Published in final edited form as:

J Allergy Clin Immunol. 2013 September ; 132(3): 704–712.e10. doi:10.1016/j.jaci.2013.03.033.

Efficient cytokine-induced IL-13 production by mast cells requires both IL-33 and IL-3

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Abstract

Background—IL-13 is a critical effector cytokine for allergic inflammation. It is produced by several cell types, including mast cells, basophils, and T_H2 cells. In mast cells and basophils its induction can be stimulated by cross-linkage of immunoglobulin receptors or cytokines. The IL-1 family members IL-33 and IL-18 have been linked to induction of IL-13 production by mast cells and basophils. In CD4 T_H2 cells IL-33-mediated production of IL-13 requires simultaneous signal transducer and activator of transcription (STAT) 5 activation.

Objective—Here we have addressed whether cytokine-induced IL-13 production in mast cells and basophils follows the same logic as in T_H2 cells: requirement of 2 separate signals. Methods: By generating a bacterial artificial chromosome (BAC) transgenic IL-13 reporter mouse, we measured IL-13 production in mast cells and basophils.

Results—In mast cells harvested from peritoneal cavities, 2 cytokine signals are required for IL-13 production: IL-33 and IL-3. In bone marrow mast cells IL-13 production requires IL-33, but the requirement for a STAT5 inducer is difficult to evaluate because these cells require the continuous presence of IL-3 (a STAT5 activator) for survival. Poorer STAT5 inducers in culture (IL-4 or stem cell factor) result in less IL-13 production on IL-33 challenge, but the addition of exogenous IL-3 enhances IL-13 production. This implies that bone marrow-derived mast cells, like peritoneal mast cells and T_H2 cells, require stimulation both by an IL-1 family member and a STAT5 inducer to secrete IL-13. Basophils follow the same rule; splenic basophils produce IL-13 in response to IL-18 or IL-33 plus IL-3.

Conclusion—Optimal IL-13 production from mast cells and basophils requires 2 cytokine signals.

Keywords

Mast cells; basophils; allergic inflammation; cytokines; signal transducer and activator of transcription 5; tyrosine phosphorylation; BAC transgenic mouse; DsRed fluorochrome

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The rest of the authors declare that they have no relevant conflicts of interest.

Mast cells are tissue-based effector cells of innate immunity. They participate in tolerance induction and immune/allergic inflammatory responses by secreting soluble factors that regulate responses of cells in tissues.^{1,2} Among these factors are the type I cytokine IL-13 and, to a lesser extent, IL-4. Both these cytokines initiate airway hypersensitivity.^{3,4} However, in mice that are actively immunized and then challenged with the immunogen, IL-13 is the principal inducer of allergic inflammation; IL-4 appears to play its major role in the induction of T_H2 responses.⁵

In keeping with IL-13's role as a major mediator of allergic inflammation, mast cells produce more IL-13 than IL-4 in response to cross-linkage of Fc RI⁶ or treatment with ionomycin.⁷ Importantly, mast cells also secrete IL-13 when challenged with certain cytokines.⁸ IL-13 and IL-4 are also expressed in basophils independently of Fc RI activation in response to cytokines.^{9,10} Such cytokine-induced cytokine production could be of importance in propagating allergic inflammation after an inciting allergen has been eliminated.

Cytokine-induced cytokine production appears to be a general phenomenon seen in CD4 T_H cells, natural killer T cells, and innate lymphoid cells, as well as mast cells and basophils.^{11,12} In T_H1/T_H2/T_H17 cells cytokine-induced cytokine production depends on stimulation by 2 agents, one of which activates a member of the IL-1 family of receptors, with the other inducing signal transducer and activator of transcription (STAT) activation.¹¹ In mast cells IL-33 appears to be the critical IL-1 family member for functional modulation. In vivo injection of IL-33 in the mouse ear induces an inflammatory skin lesion that is significantly reduced in mice that lack mast cells.¹³ Furthermore, in vivo injection of IL-33 in *Rag-2*^{-/-} mice induces airway hyperreactivity and goblet cell hyperplasia and increases the production of IL-4, IL-5, and IL-13 independently of lymphocytes.¹⁰ In vitro type I cytokine production by mast cells has been reported to be mediated simply by the addition of the IL-1 family member IL-33.⁸ Here we show that mast cells and basophils also follow the "2-signal model," with IL-13 production dependent on an IL-1 family member and an inducer of STAT5, mainly IL-3, in both mast cells and basophils. In keeping with the basophil's expression of both IL-18 receptor (IL-18R)¹⁴ and IL-33 receptor, both IL-18 and IL-33 are active in these cells; mast cells, expressing very low levels of IL-18R but high levels of IL-33 receptor, respond to IL-33 but not IL-18.

METHODS

DsRed transgenic mice

Transgenic mice expressing DsRed under IL-13 regulatory elements were prepared by using bacterial artificial chromosome (BAC) recombineering technology with *galK*-selection.¹⁵ The BAC clone (BAC172) contains the *Il4*, *Il13*, and *Il5* genes and the T_H2 locus control region. The ATG of the *Il13* gene in the BAC172 clone was targeted with a *galK* construct containing homology arms at both the 5' and 3' ends of the *galK* gene that had *Bam*HI restriction sites. *galK* was subsequently targeted with a DsRed (Clontech, Mountain View, Calif) construct. The new BAC172 construct was fully sequenced between the 5' and 3' homology regions. Microinjection of the construct into B6 oocytes was followed by transfer into pseudopregnant foster mothers. Genomic DNA of 121 tentative IL-13/DsRed-transgenic pups was digested with *Bam*HI, separated on 0.8% agarose gel, transferred to a nylon membrane, and probed with a 916-bp PCR fragment spanning the 5' and 3' homology arms of the *Il13*/DsRed construct.

For correlation of DsRed expression with simultaneous IL-4 expression in T_H2 cells, mice born from the founders were bled, blood cells were plated under T_H2 conditions for 3 days

and stimulated with phorbol 12-myristate 13-acetate/ionomycin for 4 hours, and cells were stained for CD4 and IL-4.

Mice

Wild-type (WT) C57BL/6 mice were from Jackson Laboratories (Bar Harbor, Me) or Taconic Farms (Hudson, NY). B6 *MyD88*^{-/-} mice were from Dr R. Caspi (National Eye Institute, Bethesda, Md), with permission from Professor S. Akira (Osaka University, Osaka, Japan).¹⁶ Mice were housed at the National Institute of Allergy and Infectious Diseases pathogen-free animal facility, and all experiments were done under a protocol approved by the National Institute of Allergy and Infectious Diseases Animal Care and Use Committee.

Cell culture

Bone marrow-derived cells were prepared by culturing red cell-depleted bone marrow cell suspensions in 20 ng/mL IL-3 for 10 to 40 days. The cells were used between days 10 and 12 to obtain basophils. In IL-33 receptor blocking experiments unlabeled T1-ST2 antibody (10 µg/mL; MD Bioscience, St Paul, Minn) was added to the culture. Peritoneal cavities of mice were flushed with PBS containing 2 mmol/L EDTA; plated in 6-well plates in RPMI containing 2% FBS, penicillin/streptomycin, and L-glutamine (2 mmol/L); and maintained at 37°C with 5% CO₂ to study peritoneal cells. T_H2 and T_H17 *in vitro* differentiation was performed and evaluated, as previously described.¹¹ Briefly, naive CD4 T cells were purified, cultured under specific T_H2 or T_H17 culture conditions for 4 days, and rested for 3 days, and then after restimulation with phorbol 12-myristate 13-acetate/ionomycin, the production of IL-17 or IL-4 was measured by using RNA analysis, cytoplasmic anticytokine staining, and ELISA.

Cell stimulation, flow cytometry, and quantitative PCR

Cells were stimulated with IL-1, IL-33, IL-3, IL-5, GM-CSF, stem cell factor (SCF; PeproTech, Rocky Hill, NJ), thymic stromal lymphopoietin, IL-7 (R&D Systems, Minneapolis, Minn), or IL-18 (MBL International, Woburn, Mass), as indicated. All stainings for CD4, c-Kit, FcγRI (eBioscience, San Diego, Calif), IL-18Rα (BioLegend, San Diego, Calif), and IL-33 receptor/T1-ST2 (MD Biosciences) were performed in the presence of the FcγRII and III blocking antibody 2.4G2 and 0.1% mouse serum. Phospho-STAT5 staining was performed, as previously described,¹⁷ IL-6 and IL-13 staining was performed with 0.5% Triton X in staining buffer. Anti-IL-6 and anti-IL-13 antibodies were purchased from eBioscience, and anti-phospho-STAT5 was purchased from BD (Franklin Lakes, NJ). For analysis of DsRed expression in bone marrow-derived mast cells (BMMCs) and bone marrow-derived basophils, the cultures were washed twice with PBS and plated with indicated cytokines for 16 hours. The unstimulated wells contained a basal level of IL-3 to maintain cell viability. For IL-4 and SCF cultures of bone marrow cells, cells were washed with PBS 3 times and grown thereafter either in IL-3-, IL-4-, or SCF-containing media, as indicated. In subsequent analysis a live cell gate was used.

Cell sorting was performed with FACSDiva software (BD). DsRed and IL-13 mRNA was measured after cell sorting by using isolating total RNA with an RNeasy kit (Qiagen, Hilden, Germany), and IL-13 protein was measured by using ELISA (R&D Systems). For measurement of RNA expression, total RNAs were reverse transcribed to cDNA by using SuperScript II First Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, Calif). Quantitative PCR reactions were performed with a 7900HT sequence detection system (Applied Biosystems, Foster City, Calif). The probe sets for IL-3, IL-13 (FAM-MGB probe), and 18S ribosomal RNA (VIC-MGB-probe) were from Applied Biosystems. All mRNA levels were normalized to 18S ribosomal RNA.

RESULTS

Construction of an IL-13 reporter mouse

We used the 120-kbp BAC172/pBACBelo11 construct (kindly provided by Gap Lee and Richard Flavell) containing the *Il4*, *Il13*, and *Il5* genes, as well as the *Il4/Il13* locus control region. We inserted a DsRed-encoding construct immediately after the translation-initiating ATG for *Il13* using recombineering technology (Fig 1, *A*, and see the Methods section).¹⁵ BAC-containing mice were screened by means of Southern blotting of their genomic DNA (Fig 1, *B*). CD4 T cells from distinct founder lines were cultured under T_H2 conditions, revealing a good correlation between the proportion of cells that were DsRed⁺ and those that produced IL-4 on restimulation (see Fig E1 in this article's Online Repository at www.jacionline.org), implying a direct relationship between the number of copies of the transgene and expression of IL-4.

We chose one line, K-1, for further study. It had approximately 5 copies of the recombineered BAC. We analyzed both IL-13 protein production and RNA expression as a function of DsRed brightness of the cells. For protein analysis, we independently stimulated BMMCs from 3 different K-1 mice on day 40 of the culture for 4 hours with IL-33 and then sorted DsRed^{hi}, DsRed^{int}, and DsRed^{lo} BMMCs (c-Kit⁺/Fc RI⁺) and continued these separated cultures o/n. We then measured IL-13 concentration in the supernatants (Fig 1, *C*). For RNA expression, we sorted BMMCs from 2 K-1 mice after a 5-hour *in vitro* stimulation with IL-33 on day 21 of culture and measured the relation of IL-13 mRNA expression to DsRed mRNA expression, as determined by using quantitative PCR (Fig 1, *D*). On the basis of the good correlation of both IL-13 RNA and protein to DsRed brightness, we regard DsRed expression as a reliable surrogate for IL-13 expression.

IL-3 is required for IL-33–induced IL-13 production in peritoneal mast cells

It has been reported that addition of IL-33 alone strongly upregulated IL-13 expression in BMMCs.^{8,10} IL-33 participates in inducing IL-13 production in CD4 T cells, but a STAT5 signal is also required in T cells.¹¹ BMMCs are traditionally elicited by culturing bone marrow progenitors in IL-3, a STAT5 activator, and thus the possibility existed that such basal IL-3/STAT5 synergized with IL-33 in inducing IL-13.

We tested the cytokine requirements for IL-13 production in mast cells directly harvested from the peritoneal cavity (see Fig E2 in this article's Online Repository at www.jacionline.org). Peritoneal cells were stimulated with nothing or with the IL-1 family members IL-1, IL-18, or IL-33 (10 ng/mL) with or without 10 ng/mL IL-3 for 16 hours (Fig 2, *A*). WTB6 mice showed a background frequency (1% to 3%) of apparently DsRed-expressing cells under all stimulation conditions. Unstimulated c-Kit⁺/Fc RI⁺ peritoneal cells from K-1 mice showed no DsRed⁺ cells above background ($n = 3$; mean $\pm$ SEM, 1.3% $\pm$ 0.3%). IL-1 or IL-18 alone ($n = 2$) resulted in no induction of DsRed⁺ cells above the WT background (2% each), and IL-33 ($n = 3$) alone caused essentially no induction of DsRed⁺ cells (mean $\pm$ SEM, 3% $\pm$ 1.5%). IL-3 alone ($n = 2$) induced a very small proportion of cells to be DsRed⁺ (approximately 5%). The combination of IL-3 plus IL-18 produced no more DsRed⁺ cells than IL-3 ($n = 2$) alone, whereas IL-3 plus IL-1 caused approximately 10% of the c-Kit⁺/Fc RI⁺ cells to express DsRed ($n = 2$). However, the combination of IL-3 plus IL-33 ($n = 3$) led to a striking induction of DsRed⁺ cells; on average, approximately 47% of the c-Kit⁺/Fc RI⁺ cells were DsRed⁺ (mean $\pm$ SEM, 46.7% $\pm$ 4.8%).

We then asked whether IL-33 induced substantial numbers of DsRed⁺ cells in the presence of other STAT5-inducing cytokines. We cultured peritoneal mast cells in 2 independent experiments with 20 ng/mL IL-33 and 20 ng/mL of several STAT5-inducing cytokines (IL-5, GM-CSF, thymic stromal lymphopoietin, and IL-7). Only IL-3 combined with IL-33

caused a major fraction of the peritoneal mast cells to express DsRed (Fig 2, *B*). In parallel, of the tested STAT5 inducers, only IL-3 induced STAT5 phosphorylation in peritoneal mast cells ($n = 2$; Fig 2, *C*).

BMMCs and bone marrow–derived basophils produce IL-13 in response to addition of IL-33

We prepared bone marrow cultures from DsRed transgenic mice to determine BMMC and basophil cytokine requirements for IL-13 production. Approximately 11% (SEM, $\pm 0.6\%$) of BMMCs ($n = 3$) from 10- to 12-day cultures in IL-3 were DsRed⁺ without further activation (Fig 3, *A*, left panel); this probably represents a low level of IL-13 expression because staining with anti-IL-13, which is relatively insensitive, did not indicate “background” (Fig 4, *A*, upper panel). On 16-hour culture with IL-1 family members, we observed that IL-33 was capable of strongly inducing DsRed expression (approximately 69% [SEM, 69%] of cells, $n = 3$), whereas neither IL-1 ($n = 3$) nor IL-18 ($n = 3$) caused any significant increase in the frequency of DsRed⁺ cells (Fig 3, *A*, and see Fig E3 in this article’s Online Repository at www.jacionline.org). The mean fluorescence intensity of DsRed in the positive gate was approximately 3-fold higher in cells stimulated with IL-33 as opposed to IL-3 alone, and thus not only did IL-33 increase the frequency of DsRed⁺ cells approximately 7-fold, it also enhanced the degree of expression of DsRed approximately 3-fold on a single-cell basis. These results were obtained in 3 independent BMMC cultures grown in IL-3 for 40 days. Such cultures are essentially pure mast cells, ruling out the role of other cell types in DsRed induction. An important difference between the “short” and “long” culture durations was that prolonged culture decreased the background of DsRed⁺ cells to approximately 5%. In addition, both IL-1 and IL-18 consistently showed modest (10% to 20%) induction of DsRed expression, whereas IL-33 induced striking DsRed production in these cells (see Fig E3).

For basophils (Fc RI⁺/c-kit⁺ cells) in the 10- to 12-day cultures, IL-18 was the best inducer of IL-13 production. Approximately 19% of basophils became DsRed⁺ when cultured in IL-3 alone. Addition of IL-18 resulted in 37% DsRed⁺ cells. IL-33 was a slightly poorer inducer of IL-13 than IL-18, whereas IL-1 did not induce any IL-13 expression above baseline (Fig 3, *B*). In splenic basophils from 2 DsRed mice (see Fig E4 in this article’s Online Repository at www.jacionline.org), stimulation for 4 hours with IL-3 alone induced some DsRed expression (22% to 24%, with backgrounds of 4% to 8% of cells); addition of either IL-18 or IL-33, in combination with IL-3, increased DsRed production to 35% to 47% of cells; and IL-1 plus IL-3 also caused an increase in the frequency of DsRed-expressing cells. In contrast to basophils from IL-3 bone marrow cultures, IL-18, or IL-33 alone caused little or no induction of DsRed expression in splenic basophils.

IL-6 is also upregulated by IL-33 in BMMCs.⁸ Not surprisingly, unstimulated WT BMMCs (but responding to IL-3 as a result of its presence in culture medium) or cells to which further IL-3 was added showed essentially no IL-6 expression. Addition of IL-33 resulted in approximately 20% of BMMCs expressing IL-6 (see Fig E5 in this article’s Online Repository at www.jacionline.org).

What is responsible for the background of DsRed⁺ cells in the BMMCs and basophils obtained from the 10- to 12-day cultures? This background expression was observed in all experiments described here (Fig 3, *C*). It presumably resulted from stimulation by IL-3 (added as a growth/differentiation factor) and possibly from an endogenous source of IL-33. Interestingly, in another IL-13 indicator mouse (IL-13-GFP-KI), approximately 5% of BMMCs from heterozygous or homozygous reporter mice cultured in IL-3 were reported to be green fluorescent protein positive.¹⁸

To test whether a source of IL-33 existed in the cultures, we added an unlabeled neutralizing anti-IL-33 receptor (T1-ST2) antibody (clone DJ8)¹⁹ from the outset of culture to 2 independent BMMC cultures from different DsRed mice. The presence of T1-ST2 decreased DsRed expression by the unstimulated mast cells (ie, BMMCs that received only IL-3) by approximately 50% (Fig 3, *D*). This implies that a source of IL-33 exists in the early bone marrow cultures.

Both MyD88 expression and STAT5 phosphorylation are needed for IL-33–induced IL-13 production by BMMCs

It was reported that BMMCs from *MyD88*^{−/−} mice did not make IL-13 when stimulated with IL-33⁸ and that IL-1 and IL-18 responses were impaired in these mice.¹⁶ Similarly, bone marrow–derived basophils from these mice are unable to produce IL-13 in response to IL-33.¹⁰ We prepared BMMCs from 2 WT and 2 *MyD88*^{−/−} mice. Because these animals lacked the DsRed BAC transgene, we relied on intracellular staining for IL-13 rather than expression of DsRed as a marker of IL-13 production. Our experience is that staining for IL-13 is much less sensitive than DsRed expression, and in consequence, cells harvested from an IL-3–only culture show no detectable IL-13 by using intracellular staining. As anticipated, BMMCs from *MyD88*^{−/−} donors did not express IL-13 when cultured with IL-33 plus the IL-3 that was in the bone marrow culture or IL-33 plus additional IL-3. By contrast, 17% to 19% of WT BMMCs expressed intracellular IL-13 within 5 hours of stimulation with 10 ng/mL IL-33 or IL-33 plus additional IL-3 (Fig 4, *A*). For basophils in the same cultures, IL-33 induced 14% to 15% IL-13–expressing cells, and as expected,¹⁰ *MyD88*^{−/−} basophils did not respond to IL-33 with any additional IL-13–expressing cells (see Fig E6 in this article's Online Repository at www.jacionline.org).

A major difficulty in determining the need for IL-3–mediated STAT5 phosphorylation in IL-33 induction of IL-13 production is that cultured BMMCs and basophils are dependent on IL-3 for survival, and accordingly, STAT5-deficient mice lack mast cells.²⁰ However, for short-term culture, IL-4 or SCF can replace IL-3 and will maintain the viability of the cells. To test the importance of STAT5 phosphorylation in IL-13 production, we performed 2 independent experiments in which we compared the expression of DsRed in K-1 BMMCs that had been cultured for the last 32 hours only with IL-4 or IL-3, as well as the degree of STAT5 Y594 phosphorylation in these cells. Substantially fewer (approximately 50%) of the cells grown in IL-4 stained with anti-phospho-STAT5 than did the cells grown in IL-3 for the same length of time. We then exposed these cells to no additional cytokines or to IL-3, IL-33, or both. Cells grown in IL-4 displayed substantially fewer DsRed⁺ BMMCs in response to IL-33 than did cells grown in IL-3 (45% vs 64%). This difference disappeared when IL-3 was added to the IL-4/IL-33 culture (Fig 4, *C*). Basophils tolerated replacement of the IL-3 with IL-4 much less well so that we were unable to analyze IL-13 production using the few basophils that remained viable after a 32-hour culture in IL-4. We also tested the capability of IL-33 to induce DsRed expression if IL-3 was replaced by SCF as a mast cell growth factor. We cultured BMMCs from 2 WT and 3 DsRed transgenic mice for 40 days in IL-3, washed out the IL-3, and cultured the cells in the presence of IL-3 or SCF for 48 hours, after which the cells were either left unstimulated or stimulated o/n with IL-33 alone or IL-3 plus IL-33. We found that SCF-cultured cells that were stimulated with IL-33 were poorly induced to produce DsRed. However, if these cells received IL-3 also, they induced DsRed strongly (see Fig E7 in this article's Online Repository at www.jacionline.org). In line with this, cells cultured in SCF for 48 hours had a substantially lower level of phospho-STAT5 than did cells that were cultured in IL-3 (see Fig E7).

We then studied the possibility that the failure of *MyD88*^{−/−} cells to produce IL-13 in response to IL-3 plus IL-33 could be explained by diminished STAT5 phosphorylation in the *MyD88*^{−/−} cells. As shown in Fig 4, *D*, there was no difference in STAT5

phosphorylation between WT and *MyD88*^{-/-} BMMCs or basophils; this experiment was performed twice (see Fig E8 in this article's Online Repository at www.jacionline.org).

Role of IL-3 and IL-33 in IL-33 receptor expression

One possible mode of action of IL-3/STAT5 in IL-13 production could be through increasing the degree of expression of the IL-33 receptor. Indeed, in resting T_H2 cells, where IL-33 receptor expression is low, STAT5 activation is essential for upregulation of the IL-33 receptor.¹¹ We first confirmed the specificity of the T1-ST2 antibody (clone DJ8; Fig 3, *D*) by staining *in vitro* differentiated CD4 T_H17 or CD4 T_H2 cells with this antibody. As expected, we found high expression on T_H2 cells but none on T_H17 cells (Fig 5, *A*). We then tested whether peritoneal mast cells would increase T1-ST2 expression in response to IL-3 or IL-33. Peritoneal mast cells expressed high levels of T1-ST2 without stimulation (see Fig E9 in this article's Online Repository at www.jacionline.org). Stimulation of these cells for 16 hours with IL-3 resulted in approximately 1.5-fold induction of receptor expression (Fig 5, *B* and *C*), whereas IL-33 alone had no effect on T1-ST2 expression, and the combination of IL-3 and IL-33 was no better than IL-3 alone.

Exogenous IL-3 did not further enhance T1-ST2 expression on IL-3–cultured BMMCs. The addition of IL-33 increased receptor expression approximately 1.4-fold; no further enhancement was obtained by adding exogenous IL-3 to IL-33 (Fig 5, *B* and *C*). Thus the presence of IL-3 modestly enhanced T1-ST2 in peritoneal mast cells; whether it was important in BMMCs could not be ascertained, but additional IL-3 did not enhance receptor expression. Furthermore, BMMCs grown in IL-4 for the last 48 hours of the culture did not downregulate IL-33 receptor expression despite their diminution in STAT5 phosphorylation (Figs 4, *B*, and 5, *D*). We also tested the possibility that *MyD88*^{-/-} BMMCs might express lower levels of T1-ST2 and thus be less responsive to IL-33 by comparing staining with anti-T1-ST2 in 2 WT and 2 *MyD88*^{-/-} BMMC cultures (Fig 5, *E*). No difference was observed.

IL-18R expression in basophils and BMMCs

Because IL-18 is superior to IL-33 in stimulating IL-13 production by basophils while only IL-33 is active in BMMCs, we examined expression of receptors for these cytokines in bone marrow–derived basophils and BMMCs. In line with previous work,¹⁰ we could not detect expression of IL-18R on BMMCs, whereas basophils reproducibly expressed IL-18R (Fig 6, *A*). We also observed that mature basophils from spleens expressed higher levels of IL-18R than did bone marrow–derived basophils (Fig 6, *A*, and see Fig E10 in this article's Online Repository at www.jacionline.org). In contrast, BMMCs showed very strong staining (mean fluorescence intensity, approximately 200) with anti-T1-ST2, whereas basophils stained far more weakly (mean fluorescence intensity, approximately 10; Fig 6, *A* and *B*). We quantitated these data by measuring the ratio of isotype control staining to the specific receptor antibody staining in 3 independent experiments (Fig 6, *C*).

DISCUSSION

Production of IL-13 is often of crucial importance for elimination of parasites in infection models, although this requirement varies for different parasites.²¹ IL-13 plays a major role in many allergic inflammatory systems, particularly in airway hypersensitivity models.⁴ Furthermore, because the functionality of IL-13 is related to the amount of the cytokine,²² understanding the mechanisms controlling IL-13 production is of importance. To overcome technical limitations in measuring intracellular IL-13 production, we constructed a BAC transgenic indicator mouse in which the DsRed fluorochrome is controlled by IL-13 genetic regulatory elements.

We observed that peritoneal mast cells did not become DsRed⁺ (ie, to become IL-13 producers) in response to IL-33 alone. The need for stimulation by an IL-1 family member and a STAT activator to induce cytokine-induced cytokine production is in keeping with the requirements of primed CD4 T cells for such responses. Superficially, BMMCs appeared to respond differently. They produced IL-13 with only the addition of IL-33. However, we believe that this difference is purely technical. For BMMCs to survive, a STAT5-activating factor is essential.²⁰ That factor is typically IL-3. Indeed, without IL-3, these cultures rapidly lose viability. IL-4 or SCF, weaker STAT5 inducers, can maintain BMMC cultures. BMMCs from IL-4 or SCF cultures show weaker induction of DsRed in response to addition of IL-33 alone, which is in keeping with their poorer activation of STAT5. The addition of IL-3 to the stimulatory “cocktail” restores IL-13 production levels to those obtained when IL-33 was added to cells that had been stimulated with IL-3 from the beginning of the culture. Because BMMCs cannot be obtained from STAT5^{-/-} knockout⁴ bone marrow,²⁰ we cannot unequivocally establish that STAT5 activation is essential for IL-13 production, but our results are certainly consistent with the conclusion that cytokine-induced IL-13 production by both tissue mast cells and BMMCs requires both IL-33 and IL-3.

For splenic basophils, IL-18 or IL-33 alone did not upregulate DsRed production, whereas their combination with IL-3 clearly did. Effectively, this means that IL-3-mediated STAT5 activation is important for IL-13 production by basophils as well. On the basis of the rapid expression of IL-13 and IL-6 in BMMCs (Fig 4, A, and see Fig E5), it seems likely that the effect of IL-33 is mediated through the direct action of its signaling intermediates on the *Il13* promoter; it is known that functional inhibition of nuclear factor κ B results in decreased immediate IL-13 production by IL-33 in T_H2 cells.¹¹

Our finding that IL-33 was not a better inducer of IL-13 than IL-18 in basophils in our experiments is somewhat different from published work. Kroeger et al⁹ found that IL-33 induces IL-13 more strongly than does IL-18 in bone marrow-derived basophils. We cannot fully explain the discrepancy of our results. Although the expression of T1-ST2 is clearly less in basophils than in mast cells, tissue basophils and bone marrow-derived basophils both express T1-ST2 and respond to IL-33. One explanation might be that we did not use cell sorting to purify the cells, which might activate basophils and possibly upregulate their T1-ST2 expression. A further complication in the evaluation of IL-18's effect on basophils is the clearly higher IL-18R expression in splenic basophils compared with bone marrow-derived basophils.

There are interesting differences between the cytokine regulation of IL-13 production by resting T_H2 cells and mast cells. In BMMCs levels of the IL-33 receptors are inherently high and not altered by changes in STAT5 phosphorylation, nor does the absence of MyD88 impair the receptor expression. By contrast, in resting CD4 T_H2 cells, IL-33 receptor levels are quite low, and their upregulation requires both an IL-33-mediated signal and the presence of pSTAT5.¹¹ Although BMMC IL-33 receptor levels are not altered by diminishing or increasing the concentration of IL-3, peritoneal mast cells cultured in the presence of IL-3 show a 1.5-fold increase in IL-33 receptor expression compared with those cultured without IL-3, although T1-ST2 expression is very high on both populations. These results suggest that the main function of the IL-3-STAT5 axis in mast cells is in enhancing IL-13 transcription rather than increasing expression of IL-33 receptors. STAT5 is bound to STAT5 sites within the *Il13* gene in T_H2 cells, and therefore a role for direct regulation of transcription seems quite reasonable. Similarly, mast cells do not require an IL-33 stimulation event to maintain expression of the IL-33 receptor, as judged by the lack of difference in IL-33 receptor expression in WT and *MyD88*^{-/-} BMMCs. Furthermore, IL-33 did not upregulate T1-ST2 in peritoneal mast cells, confirming that IL-33 signal does not regulate the expression of its receptor in these cells.

Our findings are consistent with a general requirement of 2 cytokine signals for Fc γ R-independent or T-cell receptor-independent IL-13 production by mast cells/basophils and T_H cells. One signal is delivered by an IL-1 family member and the other by a STAT5 activator.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by the National Institute of Allergy and Infectious Diseases Intramural Program (to I.S.J., C.W., X.C., J.H.-L., L.G., R.Y., and W.E.P.), the Finnish Medical Foundation (to I.S.J.), Competitive Research Funding of the Tampere University Hospital (grant 9N018, to I.S.J.), the Sigrid Juselius Foundation (to I.S.J. and L.K.), and Fimlab laboratories (grant X51409, to I.S.J.).

Disclosure of potential conflict of interest: I. S. Junttila has been supported by one or more grants from Competitive Research Funding of Tampere University Hospital, Fimlab Laboratories, and the Sigrid Juselius Foundation. L. Kummola has been supported by one or more grants from the Sigrid Juselius Foundation.

We thank Julie Edwards for advice and expert cell sorter operation and Dr Lionel Feigenbaum (National Cancer Institute) for assistance in generating DsRed mice. We also thank Sanna Hämmäläinen for help with IL-6 staining.

Abbreviations used

BAC	Bacterial artificial chromosome
BMMC	Bone marrow-derived mast cell
IL-18R	IL-18 receptor
SCF	Stem cell factor
STAT	Signal transducer and activator of transcription
WT	Wild-type

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Key message

- Mast cells and basophils follow the general rule observed in CD4 T cells for cytokine-induced IL-13 production: joint stimulation by an IL-1 family member and a STAT activator.

FIG 1.

Generation and characterization of IL-13 DsRed reporter mice. **A**, Strategy and transgenic construct used to generate IL-13 DsRed mice. **B**, Southern blot analysis identifying DsRed⁺ pups. **C**, Correlation of IL-13 protein production with DsRed intensity. **D**, Correlation of IL-13 and DsRed mRNA expression in purified BMMCs.

FIG 2.

Peritoneal cavity mast cell IL-33 – mediated IL-13 production requires an IL-3–STAT5 signal. **A**, DsRed expression in c-Kit⁺/Fc RI⁺ cells from peritoneal cavities of WT and DsRed mice on cytokine stimulation. **B**, DsRed expression in response to various STAT5 activators among c-Kit⁺/Fc RI⁺ cells. **C**, STAT5 activation in response to different cytokines in c-Kit⁺/Fc RI⁺ cells of the B6 peritoneal cavity. The *red line* indicates the basal level of STAT5 phosphorylation in unstimulated cells. *TSLP*, Thymic stromal lymphopoietin.

FIG 3.

BMMCs and bone marrow-derived basophils respond to exogenous IL-18 and IL-33. **A**, DsRed expression in BMMCs from DsRed transgenic mice on cytokine stimulation. **B**, DsRed expression in bone marrow-derived basophils from DsRed transgenic mice on cytokine stimulation. **C**, DsRed background in bone marrow cultures is located in basophils and mast cells. **D**, T1-ST2 mAb decreases DsRed expression in BMMCs. *SSC*, Side scatter.

FIG 4. MyD88 expression coupled with strong STAT5 phosphorylation is required for optimal IL-13 expression from BMMCs in response to IL-33. **A** *MyD88*^{-/-} BMMCs do not express IL-13 in response to IL-33. **B**, IL-3 replacement with IL-4 decreases STAT5 phosphorylation of BMMC STAT5. **C**, IL-3 replacement with IL-4 decreases IL-33-induced DsRed expression. **D**, STAT5 phosphorylation is not affected by deletion of MyD88.

FIG 5.

Expression of T1-ST2 in mast cells. **A**, T1-ST2 surface staining is specific because T_H2 cells express high levels of T1-ST2. **B**, Effect of cytokines on T1-ST2 expression in peritoneal cavity mast cells (PCMCs) and BMMCs. **C**, Quantitation of T1-ST2 expression in BMMCs and PCMCs. **D**, IL-3 replacement with IL-4 does not affect T1-ST2 expression in BMMCs. **E**, MyD88 does not regulate T1-ST2 expression.

FIG 6. IL-18 and IL-33 receptor expression in BMMCs and bone marrow-derived basophils. **A**, IL-18R expression in bone marrow-derived basophils and BMMCs. **B**, T1-ST2 expression in bone marrow-derived basophils and BMMCs. **C**, Quantitation of IL-18R α and T1-ST2 expression in bone marrow-derived basophils and BMMCs from 4 independent experiments.