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IL-33 Induces a Hyporesponsive Phenotype in Human and Mouse Mast Cells

Mi-Yeon Jung,* Daniel Smrž,* Avanti Desai,* Geethani Bandara,* Tomonobu Ito,* Shoko Iwaki,† Jeong-Han Kang,‡ Marcus V. Andrade,§ Susana C. Hilderbrand,∥ Jared M. Brown,¶ Michael A. Beaven,† Dean D. Metcalfe,* and Alasdair M. Gilfillan*  

IL-33 is elevated in afflicted tissues of patients with mast cell (MC)–dependent chronic allergic diseases. Based on its acute effects on mouse MCs, IL-33 is thought to play a role in the pathogenesis of allergic disease through MC activation. However, the manifestations of prolonged IL-33 exposure on human MC function, which best reflect the conditions associated with chronic allergic disease, are unknown. In this study, we found that long-term exposure of human and mouse MCs to IL-33 results in a substantial reduction of MC activation in response to Ag. This reduction required >72 h exposure to IL-33 for onset and 1–2 wk for reversion following IL-33 removal. This hyporesponsive phenotype was determined to be a consequence of MyD88-dependent attenuation of signaling processes necessary for MC activation, including Ag-mediated calcium mobilization and cytoskeletal reorganization, potentially as a consequence of downregulation of the expression of phospholipase Cγ1 and Hck. These findings suggest that IL-33 may play a protective, rather than a causative, role in MC activation under chronic conditions and, furthermore, reveal regulated plasticity in the MC activation phenotype. The ability to downregulate MC activation in this manner may provide alternative approaches for treatment of MC-driven disease. The Journal of Immunology, 2013, 190: 000–000.

Mast cells (MCs) are tissue-resident cells of hematopoietic origin that participate in innate and acquired immune defense mechanisms through the release of an array of inflammatory mediators following receptor-dependent activation (1). These mediators, however, are also responsible for initiating the cellular and pathological manifestations of allergic disorders, including anaphylaxis, asthma, rhinitis, and atopic dermatitis (2). MCs are predominantly activated through Ag/IgE-mediated aggregation of the high-affinity IgE receptor FcεRI (3). However, it is apparent that cytokines and other molecules present in the surrounding milieu can acutely amplify MC signaling and responses to Ag (4).

IL-33 levels are reported to be elevated in lungs of patients with chronic asthma (5) and, based on the reports that IL-33 enhances MC activation (6–9), it has been proposed that IL-33 may play a role in the pathogenesis of allergic disorders. The effects of IL-33 on MC activation have, however, been controversial. Although it is recognized that IL-33 induces cytokine release from MCs in culture (7, 10–12) and markedly enhances the ability of Ag to elicit this response (6–9), some studies report that IL-33 also stimulates or enhances degranulation and production of PGD2 (9, 12), whereas others report that IL-33 neither induces or enhances FcεRI-mediated degranulation and eicosanoid production (7, 8, 10, 11).

The amplification of cytokine release by IL-33 in Ag-stimulated MCs in culture was noted after acute exposure to IL-33, conditions that may not mimic the long-term effects of IL-33 on MC function associated with pathologic conditions of atopic or rheumatoid arthritis. We thus investigated the long-term effects of IL-33 on the ability of Ag to activate human and mouse MCs. In this study, we report that, following prolonged IL-33 exposure, MCs become refractory to stimulation via FcεRI, with respect to degranulation, as a consequence of the attenuation of critical signaling processes required for MC activation. To our knowledge, these findings provide the first evidence that human (hu)MCs can be reprogrammed to a hyporesponsive phenotype in this manner and thus may have important implications for how MC activation may be regulated in health and disease. The ability to manipulate MC activation in this manner may also provide a basis for the development of novel approaches for therapeutic intervention of MC-driven disease.

Materials and Methods  
Cell culture  
Primary huMCs were prepared from CD34+ peripheral blood progenitors isolated from healthy volunteers following informed consent under a protocol (NCT00001756) approved by the National Institutes of Health Internal Review Board. Human MCs were prepared and cultured as described (13) with or without recombinant human IL-33 (10 ng/ml) and used for studies between 7 and 9 wk age. All mouse studies, with the exception of those involving the use of ST2−/− mice, were conducted under a protocol approved by the National Institute of Allergy and Infectious Diseases, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892;†Laboratory of Molecular Immunology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892;*Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892;Department of Internal Medicine, School of Medicine, Federal University of Minas Gerais, Belo Horizonte, MG 30130-100, Brazil; and∥Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, Greenville, NC 27834

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The sequences presented in this article have been submitted to the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE59382.

Address correspondence and reprint requests to Dr. Alasdair M. Gilfillan, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 10 Center Drive, MSC 1881, Building 10, Room 11C206, Bethesda, MD 20892. E-mail address: agilfillan@niaid.nih.gov

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Abbreviations used in this article: BMMC, bone marrow–derived mast cell; HSA, human serum albumin; hu, human; MC, mast cell; MFI, mean fluorescence intensity; PLC, phospholipase C; SCF, stem cell factor; WT, wild-type.
Institute of Allergy and Infectious Diseases Institutional Animal Care and Use Committee at the National Institutes of Health. Studies involving the use of \( \text{S2}^{-/-} \) mice were approved by East Carolina University’s Institutional Animal Care and Use Committee. These studies were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Mouse bone marrow–derived MCs (BMMCs) were prepared from wild-type (WT; C57BL/6 background; The Jackson Laboratory, Bar Harbor, ME), \( \text{S2}^{-/-} \), and \( \text{MyD88}^{-/-} \) mice on a C57BL/6 genetic background were obtained from Dr. Robert B. Fick at Merck Research Laboratories (Division of Biologics, Palo Alto, CA). Homozygous \( \text{MyD88}^{-/-} \) mice on a C57BL/6 genetic background (14, 15) were obtained from Dr. Shizuo Akira (Osaka University, Osaka, Japan) by way of Dr. Helene Rosenberg (National Institute of Allergy and Infectious Diseases, National Institutes of Health). WT mice on an identical genetic background were sex and age matched. BMMCs were developed and cultured as described (16, 17) with or without mouse IL-33 (10 ng/ml) and used for studies between 4 and 6 wk culture. The endotoxin content of the IL-33 was <0.1 ng/ml of IL-33, well below (>10-fold less than) that required to substantially influence MC activation (100 ng/ml) (18).

Cell activation and degranulation

For degranulation and cytokine release, huMCs and BMMCs were incubated overnight in cytokine-free media containing biotinylated myeloma human IgE (100 ng/ml) (19) or mouse monoclonal DNP-IgE (100 ng/ml; Sigma-Aldrich, St. Louis, MO), respectively. The following day, the cells were rinsed with HEPES buffer (10 mM HEPES, 137 mM NaCl, 2.7 mM KCl, 0.4 mM NaHPO4, 7 H2O, 5.6 mM glucose, 1.8 mM CaCl2·2H2O, 1.3 mM MgSO4·7H2O [pH 7.4]) containing 0.04% BSA (Sigma-Aldrich) then treated as described (19) and as in the figure legends. Degranulation was calculated as the percentage of total \( \beta \)-hexosaminidase recovered from the supernatant (19, 20).

**FIGURE 1.** The effect of IL-33 exposure on huMCs. (A) huMCs were cultured with or without IL-33 for 7–9 wk and cytospins were stained with toluidine blue and analyzed by light microscopy. Scale bar, 10 \( \mu \)m. (B) FcεRI and KIT expression in cells from (A) were analyzed by flow cytometry. (C) Naive huMCs were sensitized overnight with biotinylated IgE (100 ng/ml) (19) or mouse monoclonal DNP-IgE (100 ng/ml; Sigma-Aldrich, St. Louis, MO), respectively. The following day, the cells were rinsed with HEPES buffer (10 mM HEPES, 137 mM NaCl, 2.7 mM KCl, 0.4 mM NaHPO4·H2O, 5.6 mM glucose, 1.8 mM CaCl2·2H2O, 1.3 mM MgSO4·7H2O [pH 7.4]) containing 0.04% BSA (Sigma-Aldrich) then treated as described (19) and in the figure legends. Degranulation was calculated as the percentage of total \( \beta \)-hexosaminidase recovered from the supernatant (19, 20).

**In vivo studies and isolation of peritoneal MCs**

The acute effects of IL-33 (IL-33–induced anaphylaxis) were examined in 6 wk old C57BL/6 mice (The Jackson Laboratory). The mice were sensitized with anti-DNP IgE mAb (21) (3 \( \mu \)g i.v.), a gift from Juan Rivera (National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health), and isolated from ascites supplied by Dr. Fu-Tong Liu (Davis School of Medicine, University of California, Sacramento, CA). After 24 h, the mice were injected with rIL-33 (2 \( \mu \)g in 200 \( \mu \)l) or PBS retro-orbitally (i.l.), and the anaphylactic response was monitored by recording changes in core body temperature every 5 min for 2 h using an implantable electronic transponder (IPTT-300; Bio Medic Data Systems, Seaford, DE). As a positive control, mice were challenged with Ag (200 \( \mu \)g DNP-human serum albumin [HSA]; Sigma-Aldrich, St. Louis, MO).

To examine the consequences of prolonged exposure to IL-33 on the MC compartment in vivo, mice were injected i.p. with 1 \( \mu \)g IL-33 (500 \( \mu \)l) or PBS every second day for a total of 12 d. The fifth and sixth injections were supplemented with 1 \( \mu \)g anti-DNP-IgE to sensitize the mice. Two days after the sixth injection, blood-free peritoneal cells were recovered by lavage with HEPES buffer containing 0.2% (w/v) BSA. Peritoneal cells were immediately labeled with 10 \( \mu \)M Fluo-4 AM (Invitrogen, Carlsbad, CA) and allophycocyanin-labeled KIT-specific Ab (BD Biosciences, San Jose, CA) in the presence of 5 mM probenecid (Sigma-Aldrich) for 30 min at room temperature in HEPES/BSA (0.2%). The cells were then washed with HEPES/BSA (0.2%/2.5 mM probenecid. Before measurement, the cells were sedimented, resuspended in prewarmed (37˚C) HEPES/BSA (0.2%), and analyzed at 37˚C by a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) to obtain a time course of Fluo-4 AM fluorescence of the gated KIT+ cell population. A 2-s averaged geometric mean of the recorded Fluo-4 AM fluorescence (mean fluorescence intensity [MFI]) was subsequently analyzed. To evaluate the data, the difference in the averaged MFI within the interval 30–50 s after (maximal fluorescence) and 20–0 s prior (basal fluorescence)
fluorescence) each challenge (ΔMFI) was determined. The ΔMFI for control (vehicle-challenged cells) was subtracted from ΔMFI for Ag-challenged cells and the resultant values were normalized to maximal response.

**Immunoblotting and intracellular Ca\(^{2+}\) determination**

For immunoblotting, cell lysates from BMMCs and huMCs were prepared as described (22). The following Abs were used: p–phospholipase C (PLC) \(\gamma_1\) (Tyr\(^{308}\)) (Biosource International, San Diego, CA), Hck, LAT, PLC\(\gamma_1\), Syk (Santa Cruz Biotechnology, Santa Cruz, CA), b–actin (Sigma-Aldrich), p-LAT (Tyr\(^{316}\)) (Millipore, Billerica, MA), p-Btk (BD Biosciences). All other Abs (p–p38 [Thr\(^{180}\)/Tyr\(^{182}\)], p38, p-JNK [Thr\(^{183}\)/Tyr\(^{185}\)], JNK, p-ERK [Thr\(^{202}\)/Tyr\(^{204}\)], ERK, p-AKT [Ser\(^{473}\)], p-AKT [Thr\(^{308}\)], AKT) were from Cell Signaling Technology (Danvers, MA). Intracellular Ca\(^{2+}\) was determined utilizing Fura-2 as described (23).

**Real-time PCR**

Total RNA was isolated from 3 \(\times\) 10\(^{6}\) cells using an RNeasy Plus Mini kit (Qiagen, Valencia, CA). One microgram RNA was used for reverse transcription reaction using random hexamers and SuperScript III reverse transcriptase (Invitrogen) in a 20 \(\mu\)l reaction. One microlitre of the resulting cDNA was used for real-time PCR using TaqMan gene expression assay for PLC\(\gamma_1\) (Mm01247293_m1) or Hck (Mm01241463_m1) according to the manufacturer’s instructions.

**Actin polymerization**

After treating the cells as described in the figure legends, cells were fixed for 15 min at room temperature using 4% paraformaldehyde in PBS containing 5 mM EDTA and 5 mM EGTA, washed with 2% BSA in PBS containing EGTA, and attached to glass slides. Cells were stained with FITC-labeled phalloidin (Sigma-Aldrich) (1:100) in 0.1% saponin, 2% BSA, in PBS/5 mM EGTA for 1 h at room temperature. After washing, slides were mounted using mounting solution containing DAPI (Molecular Probes). Imaging was obtained using a Zeiss LSM 510 UV confocal microscope.

**Affymetrix GeneChip analysis**

These studies were conducted as part of a three-way analysis where nontreated BMMCs were compared with one group in which the cells were treated with stem cell factor (SCF; 100 ng/ml) and with one group where the cells were treated with IL-33. The SCF/control group comparison data are included in the supplemental material to Ito et al. (24). The methods are also described in that study. In this study, we report the comparison data between the control and IL-33 groups. The data are deposited in the Gene Expression Omnibus (GSE39382; http://www.ncbi.nlm.nih.gov/geo/).

**Statistical analysis**

Data are represented as the means \(\pm\) SEM from experiments conducted on separate animals or cell preparations (n values are indicated in figure legends). The statistical analyses between two sets of data were performed by an unpaired or paired Student t test or between multiple sets by ANOVA \((p < 0.05)\). Differences were considered significant when \(p < 0.05\).

**Results**

**IL-33 promotes a hyporesponsive huMC phenotype**

Human MCs were generated in the presence or absence of IL-33 for the duration of the culture period (7–9 wk). There was little difference in the morphological characteristics and purity (>99%) between the two cell populations (Fig. 1A) and no detectable difference in surface expression of FcεRI or KIT (Fig. 1B). In huMCs cultured in the absence of IL-33, acutely added IL-33 neither induced degranulation or enhanced FcεRI-mediated degranulation (Fig. 1C). In contrast, but consistent with previous studies (23), Ag-induced degranulation was enhanced by the KIT ligand, SCF, in these cells. However, huMCs exposed to IL-33 for the duration of the culture exhibited markedly reduced FcεRI-mediated degranulation in the absence (Fig. 1D) or presence of SCF (Fig. 1E) when compared with cells not exposed to IL-33. MC degranulation is dependent on an increase in cytosolic calcium regardless of the type of stimulant (4, 25). Consistent with this requirement and with a previous study (8), IL-33 did not induce an increase in cytosolic calcium nor did it enhance that elicited by Ag, whereas SCF could do so in IL-33–naive huMCs (Fig. 1F). Nevertheless, the Ag-mediated calcium signal and the ability of SCF to enhance this signal were significantly impaired in cells chronically exposed to IL-33 (Fig. 1G).

 Taken together, these data revealed that prolonged exposure of huMCs to IL-33 downregulated FcεRI-mediated and KIT-enhanced degranulation, and that this appeared to be a manifestation of IL-33–mediated attenuation of signaling events downstream of the FcεRI or KIT.

**Onset and reversibility of IL-33 suppressive actions**

During maturation of huMCs, we observed that IL-33 induced an identical deficiency in degranulation in huMCs whether IL-33 was added 2, 4, or 6 wk prior to examining degranulation in mature (8 wk) cells (Fig. 2A). When cells were incubated with IL-33 for periods of 4–12 h before the addition of streptavidin, degranulation was moderately enhanced (Fig. 2B, 2C). However, with more sustained exposure to IL-33, the extent of degranulation progressively declined to levels well below those observed before addition of IL-33 (Fig. 2B, 2C).
To examine whether the changes induced by IL-33 were reversible, huMCs were maintained in media containing IL-33 for 8 wk and then IL-33 was removed from the media and degranulation assessed weekly. The hyporesponsive phenotype gradually reverted to normal and, within 2 wk, the cells previously exposed to IL-33 showed similar FcεRI-mediated degranulation to huMCs not exposed to IL-33 (Fig. 2D, 2E).

These data suggest that the ability of IL-33 to promote a hypoactive MC phenotype occurs irrespective of the stage of MC development, requires at least 72 h exposure, and is slowly reversible.

Elevated IL-33 levels in vivo suppress Ag-induced MC activation

We next examined whether elevated IL-33 levels in vivo similarly promote a hypoactive MC compartment. We first established that degranulation in mouse BMMCs grown in the presence of IL-33 were also refractory to Ag challenge (Fig. 3A), and that this phenotype was also not associated with reduced KIT and FcεRI expression (Fig. 3B) but was accompanied by reduced cell activation as assessed by a decrease in the Ag-mediated calcium signal (Fig. 3C).

Consistent with the lack of effect on degranulation (Fig. 1), a single injection of IL-33 failed to induce an anaphylactic response in mice (Fig. 3D). To examine chronic effects of IL-33 on the MC compartment, mice were injected i.p. with IL-33 (1 µg) or PBS every 2 d for a total of 12 d. Two days after the last injection and sensitization with IgE, peritoneal cells from the control and IL-33-treated mice were collected and the activation capacity of KIT+ MCs to Ag was determined by examining the calcium signal by flow cytometry. As shown in Fig. 3E–G, MCs collected from the control mice responded as expected to Ag, whereas cells collected from IL-33–treated mice were refractory to such stimulation. Thus, as was observed for BMMCs, long-term exposure of MCs to IL-33 in situ resulted in a marked decrease in the ability of Ag to induce MC activation.

Having established that mouse BMCCMs exhibited the same IL-33–induced hyporesponsive phenotype as that observed in the huMCs, we used mouse BMCCMs for further mechanistic studies.

Absence of IL-33–induced phenotypic changes in ST2−/− and MyD88−/− BMCCMs

The IL-33 receptor, ST2, signals through the MyD88 adaptor molecule (26). We therefore investigated whether the inhibitory

FIGURE 3. The effect of prolonged IL-33 exposure on mouse MCs. (A) BMMCs were cultured with or without IL-33 during the 4–6 wk development, sensitized with DNP-specific mouse IgE overnight, and then stimulated with the indicated concentrations of DNP-HSA (Ag). (B) BMMCs were cultured with or without IL-33 for 4–6 wk and expression of FcεRI and KIT was analyzed by flow cytometry. (C) Cells were sensitized and cultured as in (A) and then stimulated with DNP-HSA (Ag) or SCF alone or in combination and calcium response was determined. (D) IgE-sensitized mice were injected with IL-33 (2 µg), DNP-HSA (200 µg), or PBS retro-orbitally and the change in core body temperature was monitored. (E and F) Peritoneal cells from PBS-treated mice (E) and from mice treated for 12 d with IL-33 (six doses of 1 µg on alternative days) and sensitized with DNP-specific mouse IgE (two doses of 1 µg on alternative days prior to stimulation) (F) were labeled with a KIT-specific Ab and Fluo-4 AM. The calcium responses of the KIT+ population of these cells to vehicle alone (Ctrl) or Ag (DNP-HSA; 100 ng/ml) were determined by acquisition of their Fluo-4 AM fluorescence using flow cytometry, and 2-s averaged geometric mean fluorescence (MFI) was analyzed. (G) ΔMFI values (see Materials and Methods) for PBS- (E) and IL-33–treated (F) cells were calculated and normalized responses were evaluated. Data in (A) are the means ± SEM (n = 14) and in (B) and (C) are representative of three experiments conducted on separate cell preparations. In (D), data are presented as the means ± SEM of n = 4 and the difference among the groups of mice is indicated. *p < 0.05, two-way ANOVA with Bonferroni post hoc test. Data in (G) are presented as the means ± SEM of n = 4. The arrows in (E) and (F) indicate the time of cell challenge. *p < 0.05 for comparison with cells not exposed to IL-33, Student t test.
effects of IL-33 were similarly dependent on MyD88 by employing BMMCs developed from MyD88−/− mice. As shown in Fig. 4A, as expected, the ability of chronic IL-33 exposure to downregulate Ag-mediated degranulation was significantly impaired in ST2-deficient BMMCs. Furthermore, such downregulation was also not observed in the MyD88−/− BMMCs upon stimulation with Ag in the absence (Fig. 4B) or presence (Fig. 4C) of SCF. Note that in this series of experiments we observed lower levels of degranulation than those shown in Fig. 3; however, the results were within the range of variability noted among different experiments with BMMCs. Similarly, the ability of chronic IL-33 exposure to reduce the Ag-mediated calcium signal was not observed in the MyD88−/− BMMCs (Fig. 4D).

The MyD88−/− cells were then used to exclude the possibility that the effects of IL-33 were dependent on the secondary release of other cytokines. Conditioned medium was taken from WT BMMCs incubated for 2 wk in IL-33 and then its ability to reduce degranulation were examined. The MyD88−/− BMMCs were thus used to avoid any potential effects of IL-33 carried over in the conditioned media. As shown in Fig. 4E, the conditioned media failed to recapitulate the hyporesponsive phenotype. From these data we conclude that the ability of IL-33 to induce a hyporesponsive MC phenotype was due to a direct action of IL-33 mediated through MyD88.

**Diminished PLCγ1 and Hck expression in IL-33-cultured MCs**

Multiple early events contribute to the signaling pathways leading to FceRI-mediated degranulation of MCs (27). These events culminate in the calcium signal and cytoskeletal reorganization that are required for exocytosis. As shown in Figs. 1G and 3C, the IL-33–induced hyporesponsive phenotype was associated with a significantly reduced calcium signal. To identify underlying defects that could account for this attenuated calcium signal, two approaches were adopted: gene arrays to examine changes in expression of potentially responsible signaling molecules at the mRNA level, and immunoblot analysis to determine IL-33–dependent alterations not only in expression but also activation of relevant signaling molecules.

The microarray analysis was conducted on BMMCs cultured in the presence or absence of IL-33 for 4 wk. As expected, an extensive number of genes were either upregulated or downregulated following IL-33 treatment (Supplemental Table I). However, there were little obvious changes in ST2 (also by immunoblot analysis; data not shown) or any of the genes encoding signaling molecules that would be predicted to impact calcium mobilization (27). Nevertheless, the array analysis did reveal a significant downregulation of Hck, a Src kinase that has been demonstrated to be critical for Ag-mediated cytoskeletal rearrangement through the regulation of actin/tubulin depolymerization/polymerization as well as degranulation (28). The downregulation of Hck expression was confirmed by quantitative PCR and at the protein level (Fig. 5A).

To further investigate the underlying cause for the attenuated calcium signal in IL-33–treated cells, we explored whether PLCγ activation and other signals required for calcium mobilization (27) were similarly affected at the protein level. IL-33 indeed markedly decreased the inducible phosphorylation of PLCγ1 and this reflected a similar decrease in PLCγ1 expression (Fig. 5B, 5C). Similar reductions were observed in phosphorylation of LAT and LAT2 (Supplemental Figs. 1, 2), but without apparent reduction in their expression. In contrast to PLCγ1, the expression of Btk, AKT, Syk, and the MAPKs p38, JNK, and ERK1/2 were relatively unaffected (Supplemental Figs. 1, 2). Correlating with the lack of a calcium response, the IL-33–induced reduction in PLCγ1 expression and phosphorylation was not observed in the MyD88−/− BMMCs (Fig. 5D).

**Inefficient actin depolymerization/polymerization in MCs following prolonged IL-33 exposure**

Because of the recognized role of Hck in cytoskeletal rearrangement (29) required for MC degranulation, we examined whether the diminished Hck expression in IL-33–treated MCs also resulted in attenuated Ag-mediated actin polymerization/depolymerization. As expected, Ag challenge of untreated BMMCs resulted in initial actin depolymerization followed by repolymerization (Fig. 6). However, as for calcium signaling, actin and hence cytoskeletal rearrangement were substantially attenuated in the cells pre-exposed to IL-33.

We conclude from the above data that IL-33 promotes a hyporesponsive MC phenotype and that this is at least in part a consequence of the parallel attenuation of the calcium signal, as a consequence of decreased PLCγ1 expression, and cytoskeletal reorganization, as a consequence of downregulation of Hck expression (25, 27–29).
The ability of IL-33 to modify MC activation has recently been the topic of active investigation owing to reports of elevated IL-33 levels in the lungs of asthmatics and affected tissues of patients with rheumatoid arthritis, Crohn's disease, atopic dermatitis, and psoriasis, diseases that are associated with MC activation (5, 30–32). A role for IL-33 in MC-driven allergic reactions had also been suggested by the report that IL-33 alone induces anaphylaxis in a mouse model, as well as degranulation of MCs in culture (33), now retracted. However, as described by others in mouse BMMCs (8, 11, 12), we observed that IL-33 itself failed to induce calcium mobilization and thus was unable to induce degranulation (Fig. 1). Furthermore, we observed no anaphylactic response in the mouse following direct challenge with IL-33 (Fig. 3). From these data, we conclude that, under acute conditions, IL-33 does not influence MC degranulation. Nevertheless, our data clearly demonstrate that prolonged exposure to IL-33 (Fig. 2) markedly reduces FcεRI-mediated MC degranulation and that this was associated with MyD88-dependent (Figs. 4, 5) IL-33-induced attenuation of the FcεRI-induced calcium signal (Fig. 1) and cytoskeletal rearrangement, which are required for effective degranulation (Fig. 6).

The observed IL-33-dependent downregulation of PLCγ1 and Hck likely accounted for, or at least contributed to, the impaired calcium signal and cytoskeletal rearrangement in the hyporesponsive phenotype. Of note, Hck-deficient BMMCs display impaired degranulation and associated actin reorganization (28), and we have recently documented that a hyporesponsive mouse BMMC phenotype induced by prolonged exposure to SCF is also associated with downregulation of Hck (24). In contrast to the effects of IL-33, however, the SCF-induced hyporesponsive phenotype was not associated with similar defects in PLCγ1 expression and calcium signaling (24). This difference may be related to the distinctly different signaling pathways through which IL-33 and SCF operate (4).

PLCγ1 is required for induction of the calcium signal (25) and, as for Hck (28), the associated actin reorganization (34). Both of these signals are necessary for MC degranulation. Thus, the

**Discussion**

The ability of IL-33 to modify MC activation has recently been the topic of active investigation owing to reports of elevated IL-33 levels in the lungs of asthmatics and affected tissues of patients with rheumatoid arthritis, Crohn’s disease, atopic dermatitis, and psoriasis, diseases that are associated with MC activation (5, 30–32). A role for IL-33 in MC-driven allergic reactions had also been suggested by the report that IL-33 alone induces anaphylaxis in a mouse model, as well as degranulation of MCs in culture (33), now retracted). However, as described by others in mouse BMMCs (8, 11, 12), we observed that IL-33 itself failed to induce calcium mobilization and thus was unable to induce degranulation (Fig. 1). Furthermore, we observed no anaphylactic response in the mouse following direct challenge with IL-33 (Fig. 3). From these data, we conclude that, under acute conditions, IL-33 does not influence MC degranulation. Nevertheless, our data clearly demonstrate that prolonged exposure to IL-33 (Fig. 2) markedly reduces FcεRI-mediated MC degranulation and that this was associated with MyD88-dependent (Figs. 4, 5) IL-33-induced attenuation of the FcεRI-induced calcium signal (Fig. 1) and cytoskeletal rearrangement, which are required for effective degranulation (Fig. 6).

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IL-33–dependent downregulation of Hck and PLCγ1 likely accounted for the impaired cytoskeletal rearrangement and marked attenuation of the FcεRI-mediated calcium signal in the hyporesponsive phenotype. Additionally, because the Ag-induced PLCγ1-dependent calcium signal interacts synergistically with weak calcium signals generated by SCF (23) to further enhance Ag-mediated degranulation, this could explain why IL-33 also reduced the ability of SCF to enhance degranulation.

It is possible that IL-33 may act upon other essential signaling events even though we observed little change in the expression of other critical signaling proteins for MC activation. For example, Ag-induced LAT and LAT2 phosphorylation, but not protein levels, were substantially reduced in the IL-33–treated MCs. It has been proposed that the positive effects of Hck may in part be mediated through suppression of the inhibitory actions of Lyn (28), and such actions would likely be diminished as a consequence of the decrease in Hck expression by IL-33. Certainly, the diminished levels of Hck in IL-33–treated cells would be expected to share similar phenotypic characteristics as the hck−/− MCs.

We propose that the prolonged effects of IL-33 on MC activation through modification of the expression of critical signaling molecules can be viewed as phenotypic reprogramming. The extent to which other factors can modulate the huMC activation phenotype in the manner described in this study is currently unknown. Nevertheless, as noted above, we have recently shown that prolonged exposure to SCF also induces a hyporesponsive phenotype in the mouse (24). Similarly, we observed that prolonged exposure to IL-1, which signals in a manner similar to IL-33, also downregulates Ag-mediated degranulation in BMMCs (Supplemental Fig. 3). In contrast to the IL-33 receptor, ST2, which signals through a MyD88 and IL-1R accessory protein complex (4, 31), it has recently been shown that prolonged exposure to agonists of TLRs, which require MyD88 but not IL-1R accessory protein for signaling, enhances Ag-mediated degranulation (35). This implies that although MyD88 is essential, signals conferred by IL-1R accessory protein are also required for IL-33 induction of the hyporesponsive phenotype.

Overall, our results suggest that the secretory response of MCs to Ag in a pathophysiologic setting may not be finely regulated in an acute manner (36), but that persistent increases in factors in the surrounding milieu could conceivably dampen proinflammatory responses of MCs following an innate immune reaction and assist resolution of the immune response. In the case of IL-33, the elevated level of IL-33 in asthmatic lungs rather than contributing to the disease may actually protect by tempering further MC-dependent inflammation. This, as well as other studies (37) and our recent finding that BMMCs are rendered similarly quiescent by long-term exposure to a physiologic factor, SCF (24), points perhaps to a broad array of conditioning factors to which MCs might be exposed in health and disease states. The possible implication is that if the downregulation of signaling proteins by IL-33 or SCF is a mechanism for long-term prevention of inappropriate activation of MCs in a pathologic situation, then any disruption of such a process would render MCs hyperactive, leading to MC-driven disease. Our observations may thus have implications for the treatment of allergic disorders if, for example, receptor ligands with more restricted actions than IL-33 or SCF were found to shift MCs to a quiescent state.

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Disclosures

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References


