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

Ilkka S. Junttila, MD, PhD,[a,b,c]* Cynthia Watson, BSc,[a]* Laura Kummola, MSc,[b] Xi Chen, MD,[a] Jane Hu-Li, BSc,[a] Liying Guo, PhD,[a] Ryoji Yagi, PhD,[a] and William E. Paul, MD[a] *Bethesda, Md, and Tampere, Finland*

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. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: *Mast cells, basophils, allergic inflammation, cytokines, signal transducer and activator of transcription 5, tyrosine phosphorylation, BAC transgenic mouse, DsRed fluorochrome*

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 FcεRI[6] 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 FcεRI 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) α[14] and IL-33 receptor, both IL-18 and IL-33 are active in these cells; mast cells, expressing very low levels of IL-18Rα 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

From [a]the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda; [b]the School of Medicine, University of Tampere; and [c]Fimlab Laboratories, Pirkanmaa Hospital District, Tampere.

*These authors contributed equally to this work.

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Corresponding author: Ilkka S. Junttila, MD, PhD, Biokatu 4, Rm C-306, 33520 Tampere, Finland. E-mail: ilkka.junttila@uta.fi. Or: William E. Paul, MD, 10 Center Dr, MSC 1892, Bldg 10, Rm 11N311, Bethesda, MD 20892. E-mail: wpaul@niaid.nih.gov.

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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

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% CO_2 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-1β, 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, FcεRI (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 FcγR II 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$^+$/FcεRI$^+$) 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.