this study was to evaluate the functional responses of human mast cells to IL-33 in the context of RA.

**Methods** Human mast cells were stimulated with IL-33 combined with plate-bound IgG or IgG anti-citrullinated protein antibodies (ACPAs), and their effects on monocyte activation were evaluated. Cellular interactions of mast cells in RA synovium were assessed by immunofluorescence analysis, and the expression of messenger RNA (mRNA) for mast cell-specific genes was evaluated in synovial biopsy tissue from patients with early RA who were naive to treatment with disease-modifying antirheumatic drugs.

**Results** IL-33 induced the up-regulation of Fcγ receptor type IIa and enhanced the activation of mast cells by IgG, including IgG ACPAs, as indicated by the production of CXCL8/IL-8. Intriguingly, mast cell activation triggered with IL-33 and IgG led to the release of mediators such as histamine and IL-10, which inhibited monocyte activation. Synovial mast cells were found in contact with CD14<sup>+</sup> monocyte/macrophages. Finally, mRNA levels of mast cell-specific genes were inversely associated with disease severity, and IL-33 mRNA levels showed an inverse correlation with the levels of proinflammatory markers.

**Conclusion** When human mast cells are activated by IL-33, an immunomodulatory phenotype develops, with human mast cells gaining the ability to suppress monocyte activation via the release of IL-10 and histamine. These findings, together with the presence of synovial mast cell-monocyte interactions and the inverse association between the expression of mast cell genes at the synovial level and disease activity, suggest that these newly described mast cell-mediated inhibitory pathways might have a functional relevance in the pathogenesis of RA.

## INTRODUCTION

Mast cells are tissue-resident cells of hematopoietic origin, classically known as effector cells in IgE-mediated inflammation and as mediators of host antimicrobial defenses. In addition, mast cells have been involved in the pathogenesis of many human diseases, including autoimmune disorders (1). However, emerging evidence suggests that mast cells might also have immunoregulatory/homeostatic functions (2). Therefore, their exact role in the pathogenesis of autoimmune diseases, such as rheumatoid arthritis (RA), is still debated (3). In particular, contrasting data have been obtained from animal models of

arthritis (4-8), which could be attributable to the aforementioned double-edged functions of mast cells or to the different disease models and methods used to obtain their depletion *in vivo* (9).

In humans, several lines of evidence point toward a contributory role of mast cells in the pathogenesis of RA. First, they are constitutively present in the synovial membrane (10) and their numbers are increased in RA (11, 12), possibly correlating with parameters of disease activity and progression (13). Furthermore, activation of mast cells in rheumatoid synovial tissue has been linked to the production of proinflammatory cytokines (14), several mast cell mediators have been observed in the synovial fluid of patients with RA (15), and these mediators have been shown to have pathogenic effects on synovial fibroblasts (16). Recently, we showed that mast cells are activated by immune complexes containing anti-citrullinated protein antibodies (ACPAs), with Toll-like receptors (TLRs) augmenting the response of mast cells to ACPA-containing immune complexes (17). Despite these findings, the interaction of mast cells with other synovial immune cells has never been fully investigated, and their ability to influence immune responses during the course of RA is still uncertain.

Among the multiple factors known to activate mast cells, interleukin-33 (IL-33), a member of the IL-1 cytokine family (18), has been shown to enhance the survival, adhesion, and differentiation of these cells, and to induce the production of several proinflammatory cytokines (19, 20). Furthermore, IL-33 was found to prime mouse and human mast cells for activation by IgG immune complexes (21, 22). *In vivo*, IL-33 has been implicated in the ability of mast cells to contribute to the development of arthritis in murine experimental models (23, 24), and high levels of IL-33 have been found in the serum and synovial fluid of patients with RA and those with other inflammatory arthritides (25). In addition to these proinflammatory properties, which are consistent with the described role of IL-33 as an alarmin, recent evidence suggests that this cytokine can also mediate immunoregulatory responses in various settings, by, for example, promoting regulatory T and B cell activity and suppressing monocyte activation through its effects on basophils (26-31). Thus, similar to the findings regarding mast cells, the specific functions of IL-33 in the pathogenesis of autoimmune diseases, including arthritis, are still unclear (32, 33).

To better understand the roles of IL-33 and mast cells in RA, we examined the effects of IL-33 on human mast cells. We hypothesized a novel scenario in which activation of mast cells triggered by the alarmin IL-33 may play a homeostatic role, by contributing to the resolution of inflammation through a feedback system that leads to down-regulation of monocyte-mediated immune responses in RA synovial tissue.

## MATERIALS AND METHODS

### PERIPHERAL BLOOD-DERIVED MAST CELLS

Buffy coat cells were obtained from the peripheral blood of healthy volunteers, with samples collected from a blood bank in The Netherlands (Sanquin). CD34<sup>+</sup> hematopoietic stem cells were isolated from peripheral blood mononuclear cells (PBMCs) using CD34 microbeads (Miltenyi Biotec). Isolated CD34<sup>+</sup> stem cells were differentiated into mast cells using a previously described method (34). After 6–8 weeks, the purity of the mast cells was determined by flow cytometry analyses for the expression of CD117 (c-Kit), Ig  $\epsilon$ -class-binding Fc receptor type I (Fc $\epsilon$ RI), and CD203c; purity ranged from 90% to 99%.

### MAST CELL ACTIVATION

Plate-bound IgG was used as a model to study Fc $\gamma$ R-mediated activation of mast cells (17, 35). Briefly, culture plates (96-well or 48-well flat-bottomed plates) were coated with 100  $\mu$ g/ml of purified human IgG (Jackson ImmunoResearch) in phosphate buffered saline (PBS) for 1.5 hours at 37°C and washed 2 times with PBS. Mast cells were then cultured at a concentration of  $1 \times 10^6$ /ml in RPMI 1640 medium containing 10% fetal calf serum, glutamine, penicillin, and streptomycin (all from Invitrogen) together with 100 ng/ml of stem cell factor (Tebu-Bio), without or with 100 ng/ml of recombinant human IL-33 (PeproTech). After 24 hours, the cells were harvested and supernatants were collected and stored at –20°C until further analysis by flow cytometry.

### IgG ACPA-MEDIATED MAST CELL ACTIVATION

Cyclic citrullinated peptide 2 (CCP2) peptides were obtained from Dr. J. W. Drijfhout (Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands). Nunc Maxisorp plates (VWR Scientific Products) were coated with CCP2 peptide or arginine as a control peptide, and then incubated at 37°C for 1 hour with serum from patients with ACPA-positive RA, with the serum samples diluted 50 times in PBS–0.1% bovine serum albumin (BSA). After washing, mast cells were added to the wells and cultured in the presence or absence of 100 ng/ml of recombinant human IL-33. After 24 hours, the cells were harvested, and supernatants were collected and stored at –20°C.

### MONOCYTE ISOLATION AND STIMULATION

CD14<sup>+</sup> monocytes were isolated from buffy coat PBMCs using magnetic-labeled anti-CD14 beads (Miltenyi Biotec), according to the manufacturer's instructions. Isolated monocytes

(purity >95%) were cultured in the same medium as used for mast cells. Monocytes were incubated with mast cell supernatants (diluted 1:4 in medium) or control medium, and then stimulated with lipopolysaccharide (LPS) from *Salmonella typhosa* (Sigma-Aldrich) at a concentration of 5 ng/ml. After overnight (18-hour) incubation, the cells were harvested and supernatants were collected and stored at  $-20^{\circ}\text{C}$  until further analysis by flow cytometry.

For blocking experiments, supernatants of activated mast cells were preincubated with anti-IL-10 antibody or rat IgG2a as a matched isotype control (BD Biosciences), at 10  $\mu\text{g}/\text{ml}$  for 30 minutes at  $37^{\circ}\text{C}$  in an atmosphere of 5%  $\text{CO}_2$ , prior to being incubated with the monocytes. For inhibition of histamine, monocytes were preincubated for 30 minutes at  $37^{\circ}\text{C}$  with the histamine receptor 2 antagonist ranitidine (Sigma) at  $10^{-4}\text{M}$  (28).

### **PATIENT SAMPLES**

Serum samples were obtained from patients with RA, and the presence of total IgG ACPAs was tested by routine diagnostic enzyme-linked immunosorbent assay (ELISA). For immunofluorescence analysis, synovial tissue was obtained from 3 patients with established ACPA-positive RA (ages 59, 70, and 76 years) who had undergone surgery. For messenger RNA (mRNA) sequencing, mRNA was extracted from synovial tissue samples obtained by ultrasound-guided biopsy from patients with early active RA (<12 months' duration) who were naive to treatment with disease-modifying anti-rheumatic drugs (DMARDs) ( $n=40$ ), enrolled in the Pathobiology of Early Arthritis Cohort (details at <http://www.peac-mrc.mds.qmul.ac.uk>) at the Centre for Experimental Medicine and Rheumatology of Queen Mary University (London, UK) (36). Additional patient characteristics are listed in Supplementary Table 1 (online). All patients fulfilled the American College of Rheumatology 1987 revised criteria for RA (37). Written informed consent was obtained from the patients, and the study was approved by local human ethics committees.

### **IMMUNOFLUORESCENCE ANALYSIS**

Synovial tissue samples were fixed with 4% (weight/volume) formaldehyde (Merck) in PBS and stored in 70% (volume/volume) ethanol. The tissue samples were then embedded in paraffin, in 4- $\mu\text{m}$  sections. Slides were deparaffinized with xylene (Merck), and endogenous peroxidase activity was blocked with 1% hydrogen peroxide (Merck) in methanol for 10 minutes. After antigen retrieval with a Tris-EDTA solution (pH 9; Dako) for 30 minutes at  $96^{\circ}\text{C}$ , slides were stained with monoclonal mouse anti-human antibodies for tryptase (0.2  $\mu\text{g}/\text{ml}$ ; Millipore) or polyclonal goat anti-human antibodies for tryptase (8  $\mu\text{g}/\text{ml}$ ; Santa Cruz Biotechnology), in combination with polyclonal goat

anti-human CD14 antibodies (2.5 µg/ml; Abcam) or monoclonal mouse anti-human CD3 (2.8 µg/ml) or CD20 (0.4 µg/ml) antibodies (both from Dako) in PBS–1% BSA for 1 hour. For control sections, matching isotype control antibodies for CD14 (normal goat IgG; Merck) or for CD3 and CD20 (each mouse IgG1; Dako) were used. Detection of staining was performed using donkey anti-mouse/goat Alexa Fluor 568 and donkey anti-goat/mouse Alexa Fluor 488 (each 2 µg/ml; Invitrogen). All slides were mounted with Vectashield Hard Set mounting medium with DAPI (Vector) and visualized using a Zeiss Axio Scope A1 and AxioVision 4.9.1. Stained sections were scored by counting the number of cells in 10 high-power fields (400× magnification), with scoring performed in a blinded manner by 3 observers (FR, KH, and ALD). Interobserver agreement was evaluated using the intraclass correlation coefficient (with a cutoff value of >0.7 to indicate acceptable agreement).

### **FLOW CYTOMETRY**

For flow cytometry staining, cells were incubated with fluorochrome-conjugated antibodies diluted in PBS–0.5% BSA at 4°C for 30 minutes. To exclude dead cells, 0.2 µM DAPI (Invitrogen) was added. Flow cytometric acquisition was performed on an LSR-II flow cytometer (BD Biosciences). Analysis was performed using FACSDiva (BD Biosciences) and FlowJo software (Tree Star). The following (clonal) antibodies were used: PerCP–Cy5.5–conjugated CD14 (61D3), fluorescein isothiocyanate (FITC)–conjugated FcεRI (AER-37), phycoerythrin (PE)–conjugated mouse IgG1 (61D3) (all from eBioscience), FITC–conjugated CD16 (3G8), PE–conjugated CD63 (H5C6), PE–conjugated CD64 (10.1), PE–conjugated CD80 (L307.4), FITC–conjugated HLA–DR (L243), FITC–conjugated mouse IgG2b [27-35] (all from BD Biosciences), FITC–conjugated CD32 (IV.3) (StemCell Technologies), and Alexa Fluor 488–conjugated CD32b (ch2b6-N297Q) and a corresponding isotype control (ch4420-N297Q) (Macrogenics) (38).

### **MEASUREMENT OF CYTOKINES**

Quantitative immunoassays in mast cell culture supernatants were performed using a 42-plex cytokine Milliplex assay (Millipore). In addition, the following ELISA kits were used: human IL-8 Ready-SET-Go! ELISA kit (eBioscience), human tumor necrosis factor (TNF) ELISA set (BD Biosciences), human IL-10 PeliPair ELISA kit (Sanquin Reagents), and histamine ELISA kit (Neogen).

### **MESSENGER RNA SEQUENCING**

Total RNA from synovial tissue was extracted using a Qiagen RNeasy mini kit in accordance with the manufacturer's protocol, including on-column DNase digestion.

Quality control of samples was done to determine RNA quantity and quality, prior to their processing RNA sequencing. The concentration of total RNA samples was determined using NanoDrop 8000 (Thermo Scientific). The integrity of RNA samples was determined using both a 2100 Bioanalyzer and a 2200 TapeStation (Agilent Technologies). Where available, 1  $\mu$ g of total RNA was used as an input material for library preparation, using an Illumina TruSeq RNA Sample Preparation kit (version 2).

Generated libraries were amplified with 10 cycles of polymerase chain reaction (PCR). The size of the libraries was confirmed using a 2200 TapeStation and High Sensitivity D1K screen tape (Agilent Technologies), and their concentration was determined using a quantitative PCR-based method with a Library quantification kit (Kapa). The libraries were first multiplexed (5 per lane) and then sequenced on an Illumina HiSeq2500 to generate 50 million paired-end, 75-basepair reads (in synovial samples). For the data analysis, the Genomic Short-read Nucleotide Alignment Program (details available at <http://research-pub.gene.com/gmap/>) was used to map and assemble transcripts, using the University of California, Santa Cruz human genome reference sequence hg19 and associated transcriptome map (available at <http://genome.ucsc.edu/>).

### **STATISTICAL ANALYSIS**

Results are expressed as the mean $\pm$ SEM. For comparison between 2 groups, 2-sample t-tests were performed. For differences between multiple groups, one-way analysis of variance was performed, with the Bonferroni post hoc test to correct for multiple testing. Statistical analysis was performed using GraphPad Prism software (version 5). P values less than 0.05 were considered significant.

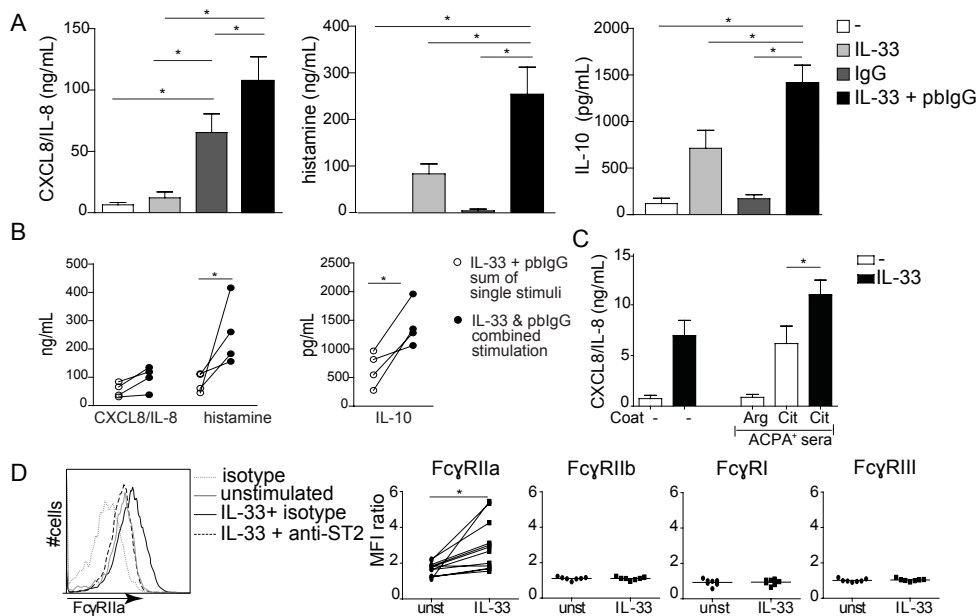
## **RESULTS**

### **MODULATION OF HUMAN MAST CELL ACTIVATION BY IL-33**

We investigated the activation of mast cells by stimuli known to be present in human synovium and implicated in the pathogenesis of RA, i.e., IL-33 and IgG. Stimulation of mast cells with plate-bound IgG mainly induced the release of CXCL8/IL-8, while stimulation with IL-33 induced higher amounts of histamine and IL-10 (Figure 1A).

Likewise, only IL-33, and not plate-bound IgG, induced the up-regulation of the mast cell activation marker CD203c. The histamine release induced by IL-33 triggering was not accompanied by an up-regulation of CD63, a marker of mast cell degranulation (Supplementary Figure 1A).





**Figure 1.** Activation of mast cells via interleukin-33 (IL-33) and immune complexes. Mast cells were left unstimulated (unst) or were activated with IL-33 and/or plate-bound IgG (pblgG) for 24 hours. A, Levels of CXCL8/IL-8, histamine, and IL-10 were measured by enzyme-linked immunosorbent assay (ELISA). Values are the mean±SEM from 4 independent experiments (n=4 donors). B, Synergy was assessed by comparing the sum concentrations of mediators (same as those in A) measured in cell supernatants with single stimuli (IL-33+IgG) and the actual values measured in cell supernatants stimulated simultaneously (IL-33 & IgG). Each symbol joined by a line represents a single mast cell donor (n=4 donors from 4 independent experiments). C, CXCL8/IL-8 levels were measured by ELISA in unstimulated or IL-33–stimulated mast cells after incubation with serum from 3 patients with anti–citrullinated protein antibody (ACPA)–positive rheumatoid arthritis bound to citrullinated (Cit) peptides or arginine (Arg) controls. Values are the mean±SEM results from 6 mast cell donor samples. –= no plate coating. D, Left, Histograms show Fcγ receptor type IIa (FcyRIIa) expression by mast cells left unstimulated or upon stimulation with IL-33 or upon antibody blocking of the IL-33 receptor ST2; matched isotype was used as control. Representative results of 4 independent experiments (n=4 mast cell donors) are shown. Right, Expression of FcγRs was examined in mast cells left unstimulated or stimulated with IL-33. Results are expressed as the ratio of mean fluorescence intensity (MFI) relative to the values for the isotype control; data are from 5 independent experiments. Each symbol represents a single mast cell donor (n=7 or more); horizontal line indicates the mean. \*= $P < 0.05$  by analysis of variance with Bonferroni post hoc test for multiple comparisons, and by Student's t-test for comparisons between 2 groups.

Combination of the 2 stimuli led to an increased activation of mast cells, as indicated by the release of significantly higher amounts of CXCL8/IL-8, histamine, and IL-10 when compared to that observed in unstimulated mast cells or mast cells incubated with either stimulus alone (Figure 1A). To distinguish whether the effect was additive or synergistic, we

compared the sum concentration of CXCL8/IL-8, histamine, and IL-10 in cell cultures incubated with each stimulus alone to the actual amounts produced upon combined stimulation with IL-33 and plate-bound IgG (Figure 1B). This comparison showed higher amounts of histamine and IL-10 in cell cultures that received combined stimulation, indicating that IL-33-triggered mediators have a synergistic effect, whereas in the production of CXCL8/IL-8, only an additive effect of each stimulus was observed.

Having found that plate-bound IgG-mediated activation of mast cells is modulated by IL-33, we wondered whether IL-33 would also enhance the activation of mast cells induced by IgG ACPAs. Similar to previous findings from our group (17), mast cell activation, evaluated by measurement of CXCL8/IL-8 levels, was observed upon triggering with IgG ACPA immune complexes, which were formed by binding of the serum of patients with ACPA-positive RA to citrullinated peptides (Figure 1C). Importantly, mast cells were not activated by ACPA-positive serum incubated with the arginine control (Figure 1C) or by serum from ACPA-negative patients (results not shown). In addition, IL-33 significantly enhanced the ACPA-induced production of CXCL8/IL-8.

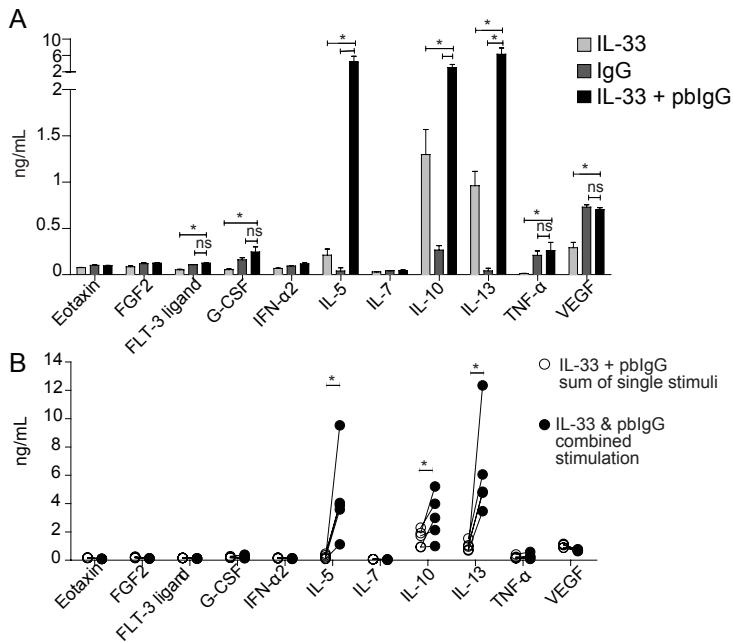
We next sought to identify the mechanism by which IL-33 enhances mast cell activation, and hypothesized that IL-33 modulates the expression of FcγRs on mast cells. Indeed, IL-33 induced a significant up-regulation of the activating FcγRIIa (Figure 1D). This effect was mediated by the IL-33 receptor ST2, as demonstrated by the finding that FcγRIIa expression could be blocked by anti-ST2 antibodies (Figure 1D). Importantly, we did not detect any notable changes in the expression of the other FcγR types by cultured human mast cells, and none of them were influenced by IL-33 stimulation (Figure 1D).

Taken together, these results indicate that IL-33, via its receptor ST2, induces the up-regulation of FcγRIIa, which thereby enhances the activation of mast cells upon triggering with plate-bound IgG, as well as IgG ACPAs, as demonstrated by the increased production of CXCL8/IL-8. More specifically, IL-33 induces IgG-activated mast cells to release histamine and IL-10, but not CXCL8/IL-8, in a synergistic manner.

### **SKEWING BY IL-33 TOWARD A TH2/IMMUNOMODULATORY PHENOTYPE IN MAST CELLS**

To further support the notion that IL-33 is able to modulate mast cell activation by IgG, we performed a multiplex assay on mast cell supernatants. Consistent with the findings in previous literature [20-22], mast cells triggered with IL-33 and plate-bound IgG secreted a wide range of mediators. When comparing the 2 stimuli, we found that plate-bound IgG induced higher amounts of classic proinflammatory mediators, whereas IL-33 induced higher levels of Th2 and immunomodulatory cytokines such as IL-5, IL-10, and IL-13 (see Supplementary Figure 1B). Interestingly, using the combination of IL-33 and

plate-bound IgG to trigger mast cell activation induced higher levels of these cytokines when compared to stimulation with IL-33 alone. In contrast, no additional effects of IL-33 (i.e., no augmentation of the effects of IgG) on the production of proinflammatory mediators such as TNF or FLT-3 ligand could be observed (Figure 2A). In addition, consistent with the data presented in Figure 1B, the combined stimulation showed a synergistic effect that was only present for those mediators whose production was induced by IL-33 (Figures 2A and B).



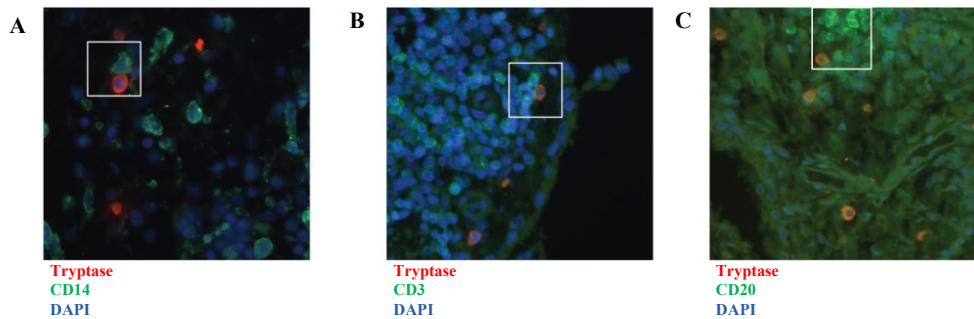
**Figure 2.** Stimulation of mast cells with IL-33 skews the cells toward an immunomodulatory phenotype. A, Production of various mediators in cell cultures was compared between single stimulations and combined stimulations with IL-33 and plate-bound IgG. Values are the mean  $\pm$  SEM from 3 independent experiments in 3 mast cell donor samples, as measured by multiplex assay. B, Synergy of the single or combined stimulations was assessed as described in Figure 1B. Symbols joined by lines represent individual donors. Only mediators exhibiting significantly increased levels upon stimulation of mast cells with either IL-33 or plate-bound IgG are shown.  $*$  =  $P < 0.05$  by analysis of variance with Bonferroni post hoc test in A and by Student's t-test in B. NS = not significant; FGF2 = fibroblast growth factor 2; G-CSF = granulocyte colony-stimulating factor; IFN- $\alpha$ 2 = interferon- $\alpha$ 2; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor (see Figure 1 for other definitions).

Taken together, these findings indicate that IL-33 is not simply enhancing mast cell activation by IgG, but is actually able to fine-tune the responses of mast cells, by

inducing the production of a specific set of Th2-associated and immunomodulatory mediators.

### INTERACTION OF MAST CELLS WITH MONOCYTES IN RA SYNOVIUM

To better understand the possible consequences of mast cell activation at the synovial level in RA, we investigated the occurrence of cellular interactions between mast cells and other immune cells *ex vivo*, by performing immunofluorescence staining for mast cells reactive with tryptase, as well as staining for the monocyte/macrophage marker CD14, the B cell marker CD19, or the T cell marker CD3, in tissue sections from the synovium of patients with RA. We found numerous tryptase-positive mast cells scattered in the synovium of patients with RA. Interestingly, tryptase-positive cells showed clear cell-to-cell interaction with CD14<sup>+</sup> cells, CD3<sup>+</sup> cells, and CD20<sup>+</sup> cells (Figures 3A–C).



**Figure 3.** Mast cell interaction with immune cells in rheumatoid arthritis (RA) synovial tissue. RA synovial tissue was analyzed by immunofluorescence staining, showing tryptase-positive mast cells (red) closely interacting with other immune cells (green), including CD14<sup>+</sup> cells (A), CD3<sup>+</sup> T cells (B), and CD20<sup>+</sup> B cells (C). Nuclear staining with DAPI (blue) is also shown. Representative images from 1 of 3 donors are shown. Original magnification  $\times 40$ . Boxed areas highlight the cellular interactions.

To quantify these interactions, we counted the number of tryptase-positive cells in close contact with each cell type. As shown in Table 1, a substantial proportion of mast cells (mean 19.5%) was found to be in contact with CD14<sup>+</sup> cells in the synovial tissue of 3 patients with RA. Similarly, the proportion of total CD14<sup>+</sup> cells interacting with mast cells was considerably higher than the proportion of B and T cells interacting with mast cells.

These results show that mast cells in the synovial tissue of patients with RA are in close contact with immune cells. In particular, a substantial number of synovial mast cells are localized in proximity to synovial CD14<sup>+</sup> cells.

**Table 1.** Interactions between mast cells (MCs) and other immune cells in RA synovia.

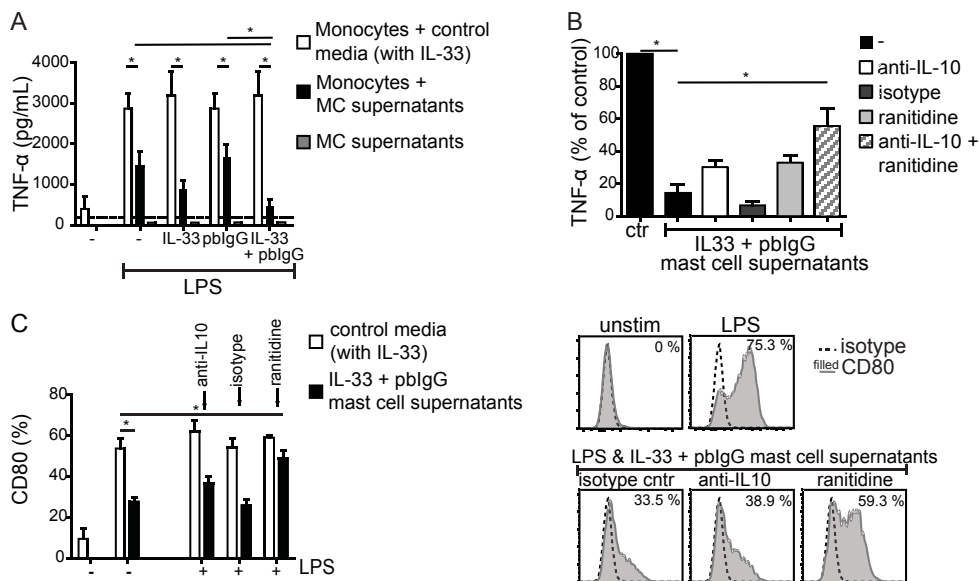
	RA 1	RA 2	RA 3	Mean*
Mast cells	31,3	11,7	30,7	24,6
Close to CD14 <sup>+</sup>	6,7 (21,4%)	1,7 (14,6%)	6 (19,6%)	4,8 (19,5%)
Mast cells	18,7	24	26,3	23
Close to CD20 <sup>+</sup>	1,7 (9,1%)	3 (12,5%)	0,3 (1,1%)	1,7 (7,3%)
Mast cells	27,3	25,6	38,3	30,4
Close to CD3 <sup>+</sup>	3,3 (12,1%)	1,7 (6,6%)	2 (5,2%)	2,3 (7,6%)
CD14 <sup>+</sup> cells	80,7	7,0	125,3	71
Close to mast cells	8,3 (10,3%)	0,3 (4,3%)	11,0 (8,8%)	6,5 (9,2%)
CD20 <sup>+</sup> cells	49,6	45,0	240,3	111,6
Close to mast cells	1 (2,0%)	0,3 (0,7%)	2,3 (1%)	1,2 (1,1%)
CD3 <sup>+</sup> cells	358,6	0	14,7	124,4
Close to mast cells	6,7 (1,9%)	0 (0%)	0 (0%)	2,2 (1,8%)

Means of the number of cells counted in 10 high power fields by 3 independent and blind observers. RA = Rheumatoid arthritis, n=3. \*Mean of the three patients.

### MODULATION OF MONOCYTE RESPONSES BY IL-33- AND IgG-ACTIVATED MAST CELLS

Since a substantial proportion of mast cells were located near CD14<sup>+</sup> cells in the RA synovium (Figure 3 and Table 1), we next examined whether IL-33-primed mast cell supernatants were able to influence the activation of CD14<sup>+</sup> cells. To this end, LPS was used to boost the proinflammatory activation of CD14<sup>+</sup> monocytes, acting as a model for TLR-4-induced activation of monocytes. Monocyte responses were evaluated in the presence or absence of mast cell supernatants. We used TNF production as the response marker because mast cells produce only minimal amounts of TNF compared to LPS-stimulated monocytes. As shown in Figure 4A, in the presence of supernatants of mast cells, the TLR-4-mediated TNF production by monocytes was inhibited. Activation of mast cells with IL-33 and plate-bound IgG significantly enhanced the inhibition of TNF production without affecting monocyte survival (as measured by DAPI staining) (results not shown). This effect was dependent, in part, on IL-10, since we found that TNF production could be partially inhibited by anti-IL-10 blocking antibodies (Figure 4B).

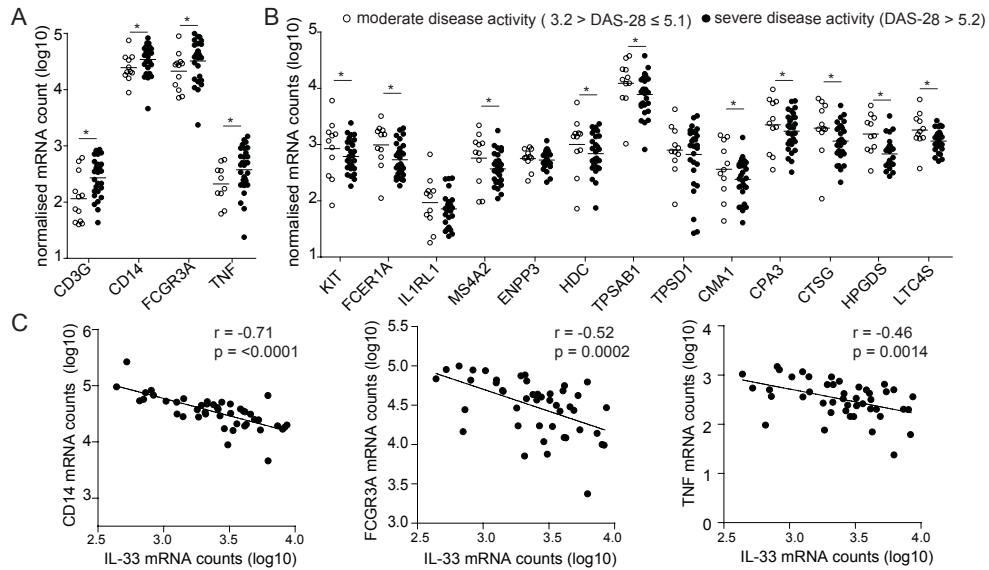
In addition, blocking of histamine receptor 2 with ranitidine also partially reverted the induced production of TNF by monocytes. When both histamine and IL-10 were blocked, the ability of monocytes to produce TNF was retained. Importantly, the possibility that IL-33 may have exerted direct effects on monocytes was excluded, since no effect on the release of cytokines by monocytes was observed after adding this cytokine to the control medium.



**Figure 4.** Mast cells (MCs) modulate monocyte activation. A, Monocytes were incubated either with control medium containing the same stimuli as used for mast cells (i.e., interleukin-33 [IL-33]) or with mast cell supernatants, followed by triggering with lipopolysaccharide (LPS). Levels of tumor necrosis factor (TNF) in cell cultures stimulated with IL-33 and/or plate-bound IgG (pblgG) were measured by enzyme-linked immunosorbent assay after 18 hours. TNF levels in mast cell supernatants were below the predetermined level of detection (dotted horizontal line). B, Monocytes were incubated with the supernatants of activated mast cells and anti-IL-10 blocking antibody, correspondent isotype control, the histamine receptor 2 antagonist ranitidine, or the latter two combined. Levels of LPS-induced TNF are shown as a percentage of the values in control (ctr) medium, which was set at 100%. Results in A and B are the mean±SEM in samples from 6 monocyte donors and 12 mast cell donors in 5 independent experiments. C, Monocytes were left unstimulated (unstim) or were triggered with LPS after incubation with mast cell supernatants (IL-33+plate-bound IgG) or control medium with IL-33 alone, together with anti-IL-10 blocking antibody, isotype control, or ranitidine. Expression of CD80 by monocytes was analyzed by flow cytometry (right; representative results shown) and quantified as the mean±SEM percentage expression in samples from 6 monocyte donors and 8 mast cell donors in 4 independent experiments (left). \* =  $P < 0.05$  by Student's t-test for comparison between 2 groups, and by analysis of variance with Bonferroni post hoc test for multiple comparisons.

Membrane markers of monocyte activation were also evaluated, and the influence of mast cell mediators was assessed with blocking experiments. Interestingly, the LPS-induced expression of the costimulatory molecule CD80 was reduced by mast cell supernatants (Figure 4C). This effect was mainly dependent on histamine, since the down-regulated expression of CD80 could be reversed by incubation with the histamine receptor 2 antagonist ranitidine.

Taken together, these findings indicate that activation of mast cells triggered by IL-33 inhibits the proinflammatory responses of monocytes, as shown by the suppression of TNF production and CD80 expression. This effect is presumably mediated through the release of IL-10 and histamine.



**Figure 5.** Gene expression in the synovial tissue of patients with early rheumatoid arthritis. A and B, Levels of mRNA for immune cell markers and tumor necrosis factor (TNF) (A) and for mast cell-specific genes (B) in patients with moderate disease activity (defined as a Disease Activity Score in 28 joints [DAS28] 3.2 to  $\leq 5.1$ ) and those with severe disease activity (DAS28  $> 5.2$ ). Symbols represent individual patients; horizontal lines indicate the mean.  $*$  =  $P < 0.05$  by Student's t-test. C, Correlation of interleukin-33 (IL-33) mRNA levels with levels of CD14, Fc $\gamma$  receptor type IIa (FCGR3A), and TNF mRNA. Each symbol represents an individual patient ( $n=40$ ), with the regression line and the Pearson's correlation coefficient. CD3G=CD3 antigen,  $\gamma$  subunit; CD14=monocyte differentiation antigen CD14; FCGR3A=Fc fragment of IgG, low affinity IIIa (receptor for CD16); KIT=c-Kit hardy-zuckerman 4 feline sarcoma viral oncogene homolog (stem cell factor receptor); FCER1A=Fc fragment of IgE, high affinity I receptor for  $\alpha$  subunit; IL1RL1=interleukin-1 receptor-like 1 (ST2/IL-33 receptor); MS4A2=membrane-spanning 4 domains, subfamily a, member 2 (Fc $\epsilon$ R1 $\beta$ ); ENPP3=ectonucleotide pyrophosphatase/phosphodiesterase 3; HDC=histidine decarboxylase; TPSAB1=trypsin,  $\alpha/\beta$ -1; TPSD1=trypsin,  $\delta$ -1; CMA1=chymase 1; CPA3=carboxypeptidase A3, mast cell; CTSG=cathepsin G; HPGDS=prostaglandin d2 synthase, hematopoietic; LTC4S=leukotriene c4 synthase.

## **INVERSE ASSOCIATION BETWEEN SYNOVIAL EXPRESSION OF MAST CELL-RELATED GENES AND DISEASE ACTIVITY IN EARLY RA**

To investigate whether the mast cell–mediated immunomodulatory/homeostatic functions observed in vitro might have a functional relevance in patients with RA, we analyzed the expression of mRNA extracted from the synovial biopsy tissue of patients with early RA (<12 months' duration) who were naive to DMARD therapy. As expected, the levels of mRNA for immune cell markers (e.g., CD3, CD14, and CD16) and TNF were significantly higher in patients with severe disease activity (measured as the Disease Activity Score in 28 joints [DAS28] (39), with severe disease activity defined as a DAS28 of >5.2) than in patients with moderate disease activity (defined as a DAS28 of 3.2 to ≤5.1) (Figure 5A). In contrast, the opposite observation was made for the mRNA expression levels of mast cell–specific genes, selected on the basis of a recent study describing highly specific genes for human mast cells (40).

Using the mRNA levels of these genes as a proxy for the presence of mast cells, we observed that most of the mast cell–related genes (such as c-Kit,  $\alpha/\beta$ 1-tryptase, and chymase, among others) displayed a significantly lower expression in patients with severe disease activity compared to patients with moderate disease activity (Figure 5B).

These findings are consistent with the observations made in our in vitro studies, as they suggest that mast cells, unlike other immune cells, are not associated with a more severe clinical phenotype. Finally, the levels of IL-33 mRNA showed an inverse correlation with the levels of proinflammatory markers, such as CD14, Fc $\gamma$ RIIIa, and TNF (Figure 5C). Supplementary Table 2 (online) provides the raw RNA sequence data on the analyzed genes in the 40 patients with early RA.

Taken together, these findings further support our hypothesis that IL-33–mediated activation of mast cells has an immunomodulatory/homeostatic role in RA and could potentially influence the level of disease severity in patients with RA.

## **DISCUSSION**

Persistency of inflammation is the hallmark of RA. In particular, an imbalance between pro- and antiinflammatory signals is presumed to be the basis of the chronic inflammation observed in the course of RA. Among the many cells and pathways potentially involved in this process, we have now identified IL-33–mediated mast cell activation as a new mechanism leading to the down-regulation of immune responses in the context of RA. To examine the involvement of mast cells in the inflammatory response in RA, we investigated



their activation by plate-bound immune complexes (plate-bound IgG) and found that activation of mast cells can be modulated by IL-33, a cytokine previously implicated in the ability of mast cells to contribute to experimental arthritis (23). It has been suggested that IL-33 induces the accumulation of proinflammatory cytokine mRNA in murine mast cells (21), whereas no clear mechanisms were proposed to explain the combined effects of IL-33 and immune complexes on human mast cells (22). Our data show that IL-33, via its receptor ST2, induces the expression of the activating receptor for the Fc fragment of IgG (FcγRIIa) in human mast cells. This, together with the observations of additional intracellular interactions, might explain the enhancement of IgG-mediated activation of mast cells in the presence of IL-33.

We recently showed that mast cells can be activated by immune complexes formed by binding of ACPAs from the sera of patients with RA to citrullinated antigens, a response that can be augmented by the triggering effects of TLRs (17). In the present study, we extended these findings by showing that IL-33 augments the activation of mast cells induced by IgG ACPAs. Overall, these observations suggest that mast cells, activated by IL-33 and IgG, both of which are known to be present in the synovial compartment, might contribute to the inflammatory response in RA, thereby supporting the longstanding hypothesis that mast cells play a deleterious role in RA. By further exploring the influence of IL-33 on mast cell activation, we found that IL-33 induces human mast cells to release immunomodulatory mediators, such as IL-10 and histamine, as well as other cytokines associated with type 2 immune responses, such as IL-5 and IL-13. The release of these IL-33-induced mediators was further enhanced by IgG triggering. In particular, the combined stimulation with IL-33 and IgG showed a synergistic effect that, intriguingly, was present for IL-33-induced mediators (such as IL-5, IL-10, IL-13, and histamine) and absent for mediators induced by IgG (such as CXCL8/IL-8). These findings are important because they indicate that IL-33 is able to prime mast cells toward a Th2/immunomodulatory phenotype, a phenotype that becomes more prominent following the concomitant activation of mast cells by IgG.

Since IL-33 is found in the synovial fluid of patients with RA, we hypothesized that the effects of IL-33 on mast cells might be relevant in modulating the immune responses in RA, by, for example, influencing the behavior of other synovial immune cells. Immunostaining of the RA synovial tissue demonstrated that human synovial mast cells were in close proximity to CD14<sup>+</sup> monocyte/macrophages, CD3<sup>+</sup> T cells, and CD20<sup>+</sup> B cells. In particular, synovial mast cells were most commonly found in proximity to CD14<sup>+</sup> monocyte/macrophages. Without implying that such interactions are specific for RA, these findings could nevertheless indicate that mast cell–monocyte interactions at the synovial level are frequent and might, therefore, have functional consequences.

To validate the hypothesis that IL-33 triggering of mast cell activation may exert immunomodulatory effects, we explored the ability of these cells to influence monocyte responses. Mast cells activated with IL-33 and plate-bound IgG were able to dampen the activation of monocytes, inhibiting both the production of the prototypic proinflammatory cytokine TNF and the up-regulation of the costimulatory molecule CD80. These effects were mediated, at least in part, by the release of IL-10 and histamine from mast cells. Among other mediators with known immunomodulatory functions, IL-4 was recently shown to be responsible for mast cell-mediated inhibition of peritoneal macrophage phagocytosis in a mouse model of sepsis (41). However, we consider the involvement of mast cell-derived IL-4 in our in vitro system to be unlikely, since we could not detect this cytokine in the supernatants of stimulated mast cells. In our experiments, monocytes were stimulated with bacterial LPS, acting via TLR-4, a commonly used model to study monocyte activation. Independent of the effects of LPS, several endogenous stimuli, ligands of TLR-4, have been implicated in the pathogenesis of RA (42), suggesting that the inhibition of TLR-4-mediated activation of monocytes by mast cells might be relevant in the context of RA.

Although several studies explored the ability of mast cells to influence both innate (41, 43) and adaptive (44, 45) immune responses, the direct cross-talk between human mast cells and monocyte/macrophages has never been investigated. This study is the first to show that mast cells and CD14<sup>+</sup> monocyte/macrophages can interact at the synovial level. Furthermore, mast cells triggered with stimuli known to be present in the inflamed synovium and classically considered to be proinflammatory (i.e., IL-33 and IgG) surprisingly gain the ability to suppress the activation of monocytes.

Taken together, these findings might help to understand the contrasting observations obtained in animal models of arthritis, in which, upon mast cell depletion, their pro- or antiinflammatory functions might be revealed or balanced, depending on the specific experimental conditions. Moreover, considering the heterogeneity of RA, the role of human mast cells might be even more complex and possibly multifaceted (2). Most of the evidence points toward a deleterious role for mast cells in patients with RA, with some reports proposing the mast cell c-Kit receptor as a therapeutic target (46-49). However, these studies used relatively nonspecific inhibitors (multitargeted tyrosine kinase inhibitors), which could have effects on other receptors on different cells (50). Similarly, the alarmin IL-33 has been proposed as a promising treatment target for RA (51), even though its involvement in the pathogenesis of RA has not been clearly established (33). Our results indicate that mast cells, in addition to their well-known proinflammatory functions, are also able to mediate regulatory/homeostatic responses, in particular when exposed to IL-33.

To confirm the relevance of the latter hypothesis, we studied a cohort of patients with early (duration <12 months) RA who were naive to DMARD therapy. Interestingly, although the presence of many types of immune cells, as determined by cell-specific gene expression, was associated with high disease activity, the presence of mast cells displayed an inverse association with disease severity. At the same time, IL-33 mRNA levels were inversely correlated with the levels of proinflammatory markers, such as CD14, CD16 (FcγRIIIA), and TNF. These observations make it tempting to speculate that mast cells, in particular when triggered by IL-33, could function in an immunomodulatory manner in the synovial tissue of patients with RA. Thus, the findings provide a rationale for future studies aimed at unravelling the complex influence of mast cells on disease activity in different stages of RA.

In conclusion, the results of this study indicate that mast cells, finely balancing between their well-known proinflammatory functions and their IL-33-triggered anti-inflammatory functions as observed herein, might play a previously unrecognized role as immunomodulatory/homeostatic cells in the pathogenesis of RA.

## REFERENCES

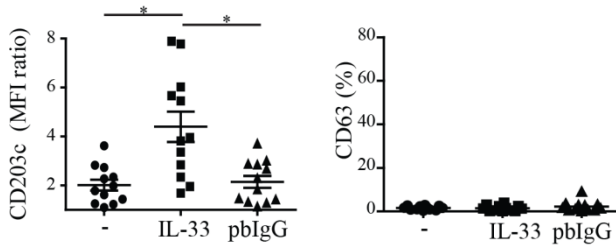
1. Voehringer D, Protective and pathological roles of mast cells and basophils. *Nat Rev Immunol* 13, 362-75 (2013).
2. Galli SJ, Grimaldeston M, Tsai M, Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. *Nat Rev Immunol* 8, 478-86 (2008).
3. Brown MA, Hatfield JK, Mast Cells are Important Modifiers of Autoimmune Disease: With so Much Evidence, Why is There Still Controversy? *Front Immunol* 3, 147 (2012).
4. Lee DM, Friend DS, Gurish MF *et al.*, Mast cells: a cellular link between autoantibodies and inflammatory arthritis. *Science* 297, 1689-92 (2002).
5. Nigrovic PA, Binstadt BA, Monach PA *et al.*, Mast cells contribute to initiation of autoantibody-mediated arthritis via IL-1. *Proc Natl Acad Sci U S A* 104, 2325-30 (2007).
6. Pitman N, Asquith DL, Murphy G, Liew FY, McInnes IB, Collagen-induced arthritis is not impaired in mast cell-deficient mice. *Ann Rheum Dis* 70, 1170-1 (2011).
7. Feyerabend TB, Weiser A, Tietz A *et al.*, Cre-mediated cell ablation contests mast cell contribution in models of antibody- and T cell-mediated autoimmunity. *Immunity* 35, 832-44 (2011).
8. Schubert N, Dudeck J, Liu P *et al.*, Mast cell promotion of T cell-driven antigen-induced arthritis despite being dispensable for antibody-induced arthritis in which T cells are bypassed. *Arthritis Rheumatol* 67, 903-13 (2015).
9. Reber LL, Marichal T, Galli SJ, New models for analyzing mast cell functions in vivo. *Trends Immunol* 33, 613-25 (2012).
10. de Paulis A, Marino I, Ciccarelli A *et al.*, Human synovial mast cells. I. Ultrastructural in situ and in vitro immunologic characterization. *Arthritis Rheum* 39, 1222-33 (1996).
11. Crisp AJ, Chapman CM, Kirkham SE, Schiller AL, Krane SM, Articular mastocytosis in rheumatoid arthritis. *Arthritis Rheum* 27, 845-51 (1984).

12. Malone DG, Irani AM, Schwartz LB, Barrett KE, Metcalfe DD, Mast cell numbers and histamine levels in synovial fluids from patients with diverse arthritides. *Arthritis Rheum* 29, 956-63 (1986).
13. Gotis-Graham I, McNeil HP, Mast cell responses in rheumatoid synovium. Association of the MCTC subset with matrix turnover and clinical progression. *Arthritis Rheum* 40, 479-89 (1997).
14. Sandler C, Lindstedt KA, Joutsiniemi S *et al.*, Selective activation of mast cells in rheumatoid synovial tissue results in production of TNF-alpha, IL-1beta and IL-1Ra. *Inflamm Res* 56, 230-9 (2007).
15. Buckley MG, Walters C, Wong WM *et al.*, Mast cell activation in arthritis: detection of alpha- and beta-tryptase, histamine and eosinophil cationic protein in synovial fluid. *Clin Sci (Lond)* 93, 363-70 (1997).
16. Sawamukai N, Yukawa S, Saito K *et al.*, Mast cell-derived tryptase inhibits apoptosis of human rheumatoid synovial fibroblasts via rho-mediated signaling. *Arthritis Rheum* 62, 952-9 (2010).
17. Suurmond J, Rivellese F, Dorjee AL *et al.*, Toll-like receptor triggering augments activation of human mast cells by anti-citrullinated protein antibodies. *Ann Rheum Dis* 74, 1915-23 (2015).
18. Liew FY, Pitman NI, McInnes IB, Disease-associated functions of IL-33: the new kid in the IL-1 family. *Nat Rev Immunol* 10, 103-10 (2010).
19. Allakhverdi Z, Smith DE, Comeau MR, Delespesse G, Cutting edge: The ST2 ligand IL-33 potently activates and drives maturation of human mast cells. *J Immunol* 179, 2051-4 (2007).
20. Moulin D, Donze O, Talabot-Ayer D *et al.*, Interleukin (IL)-33 induces the release of pro-inflammatory mediators by mast cells. *Cytokine* 40, 216-25 (2007).
21. Kaieda S, Wang JX, Shnayder R *et al.*, Interleukin-33 primes mast cells for activation by IgG immune complexes. *PLoS One* 7, e47252 (2012).
22. Kashiwakura J, Yanagisawa M, Lee H *et al.*, Interleukin-33 synergistically enhances immune complex-induced tumor necrosis factor alpha and interleukin-8 production in cultured human synovium-derived mast cells. *Int Arch Allergy Immunol* 161 Suppl 2, 32-6 (2013).
23. Xu D, Jiang HR, Kewin P *et al.*, IL-33 exacerbates antigen-induced arthritis by activating mast cells. *Proc Natl Acad Sci U S A* 105, 10913-8 (2008).
24. Xu D, Jiang HR, Li Y *et al.*, IL-33 exacerbates autoantibody-induced arthritis. *J Immunol* 184, 2620-6 (2010).
25. Talabot-Ayer D, McKee T, Gindre P *et al.*, Distinct serum and synovial fluid interleukin (IL)-33 levels in rheumatoid arthritis, psoriatic arthritis and osteoarthritis. *Joint Bone Spine* 79, 32-7 (2012).
26. Anthony RM, Kobayashi T, Wermeling F, Ravetch JV, Intravenous gammaglobulin suppresses inflammation through a novel T(H)2 pathway. *Nature* 475, 110-3 (2011).
27. Tjon AS, van Gent R, Jaadar H *et al.*, Intravenous immunoglobulin treatment in humans suppresses dendritic cell function via stimulation of IL-4 and IL-13 production. *J Immunol* 192, 5625-34 (2014).
28. Rivellese F, Suurmond J, de Paulis A *et al.*, IgE and IL-33-mediated triggering of human basophils inhibits TLR4-induced monocyte activation. *Eur J Immunol* 44, 3045-55 (2014).
29. Schiering C, Krausgruber T, Chomka A *et al.*, The alarmin IL-33 promotes regulatory T-cell function in the intestine. *Nature* 513, 564-8 (2014).
30. Zaiss MM, Kurowska-Stolarska M, Bohm C *et al.*, IL-33 shifts the balance from osteoclast to alternatively activated macrophage differentiation and protects from TNF-alpha-mediated bone loss. *J Immunol* 186, 6097-105 (2011).
31. Sattler S, Ling GS, Xu D *et al.*, IL-10-producing regulatory B cells induced by IL-33 (Breg(IL-33)) effectively attenuate mucosal inflammatory responses in the gut. *J Autoimmun* 50, 107-22 (2014).
32. Kamradt T, Drube S, A complicated liaison: IL-33 and IL-33R in arthritis pathogenesis. *Arthritis Res Ther* 15, 115 (2013).
33. Liew FY, IL-33: a Janus cytokine. *Ann Rheum Dis* 71 Suppl 2, i101-4 (2012).

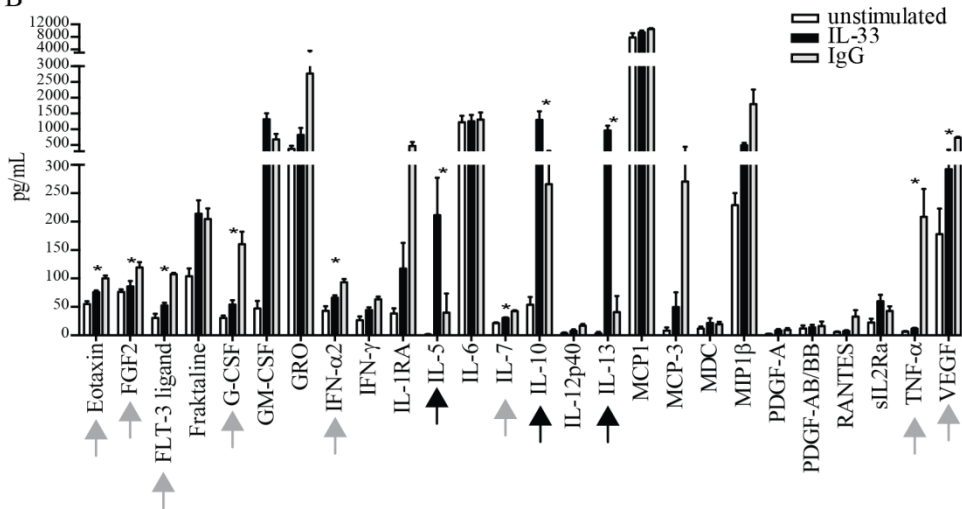
34. Radinger M, Jensen BM, Kuehn HS, Kirshenbaum A, Gilfillan AM, Generation, isolation, and maintenance of human mast cells and mast cell lines derived from peripheral blood or cord blood. *Curr Protoc Immunol* Chapter 7, Unit 7 37 (2010).
35. Boruchov AM, Heller G, Veri MC *et al.*, Activating and inhibitory IgG Fc receptors on human DCs mediate opposing functions. *J Clin Invest* 115, 2914-23 (2005).
36. Pitzalis C, Kelly S, Humby F, New learnings on the pathophysiology of RA from synovial biopsies. *Curr Opin Rheumatol* 25, 334-44 (2013).
37. Arnett FC, Edworthy SM, Bloch DA *et al.*, The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 31, 315-24 (1988).
38. Veri MC, Gorlatov S, Li H *et al.*, Monoclonal antibodies capable of discriminating the human inhibitory Fcγ-receptor IIB (CD32B) from the activating Fcγ-receptor IIA (CD32A): biochemical, biological and functional characterization. *Immunology* 121, 392-404 (2007).
39. Prevoo ML, van 't Hof MA, Kuper HH *et al.*, Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 38, 44-8 (1995).
40. Motakis E, Guhl S, Ishizu Y *et al.*, Redefinition of the human mast cell transcriptome by deep-CAGE sequencing. *Blood* 123, e58-67 (2014).
41. Dahdah A, Gautier G, Attout T *et al.*, Mast cells aggravate sepsis by inhibiting peritoneal macrophage phagocytosis. *J Clin Invest* 124, 4577-89 (2014).
42. Huang QQ, Pope RM, The role of toll-like receptors in rheumatoid arthritis. *Curr Rheumatol Rep* 11, 357-64 (2009).
43. St John AL, Abraham SN, Innate immunity and its regulation by mast cells. *J Immunol* 190, 4458-63 (2013).
44. Suurmond J, van Heemst J, van Heiningen J *et al.*, Communication between human mast cells and CD4(+) T cells through antigen-dependent interactions. *Eur J Immunol* 43, 1758-68 (2013).
45. Merluzzi S, Frossi B, Gri G *et al.*, Mast cells enhance proliferation of B lymphocytes and drive their differentiation toward IgA-secreting plasma cells. *Blood* 115, 2810-7 (2010).
46. Juurikivi A, Sandler C, Lindstedt KA *et al.*, Inhibition of c-kit tyrosine kinase by imatinib mesylate induces apoptosis in mast cells in rheumatoid synovia: a potential approach to the treatment of arthritis. *Ann Rheum Dis* 64, 1126-31 (2005).
47. Tebib J, Mariette X, Bourgeois P *et al.*, Masitinib in the treatment of active rheumatoid arthritis: results of a multicentre, open-label, dose-ranging, phase 2a study. *Arthritis Res Ther* 11, R95 (2009).
48. Eklund KK, Joensuu H, Treatment of rheumatoid arthritis with imatinib mesylate: clinical improvement in three refractory cases. *Ann Med* 35, 362-7 (2003).
49. Paniagua RT, Sharpe O, Ho PP *et al.*, Selective tyrosine kinase inhibition by imatinib mesylate for the treatment of autoimmune arthritis. *J Clin Invest* 116, 2633-42 (2006).
50. D'Aura Swanson C, Paniagua RT, Lindstrom TM, Robinson WH, Tyrosine kinases as targets for the treatment of rheumatoid arthritis. *Nat Rev Rheumatol* 5, 317-24 (2009).
51. Yuan FL, Li X, Lu WG *et al.*, IL-33: a promising therapeutic target for rheumatoid arthritis? *Expert Opin Ther Targets* 15, 529-34 (2011).

## SUPPLEMENTARY FIGURE

A



B



**Supplementary Figure 1.** FACS and multiplex analyses of IL-33 and pbIgG-triggered mast cells. Mast cells were triggered with IL-33 or plate bound IgG for 24h. A, Expression of CD203c (left) as Median Fluorescence Intensity (MFI) ratio to isotype, and CD63 (right), as % of positive cells. Each symbol represent a mast cell donor (n=12) from 5 independent experiments. \*p<0.05 determined by ANOVA with Bonferroni's post-test (for multiple comparisons) B, Of the 42 mediators included in the panel, the ones above minimal detection levels are shown\*\*. Levels of each mediators in IL-33 and IgG-triggered mast cells were compared, black arrows indicate mediators which levels were significantly higher in IL-33-triggered mast cells, grey arrows mediators which levels were significantly higher in IgG-triggered mast cells. n=3 mast cells donors, 3 independent experiments. \*p 0.05 determined by Student's t test, comparing IL-33 and IgG-triggered mast cells for each mediator. \*\*As IL-8 levels were over the detection range, this cytokine is not shown (IL-8 levels were already measured by ELISA, as shown in Figure 1).

