

Leukocyte immunoglobulin-like receptors: novel innate receptors for human basophil activation and inhibition

David E. Sloane, Nicodemus Tedla, Muiyiwa Awoniyi, Donald W. MacGlashan Jr, Luis Borges, K. Frank Austen, and Jonathan P. Arm

Basophils, recruited from the blood to tissues, have been implicated by their presence in diverse allergic disorders including bronchial asthma, allergic rhinitis, and cutaneous contact hypersensitivity. We hypothesized that like other leukocytes involved in inflammatory responses, basophils would express members of the leukocyte immunoglobulin-like receptor (LIR) family of immunoregulatory molecules on their cell surface. We identified LIR7, an activating member coupled to the common Fc re-

ceptor gamma chain, and LIR3, an inhibitory member containing cytoplasmic immunoreceptor tyrosine-based inhibitory motifs, on these cells from human peripheral blood. Cross-linking of LIR7 resulted in the concentration-dependent net release of histamine ($29.8 \pm 10.8\%$) and cysteinyl leukotrienes (cysLTs) (31.4 ± 8.7 ng/ 10^6 basophils) that were maximal at 30 minutes, and of interleukin-4 (IL-4) (410.2 ± 61.6 pg/ 10^6 basophils) that was maximal at 4 hours and comparable with the response initiated

by cross-linking of the high-affinity receptor for immunoglobulin E (Fc ϵ RI). Co-ligation of LIR3 to LIR7 or to Fc ϵ RI by means of a second monoclonal antibody significantly inhibited net histamine release, cysLT production, and IL-4 generation. That LIR3 is profoundly counter-regulatory for both adaptive and innate receptors suggests a broad role in containment of the inflammatory response. (Blood. 2004;104:2832-2839)

© 2004 by The American Society of Hematology

Introduction

Because they resemble mast cells in their panel of mediators and cell surface expression of the high-affinity receptor for the Fc portion of immunoglobulin E (Fc ϵ RI), peripheral blood basophils have in the past often been studied as mast cell surrogates due to the relative ease with which they can be isolated. However, basophils differ importantly from mast cells in their developmental pathway and limited supply of effector molecules. Developmentally, basophils originate and mature in the bone marrow, circulating as terminally differentiated cells, while mast cells circulate as precursors and complete their development after transendothelial migration to specific tissues.¹⁻³ Indeed, basophils bear a closer developmental relationship to eosinophils than to mast cells.^{4,5} A case report documents the absence of both eosinophils and basophils in a single patient,⁶ possibly secondary to destruction or dysfunction of a shared progenitor cell. Infection of *Ws/Ws* rats, which are deficient in mast cells due to a mutation in *c-kit*, with the parasite *Nippostrongylus brasiliensis* led to a more than 50-fold increase in peripheral blood basophils, equal to that observed in wild-type rats.⁷ Basophils also appear to have a limited capacity to provide inflammatory mediators in comparison with mast cells.⁸ Those currently recognized include histamine stored in preformed granules, leukotriene (LT) C₄ generated from arachidonic acid with cell activation, and interleukin (IL) 4 induced also by activation.⁹ That basophils can provide IL-4 has led to the suggestion that these cells may reinforce T helper 2 (Th2) cell polarization.¹⁰⁻¹² Basophils are also able to express CD40 ligand on their surface and have been

shown to independently support immunoglobulin E (IgE) production by B cells in vitro.¹⁰

Basophils have been implicated in a number of human diseases including bronchial asthma, allergic rhinitis, and cutaneous contact hypersensitivity. Increased numbers of basophils, as detected by immunohistochemistry using monoclonal antibody (mAb) 2D7, are found in bronchial biopsy specimens from patients with fatal asthma.¹³ Compared with nonasthmatic controls, patients with allergic asthma had more basophils in airway biopsy tissue prior to inhaled allergen challenge, and there was a significant increase in tissue basophils 24 hours after challenge.¹⁴ In patients with allergic rhinitis, allergen challenge in the nose released histamine, cysteinyl leukotrienes, and prostaglandin (PG) D₂ in the immediate phase response, and histamine and cysteinyl leukotrienes without PGD₂ in the late phase response (LPR).¹⁵ That mast cells but not basophils generate PGD₂ suggests that basophils are the likely source of histamine and cysteinyl leukotrienes in the LPR. This is supported by the 12-fold rise in Alcian blue-positive cells in nasal lavage fluids during the LPR, approximately 70% of which are basophils by morphologic criteria.¹⁶ Histologic studies of skin lesions from patients with allergic contact dermatitis to urushiol/oleoresin, responsible for poison ivy reactions, which is not IgE-dependent, demonstrate substantial infiltration of basophils, basophil degranulation, and evidence of increased vascular permeability.¹⁷⁻¹⁹ Serial biopsies of one patient showed that basophils enter the dermis after lymphocytes but prior to the arrival of

From the Department of Medicine, Harvard Medical School, Boston, MA; the Division of Rheumatology, Immunology and Allergy, and Partners Asthma Center, Brigham and Women's Hospital, Boston, MA; Johns Hopkins Asthma and Allergy Center, Baltimore, MD; and Amgen, Seattle, WA.

Submitted January 23, 2004; accepted June 21, 2004. Prepublished online as Blood First Edition Paper, July 8, 2004; DOI 10.1182/blood-2004-01-0268.

Supported by National Institutes of Health (NIH) grants AI31599, AI 07306, and HL007718; an Arthritis Foundation postdoctoral fellowship; and a GlaxoSmithKline Allergy Fellowship Award.

One of the authors (L.B.) is employed by a company whose potential product was studied in the present work.

Reprints: Jonathan P. Arm, Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, 1 Jimmy Fund Way, Room 638B, Boston, MA 02115; e-mail: jarm@rics.bwh.harvard.edu.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

© 2004 by The American Society of Hematology

eosinophils, and account for up to 1 in 6 of the inflammatory cells. Control biopsies from the same individual after antigen-independent skin trauma with liquid nitrogen did not evoke tissue basophilia. Thus, basophils accumulate and are activated in both IgE-mediated and T-cell-dependent allergic diseases.

Given their relatively low concentrations of preformed effector molecules, their ability to release immunomodulatory cytokines, their expression of costimulatory molecules, and their prominence in different classes of allergic reactions, we decided to determine the presence and function on basophils of a novel set of receptors with both activating and inhibitory members. The leukocyte immunoglobulin-like receptors (LIRs),^{20,21} also called immunoglobulin-like transcripts (ILTs)²²⁻²⁴ and recently given the CD designation CD85a-h,²⁵ are a set of Ig superfamily proteins expressed by leukocytes. The LIRs are encoded on 2 loci separated by a region of approximately 200 kb within the leukocyte receptor complex on chromosome 19q13.4.^{26,27} Genes encoding LIR3, ILT8, LIR8, LIR2, LIR4, ILT11, and ILT7 lie in the centromeric cluster and genes encoding LIR7, LIR6, LIR1, and LIR5, and the pseudogenes ILT9 and ILT10 lie in the telomeric cluster, immediately adjacent to the genes encoding the killer cell Ig-like receptors (KIRs).²⁸ The inhibitory LIRs have cytoplasmic domains containing 2 to 4 immunoreceptor tyrosine-based inhibitory motifs (ITIMs) that recruit protein tyrosine phosphatases to inhibit cell activation signals.²⁹ The activating LIRs have truncated cytoplasmic domains and possess a charged arginine residue in their transmembrane domain through which they associate with the immunoreceptor tyrosine-based activating motif (ITAM)-containing common Fc receptor gamma chain.³⁰ We found that activation of LIR7, which like FcεRI signals through the Fc receptor gamma chain, provides a comparable profile of mediators to activation through FcεRI and that coligation of LIR3 to either FcεRI or LIR7 down-regulates the response to cross-linking of each receptor.

Materials and methods

Reagents

PIPES (piperazine-*N*, *N'*-bis[2-ethanesulfonic acid]), HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), human serum albumin (HSA), cycloheximide, and protein A sepharose were from Sigma (St Louis, MO). PIPES buffer was 25 mM PIPES, 110 mM NaCl, 5 mM KCl, pH 7.4. Percoll (Pharmacia, Uppsala, Sweden) was equilibrated 9:1 (vol/vol) with 10× PIPES buffer, and then diluted with PIPES buffer to a specific gravity of 1.0863. PAG buffer was PIPES buffer with 0.003% HSA and 0.1% dextrose (Fisher Scientific, Suwanee, GA). PAB buffer was 1× phosphate-buffered saline (PBS) with 0.05% NaN₃ and 1% bovine serum albumin (BSA). RPMI medium was RPMI 1640 (Gibco, Invitrogen, Carlsbad, CA; or Cellgro, Mediatech), 10% heat-inactivated fetal bovine serum (Sigma), 100 U/mL penicillin, 100 μg/mL streptomycin, nonessential amino acids (Cellgro; Mediatech, Herndon, VA), and 25 mM HEPES. Recombinant human IL-3 was from Pierce Endogen (Woburn, MA) or from R and D Systems (Minneapolis, MN).

Monoclonal mouse IgG₁ antibodies (mAbs) against LIRs 1, 2, 3, 5, 6, and 8 were previously described^{20,31} and are designated m401, m421, m431, m451, m467, and m481, respectively. We used 2 murine IgG₁ antibodies directed against LIR7, designated m471 and m473. Mouse IgG₁ against FcεRI, clone 15A5,³² was provided by R. Chizzonite (Hoffmann-La Roche, Nutley, NJ). Mouse IgG₁ of irrelevant specificity was from BioSource International (Camarillo, CA). Fluorescein isothiocyanate (FITC)-conjugated goat F(ab')₂ anti-mouse IgG₁ (F(ab')₂-specific) with minimal cross-reactivity to human, rat, and bovine serum; normal goat serum; goat F(ab')₂ anti-mouse IgG (heavy and light chain-specific, minimal cross-reactivity to human, rat, and horse serum); and rabbit

anti-goat IgG (F(ab')₂-specific) were from Jackson ImmunoResearch Laboratories (West Grove, PA).

Isolation and LIR epitope expression of human basophils

Basophils, but not other granulocytes, cosediment with human peripheral blood mononuclear cells (PBMCs) in density gradients. Therefore, venous blood was anticoagulated with EDTA (ethylenediaminetetraacetic acid), diluted with 0.9% NaCl, layered over Percoll, and centrifuged at 300g to separate the PBMC population. Highly purified basophils were isolated from PBMCs by negative selection with antibody-coated magnetic beads (Miltenyi Biotech, Auburn, CA). Purity of basophils was assessed by Alcian blue or Toluidine blue staining and was 92.3 ± 2.5% (n = 12).

LIR expression on purified human basophils was determined by flow cytometry using mAbs (10 μg/mL) to individual LIRs and FITC-conjugated goat F(ab')₂ anti-mouse IgG₁ (F(ab')₂-specific) as described for human eosinophils.³³ m471 was used to identify LIR7 expression. Cells were analyzed on a FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ). Results were interpreted using Cell Quest software (Becton Dickinson). A unimodal shift in fluorescence intensity of cells stained with specific antibodies compared with the isotype-matched negative control IgG₁ antibody was considered positive.

Stimulation of basophils for release of histamine, cysteinyl leukotrienes, and IL-4

For experiments in which release of histamine and LTC₄ was measured, Fcγ receptors on PBMCs or purified basophils were blocked with 25% human IgG (Miltenyi Biotech) and pretreated with IL-3 (7.5 ng/mL) in PAG buffer, at 1 × 10⁷ cells/mL on ice for 1 hour. Thereafter, portions of basophils (low or high purity, as indicated in the results) were incubated with mAbs to LIR7 (m473), to FcεRI (clone 15A5), or with control IgG₁ at 5 × 10⁵ basophils/mL in PAG containing IL-3 (10 ng/mL) for 30 minutes on ice. Cells were then washed in ice-cold PAG, centrifuged at 4°C, resuspended at 5 × 10⁵ basophils/mL in PAG containing IL-3 (10 ng/mL), 1 mM CaCl₂, 1 mM MgCl₂, and 10 μg/mL goat anti-mouse IgG that was prewarmed to 37°C, and incubated at 37°C for the indicated times. Goat anti-mouse IgG was used to cross-link anti-FcεRI or anti-LIR7 and for coligation of LIR3 to either FcεRI or LIR7 for methodologic consistency. The reaction was stopped by cooling in an ice slurry for 1 minute, followed by centrifugation at 200g at 4°C for 10 minutes. The supernatants were stored at -20°C until they were assayed for histamine and LTC₄. To determine the requirement for IL-3 priming, a parallel sample of cells was processed and stimulated as described, except that IL-3 was omitted from each step. Initial experiments were performed with m471 at 1 μg/mL. Subsequent experiments performed with m473 at 0.1 μg/mL provided similar release of histamine and LTC₄.

For experiments in which release of IL-4 was measured, purified basophils were blocked and primed under the same conditions as for histamine and LTC₄ release except that RPMI medium was used instead of PAG buffer, and cells were stimulated at a 2-fold greater density of 1 × 10⁶/mL. To determine the effects of cycloheximide on IL-4 secretion, cycloheximide (1 to 10 μg/mL) was added to the RPMI medium during blocking and all subsequent steps.

Supernatant concentrations of histamine were determined by enzyme-linked immunoassay (EIA) (Beckman Coulter, Miami, FL). In parallel with the experimental samples, a portion of 5 × 10⁴ basophils (low or high purity, depending on the experiment) was blocked with IgG, incubated on ice in PAG containing IL-3 (10 ng/mL) without a primary antibody, washed, and then lysed with 1.6% perchloric acid prewarmed to 37°C. The supernatant from this acid lysate was neutralized with 0.8 M potassium tetraborate (Sigma) and assayed to determine the total basophil histamine content. The histamine released into supernatants was expressed as a percent of this value.

Cysteinyl leukotriene (cysLT) concentrations were routinely measured by EIA (Amersham Biosciences, Piscataway, NJ). To confirm the identity and absolute concentration of LTC₄ and its active metabolites, LTD₄ and LTE₄, released from basophils, some supernatants were analyzed by reverse-phase high-pressure liquid chromatography (RP-HPLC) as previously described.^{34,35}

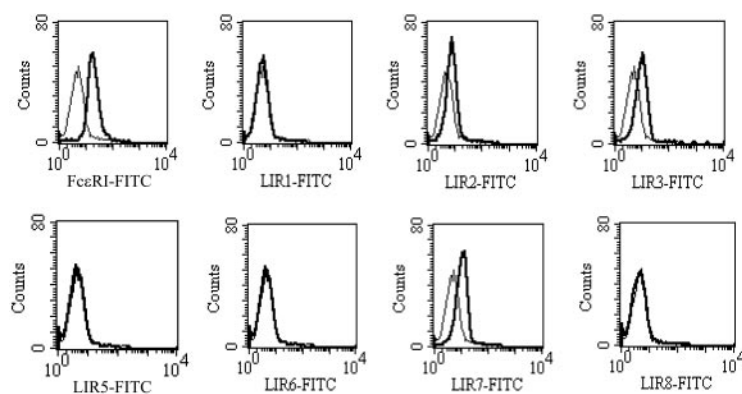


Figure 1. Cytofluorographic analysis of LIR expression on human basophils. Heavy lines depict staining with specific mAbs and light lines, their isotype controls (IgG₁). mAb directed against FcεRI was a positive control. Representative histograms from 1 of 7 donors are shown.

In the assay for IL-4, the goat anti-mouse IgG (heavy and light chain specific) used to cross-link LIR7 or FcεRI binds to both the capture and detection antibodies used in the IL-4 EIA (Beckman Coulter) leading to false high background values.³⁶ Therefore, the supernatants to be assayed for IL-4 were cleared of the goat anti-mouse IgG. Protein A sepharose beads (500 μL) were incubated for 8 to 12 hours at 4°C with 1.5 mg rabbit anti-goat IgG F(ab')₂-specific, pelleted, and resuspended in 0.02 M NaH₂PO₄, 0.15 M NaCl, pH 8.0 to make a 50% slurry. Bead slurry (40 μL) and diluent 1 (380 μL) from the IL-4 EIA kit (Immunotech) were added to each supernatant and incubated for 8 to 12 hours at 4°C with continuous mixing. Protein A sepharose beads were then pelleted by centrifugation, and IL-4 concentrations in the supernatants were measured by EIA. Separate experiments demonstrated that the goat anti-mouse IgG does not affect the histamine and cysLT EIAs.

Statistical analysis

Data are expressed as means ± SE. Statistical analyses of dose-response curves and kinetics were done by analysis of variance. Statistical comparisons between 2 sets of data were done by paired *t* tests. Differences were considered significant for *P* less than .05.

Results

Cytofluorographic analysis of LIR expression by human peripheral blood basophils

As a reference for an analysis of LIR expression on the basophil, a parallel study was conducted with monocytes (data not shown) that express each LIR for which there is a currently available mAb.^{20,33,37} Basophils from 7 of 7 donors invariably expressed a modest monophasic peak for LIR3 and LIR7, as illustrated for 1 donor (Figure 1) whose basophils also expressed minimal LIR2. Basophils from less than half the donor group of 7 individuals also expressed minimal LIR1 or LIR2. Mean fluorescence intensities for staining with control IgG₁ and mAbs to LIR-3, LIR-7, and FcεRI were 7.7 ± 1.2 , 15.4 ± 1.4 , 12.1 ± 1.5 , and 19.4 ± 1.1 , respectively (*n* = 7).

Cross-linking of LIR-7 on basophils in PBMCs or purified basophils

Because basophils are the only leukocytes in the PBMC fraction that contain histamine, the activation through LIR7 to elicit histamine release was examined using this mixed cell preparation. Basophils comprised $2.66\% \pm 0.37\%$ of PBMCs in these experiments. Cross-linking of LIR7 elicited histamine release in a concentration-dependent manner ($P < 10^{-4}$ versus IgG) with a plateau at 0.1 μg/mL (m473) (Figure 2A). The release of histamine following cross-linking of LIR7 began at the first time point studied

with gradual progression to a plateau at 30 minutes ($P < 10^{-5}$ versus IgG). These kinetics lagged the rapid histamine release observed with anti-FcεRI that occurred within 2 minutes, with an early plateau between 5 and 15 minutes (Figure 2B). The maximal net release of histamine after incubation with 0.1 μg/mL anti-LIR7 and anti-FcεRI for all 5 donors depicted in Figure 2 was $29.8 \pm 10.8\%$ and $50.1 \pm 7.6\%$, respectively.

Cross-linking of LIR7 induced cysLT generation from the PBMC population in a concentration-dependent manner ($P < .001$ versus IgG) that was near maximal at 0.1 μg/mL (Figure 3A).

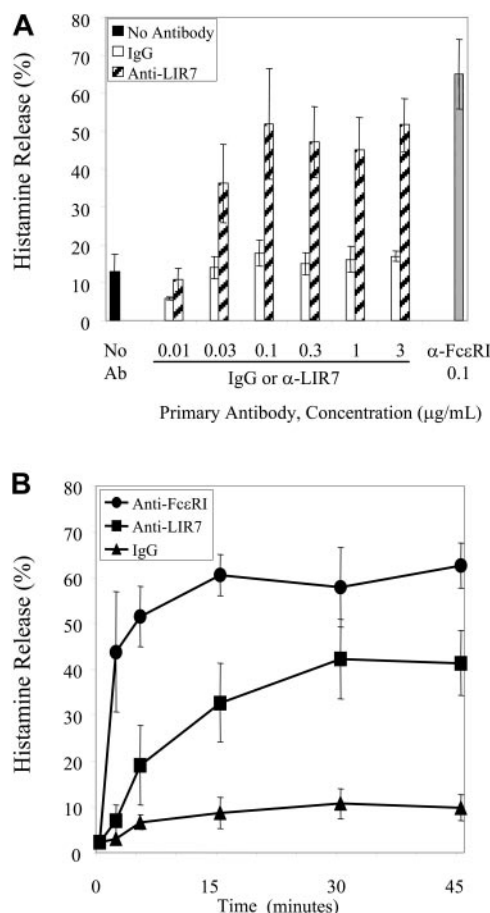


Figure 2. Concentration- and time-dependent release of histamine. (A) PBMCs were incubated with increasing concentrations of anti-LIR7 (m473) (▨), with an optimal concentration of anti-FcεRI (0.1 μg/mL) (▤), or with control IgG₁ (▥), and then stimulated by addition of goat anti-mouse IgG for 45 minutes (*n* = 3). (B) PBMCs were incubated with 0.1 μg/mL anti-FcεRI (●), anti-LIR7 (▨), or control IgG₁ (▲) and then stimulated by addition of goat anti-mouse IgG for the indicated times (*n* = 4).

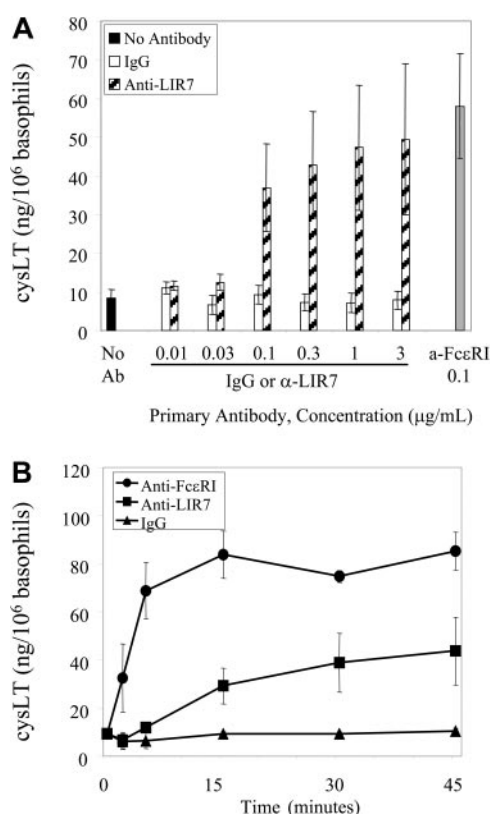


Figure 3. Concentration- and time-dependent generation of cysteinyl leukotrienes. (A) PBMCs were incubated with increasing concentrations of anti-LIR7 (m473) (▨), with an optimal concentration of anti-FcεRI (0.1 μg/mL) (▤), or with control IgG₁ (□), and then stimulated by addition of goat anti-mouse IgG for 45 minutes (n = 3). (B) PBMCs were incubated with 0.1 μg/mL anti-FcεRI (●), anti-LIR7 (■), or control IgG₁ (▲) and then stimulated by addition of goat anti-mouse IgG for the indicated times (n = 4).

Kinetic analysis revealed a definite lag for cysLT generation and release with initial detection at 5 minutes and progression to a plateau at 30 minutes ($P < .001$ versus IgG). In contrast, with anti-FcεRI, initial detection was at 2 minutes with a plateau of cysLT generation by 5 minutes (Figure 3B). The maximal net release of cysLTs from PBMCs from all donors depicted in Figure 3 after incubation with 0.1 μg/mL anti-LIR7 and anti-FcεRI was 31.4 ± 8.7 ng/10⁶ basophils (n = 6) and 52.3 ± 11.4 ng/10⁶ basophils (n = 5), respectively. As the EIA for cysLTs does not define whether the product is LTC₄, LTD₄, or LTE₄, the supernatant from activation of purified basophils with anti-LIR7 cross-linking was analyzed by RP-HPLC. In 5 separate experiments, cross-linking of LIR7 on purified human basophils (> 99% purity) with 0.1 μg/mL anti-LIR7 and goat anti-mouse IgG for 45 minutes elicited the release of 40.9 ± 12.6 ng/10⁶ basophils of only LTC₄ that was not metabolized to LTD₄ or to LTE₄. However, RP-HPLC analysis of supernatants obtained after cross-linking of LIR7 on PBMCs under identical conditions demonstrated both LTC₄ and its metabolite LTE₄ (data not shown), suggesting that nonbasophil members of the PBMC population enzymatically converted basophil-derived LTC₄.

To address the contribution of contaminating PBMCs to basophil histamine and leukotriene release, high-purity and low-purity basophils from the same donor were stimulated through LIR-7. For basophils of 98% and 5% purity the net release of histamine was 14.5% and 19.0%, respectively, and cysLT release was 5.7 and 4.6 ng/10⁶ basophils, respectively. Therefore, basophils were the major if not exclusive source of histamine and cysLTs in the PBMC

population stimulated by LIR7 cross-linking, and mediator release was due to direct stimulation of basophils through LIR-7 and not due to an indirect effect of cross-linking LIR-7 expressed on PBMCs.

Because LIR7 was expressed by mononuclear cells in the PBMC population, IL-4 release in response to stimulation through LIR7 was analyzed using high-purity basophil preparations. In concentration response experiments, the onset of IL-4 secretion over the background was observed with 0.01 μg/mL anti-LIR7 and was maximal at 0.1 to 0.3 μg/mL ($P = .004$ versus IgG). The maximal net release of IL-4 from basophils in response to stimulation through LIR7 and FcεRI was 410.2 ± 61.6 pg/10⁶ basophils (n = 3) and 246.0 ± 51.3 pg/10⁶ basophils (n = 3), respectively. Kinetic experiments showed a plateau at 4 hours (n = 3, Figure 4B). No detectable release of IL-4 was observed when LIR-7 was cross-linked on mixed PBMCs (~ 4% basophils), indicating that contaminating PBMCs are not the source of IL-4 when LIR-7 is cross-linked.

IL-3 effects

Release of mediators from basophils in response to a number of different stimuli requires pretreatment of the cells with IL-3.³⁸ We used IL-3 pretreatment routinely by convention. However, it seemed important to define whether IL-3 was necessary or just supplemental.

In 5 separate experiments, histamine release from unstimulated basophils in PBMCs was $12 \pm 3\%$ without IL-3 pretreatment, and

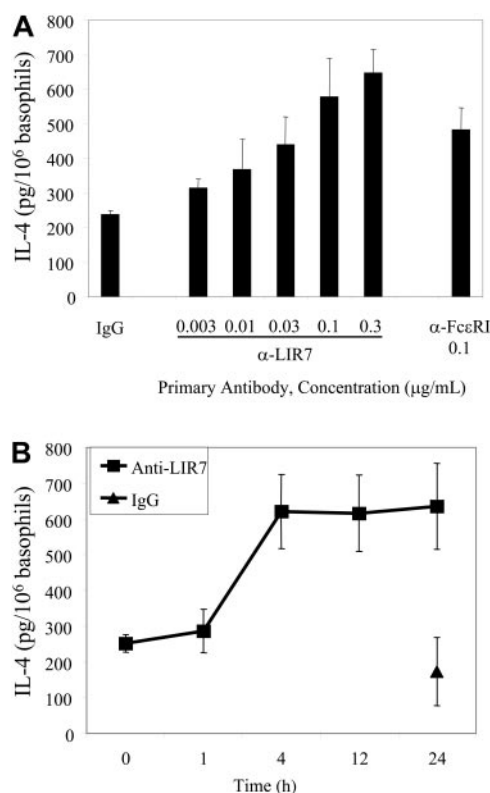


Figure 4. Concentration- and time-dependent generation of IL-4. (A) High-purity basophils were incubated with the indicated concentrations of primary mAbs and then stimulated by addition of goat anti-mouse IgG for 4 hours (n = 3). (B) High-purity basophils were incubated with 0.1 μg/mL mAb to LIR7 or control IgG₁ and stimulated by addition of goat anti-mouse IgG for 1 to 24 hours (n = 3). Supernatants were assayed for IL-4 by EIA after the goat anti-mouse IgG was cleared from the supernatants as described in "Materials and methods."

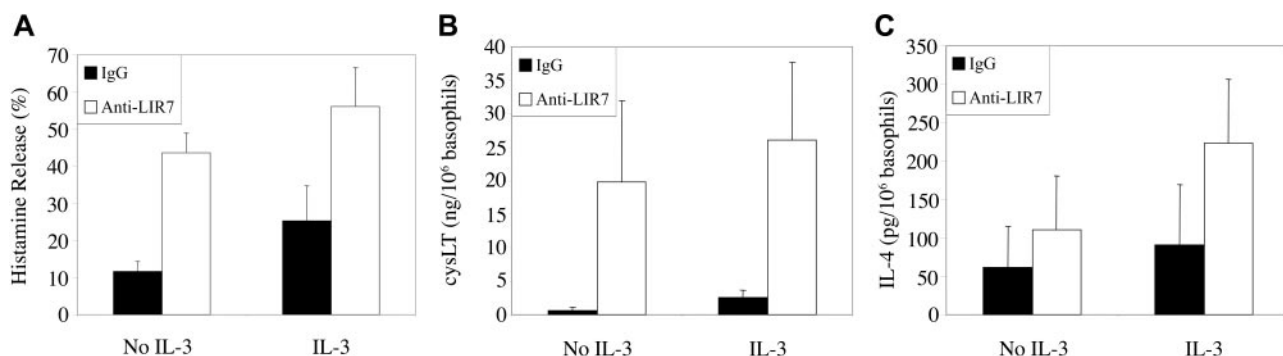


Figure 5. Histamine release, cysteinyl leukotriene generation, and IL-4 secretion after stimulation through LIR7 with and without IL-3 priming. PBMCs (A-B) or highly purified (C) basophils were either incubated with 10 ng/mL IL-3 or not, incubated with IgG₁ control antibody (■) or with mAb to LIR7 (□) and stimulated by addition of goat anti-mouse IgG for 45 minutes (A-B) or 4 hours (C). Supernatants were assayed for (A) histamine (n = 3), (B) cysteinyl leukotrienes (CysLT, n = 3), and (C) IL-4 (n = 3) by EIA.

the response to LIR7 stimulation provided a net gain of $32 \pm 7\%$ ($P < .01$; Figure 5A). IL-3 pretreatment alone increased histamine release to $25 \pm 9\%$, and the net gain with cross-linking of LIR7 was $31 \pm 7\%$ and therefore no more than additive to IL-3. For cysLT generation (Figure 5B), there was release of less than 3 ng/10⁶ unstimulated basophils in PBMCs with or without IL-3 pretreatment. Cross-linking of LIR7 elicited a net release of 19 ± 12 ng cysLT/10⁶ basophils without IL-3 (n = 7, $P = .05$), which was not significantly different from the net release of 24 ± 16 ng cysLT/10⁶ basophils with IL-3 pretreatment. In 3 separate experiments with purified basophils from different donors, the generation of IL-4 from unstimulated basophils in the absence of IL-3 was 61 ± 53 pg/10⁶ cells, and this increased to 110 ± 71 pg/10⁶ cells with cross-linking of LIR7 ($P = .06$ compared with IgG₁ control). While this just failed to reach statistical significance, in each experiment anti-LIR7 cross-linking elicited IL-4 release greater than that observed with the IgG₁-negative control. IL-3 pretreatment increased the increment in IL-4 generation (after subtraction of the value for the IgG₁ control) in response to cross-linking of LIR7 from 49 ± 19 pg/10⁶ basophils in the absence of IL-3 to 133 ± 8 pg/10⁶ basophils with IL-3 pretreatment ($P = .07$ compared with no IL-3). While this did not reach statistical significance, the net release of IL-4 was greater for each donor when basophils were pretreated with IL-3.

These findings indicate that cross-linking of LIR7, like that of FcεRI, directly elicits exocytosis, arachidonic acid metabolism, and IL-4 release without a requirement for IL-3 pretreatment.

Effect of cycloheximide on release of IL-4

In order to determine if the IL-4 released by purified basophils with and without IL-3 pretreatment following stimulation through LIR7

was preformed or newly induced, we introduced the translation inhibitor, cycloheximide (Figure 6). At 4 hours, the IL-4 released with nonspecific IgG₁, with or without IL-3 pretreatment, was unaltered by the low dose of 1 μM. The net increment in IL-4 release with LIR7 cross-linking was reduced to the baseline observed with control IgG₁, with or without IL-3 pretreatment, by 1 μM cycloheximide, indicating induction of IL-4 synthesis rather than release from preformed stores. Cycloheximide (10 μM) reduced IL-4 release observed with control IgG₁ and with cross-linking of LIR7 still further, again with no net release of IL-4 in response to LIR7 cross-linking. The findings for stimulation through FcεRI were similar (data not shown) as previously reported.^{11,12} Basophil survival at 4 hours under all conditions was more than 92% and thus the falloff in IL-4 release after cycloheximide pretreatment is not attributable to cytotoxicity. Furthermore, at the earlier time point of 45 minutes, cycloheximide treatment did not attenuate histamine release in response to cross-linking of LIR-7, $50.2 \pm 15.4\%$ and $54.6 \pm 12.6\%$ in the absence and presence of 10 μM cycloheximide, respectively (n = 3).

Effect of coligation of LIR3 to the activating receptors LIR7 and FcεRI

The ability of the ITIM-containing LIR3 to attenuate mediator release elicited by cross-linking of LIR7 or FcεRI was examined for each basophil-derived mediator. Because the window for the LIR3 counter-regulatory effect rests between baseline and the net increment over baseline, the percent inhibition must be calculated from those parameters. Data are presented for basophils stimulated in the presence of IL-3. For histamine (Figure 7A) there was no difference in baseline release when mAb to LIR3 was substituted for nonspecific IgG. The net increment with cross-linking of 0.1

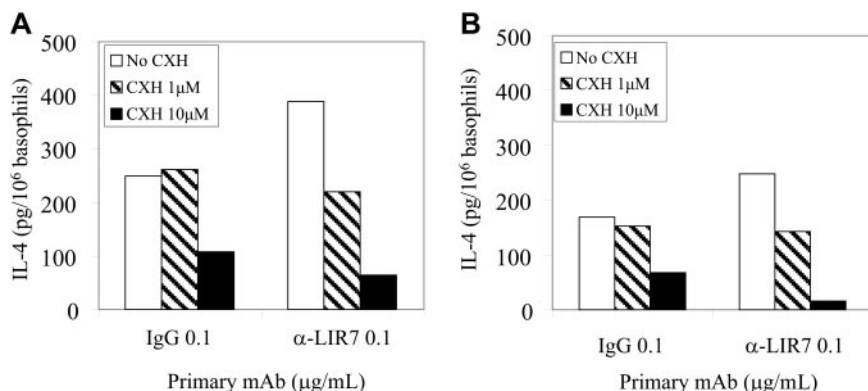


Figure 6. Effect of cycloheximide on LIR7-mediated IL-4 generation. Highly purified basophils were either incubated with IL-3 (A) or not (B), incubated with 0.1 μg/mL mAb to LIR7 or control IgG₁, and activated by addition of goat anti-mouse IgG for 4 hours in the absence (□) or presence of 1 μM (▨) or 10 μM (■) cycloheximide. Goat anti-mouse IgG was cleared from the supernatants and IL-4 was assayed by EIA. A single representative experiment of 2 is depicted. CXH indicates cycloheximide.

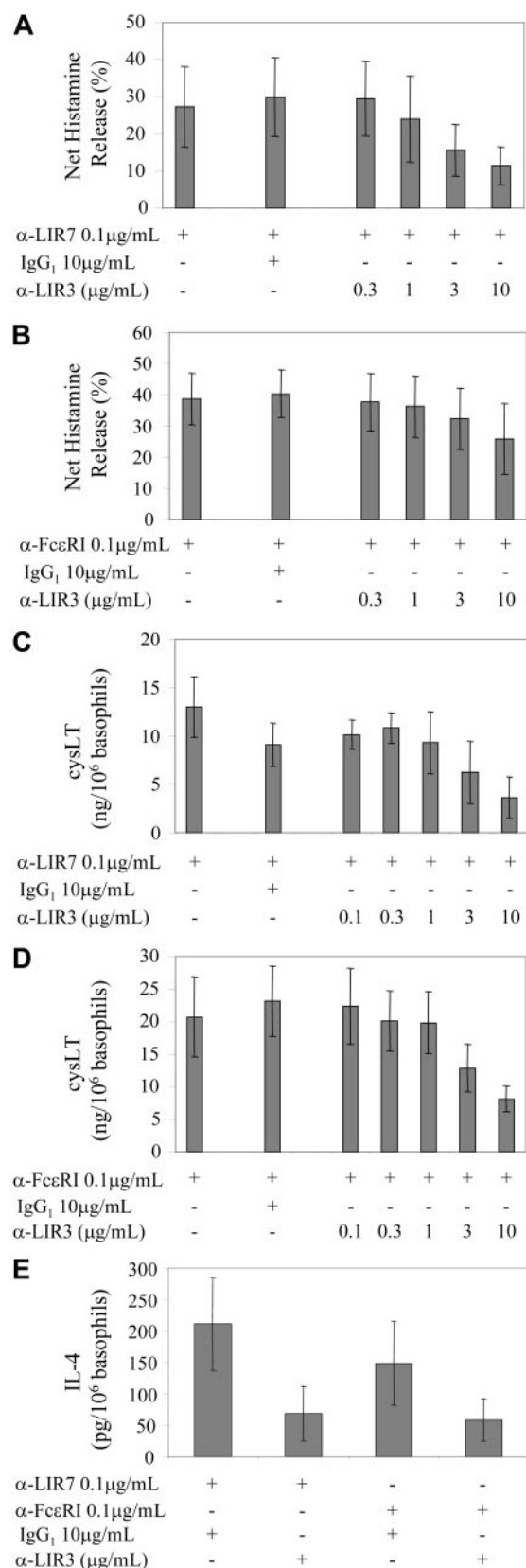


Figure 7. Effect of coligation of LIR3 with LIR7 or FcεRI on activation of human basophils. PBMCs (A-D) or purified basophils (E) were treated with IL-3 and then incubated with 0.1 μg/mL anti-LIR7 (A, C, and E), anti-FcεRI (B, D, and E), or control IgG₁ (A-E), in the presence of increasing concentrations of anti-LIR3 or 10 μg/mL IgG₁ control antibody. Basophils were then activated by the addition of 10 μg/mL goat anti-mouse IgG for 45 minutes (A-D) or 4 hours (E). Supernatants were assayed for histamine (A-B), cysteinyl leukotrienes (C-D), or IL-4 (E) by EIA.

μg/mL anti-LIR7 was $27 \pm 11\%$ of the total cellular histamine, and this was suppressed in a concentration-dependent fashion by coligation to LIR3 ($P = .005$; Figure 7A). The dose response was similar when the activating ligand was 0.1 μg/mL anti-FcεRI ($P = .017$; Figure 7B). For the 6 experiments performed with IL-3, the maximal inhibition with 10 μg/mL anti-LIR3 was $65 \pm 10\%$ and $47 \pm 14\%$ for LIR7- and FcεRI-stimulated histamine release, respectively. Control nonspecific IgG or anti-CD154 in place of anti-LIR3 was not inhibitory at concentrations up to 10 μg/mL.

For the generation of cysLTs (Figure 7C-D), the baseline with or without IL-3 pretreatment plus control IgG₁ or mAb to LIR3 was less than 5 ng/10⁶ basophils. Cross-linking of LIR7 and of FcεRI provided a net release of 13 ± 3 and 21 ± 6 ng cysLT/10⁶ basophils, respectively. Coligation to LIR3 suppressed generation in response to cross-linking of LIR7 and of FcεRI in a dose-related manner ($P = .005$ and $P = .0001$, respectively). For the experiments performed with IL-3, the maximal inhibition with 10 μg/mL anti-LIR3 was $76 \pm 9\%$ ($n = 4$) for LIR7 and $58 \pm 6\%$ ($n = 5$) for FcεRI. Control nonspecific IgG and anti-CD154 in place of anti-LIR3 were not inhibitory at concentrations up to 10 μg/mL.

Because of the difficulty in obtaining large numbers of purified basophils, experiments in which LIR3 was coligated to either LIR7 or FcεRI to inhibit release of IL-4 were performed using only the highest concentration of anti-LIR3 (10.0 μg/mL). Nonspecific IgG (10 μg/mL) provided a negative control for the mAb to LIR3. Coligation of LIR3 with LIR7 or with FcεRI provided inhibition in each of 3 separate experiments. Inhibition of LIR7-induced net IL-4 release by coligation to LIR3 was $73 \pm 8\%$ ($n = 3$, $P = .05$), while inhibition of FcεRI-induced net IL-4 release was $66 \pm 16\%$ ($n = 3$, $P = .07$) (Figure 7E).

Discussion

Using specific monoclonal antibodies we have demonstrated that human basophils consistently express LIR3 and LIR7, with additional expression of LIR1 and/or LIR2 on basophils of some donors (Figure 1). This pattern of LIR expression is identical to that on human eosinophils and neutrophils³³ and is distinct from the profile on cells of monocytic origin that express all of the LIRs examined to date,^{20,33,37} with the exception that plasmacytoid dendritic cells lack LIR7 expression.³⁹ These data suggest that the restricted expression of LIR3 and LIR7, with or without LIR1 or LIR2, is a pattern shared by myeloid cells of granulocytic lineage and are consistent with developmental regulation of LIR expression.

To examine the function of LIRs expressed on basophils, we cross-linked LIR7 using specific murine monoclonal antibodies followed by a goat anti-mouse IgG. Cross-linking of LIR7 elicited release of mediators from 3 compartments: preformed histamine from secretory granules, de novo synthesis of LTC₄ from arachidonic acid, and IL-4 (Figures 2-4). The time course of IL-4 generation and its inhibition by cycloheximide (Figure 6) indicate that IL-4 was generated de novo by gene induction. The only other "complete" stimulus identified thus far, releasing all 3 compartments of mediators, is activation through the high-affinity IgE receptor.^{11,38,40-45} A 21-kDa histamine-releasing factor, whose action appears to be IgE-dependent, is also a complete stimulus for basophil mediator release, but it is active only on basophils from approximately 50% of individuals.⁴⁶⁻⁴⁸ Our study therefore not only identifies LIR7 as a novel signaling receptor on human basophils, but also identifies activation through LIR7 as the only presently identified IgE-independent complete stimulus for human

basophils. For most adults with asthma and for many individuals with persistent asthmatic symptoms, the role of allergens and of IgE-dependent signaling is either partial or, in nonatopic asthma, nonexistent.⁴⁹ The identification of LIR7 as an IgE-independent activating receptor for basophils suggests that it could respond to a stimulus for nonatopic, Th2-driven airway inflammation. The possibility that LIR7 could contribute to the Th2 phenotype of bronchial asthma is suggested by the recent report that LIR7 is overexpressed in skin lesions of lepromatous leprosy, in which the Th2 response is dominant, compared with tuberculoid leprosy, in which the Th1 response is dominant.⁵⁰

Based entirely on *in vitro* studies, basophils respond to some stimuli *de novo* with release of only some of their potential mediators unless treated with IL-3 or are entirely dependent on IL-3 cotreatment.³⁸ Thus, C5a⁴³ and the chemokine monocyte chemoattractant protein 1 (MCP-1)^{51,52} elicit basophil histamine release without exposure to IL-3, but require IL-3 to elicit LTC₄ biosynthesis and modest IL-4 generation. While formyl-methionyl-leucyl-phenylalanine (fMLP) elicits histamine release and LTC₄ biosynthesis from basophils without exposure to IL-3, it requires cytokines to effect IL-4 generation.^{53,54} C3a, platelet-activating factor (PAF), and chemokines other than MCP-1 that act through diverse chemokine receptors including CCR3, CCR1, and CXCR1 elicit modest histamine release and LTC₄ generation only after IL-3 treatment.³⁸ Although IL-3 at a concentration of 10 ng/mL has been characterized as a basophil priming agent for diverse stimuli,³⁸ we found that IL-3 alone induced release and that costimulation of basophils by cross-linking LIR7 in the presence of IL-3 augmented mediator release from all 3 compartments, which was additive for histamine and cysLTs and possibly synergistic for IL-4 (Figure 5). Importantly, stimulation through LIR7, as through FcεRI, can effect basophil mediator release from all 3 compartments without IL-3.

Basophils express not only the activating LIR7, but also the inhibitory LIR3. Indeed coligation of LIR3 with LIR7 or with FcεRI substantially inhibited release of histamine, LTC₄, and IL-4 (Figure 7). LIR3 is therefore able to inhibit the release of mediators from all 3 basophil compartments, antagonizing both IgE-dependent and IgE-independent activation. The balance between

activating and inhibitory signals delivered by several families of receptors is critical for the regulated function of leukocytes in adaptive and innate immune responses.⁵⁵⁻⁵⁷ The functions of individual inhibitory receptors will depend in part upon their ligand(s) and sites of expression. Thus, the low-affinity inhibitory Fc receptor for IgG, FcγRIIB, terminates signaling initiated by antigen engagement of the B-cell receptor, contributing to peripheral B-cell tolerance and protection from autoimmune disease.⁵⁸⁻⁶⁰ FcγRIIB also potently down-regulates FcγRIII- and FcεRI-dependent responses in mouse mast cells⁶¹ and FcεRI-dependent responses in human basophils.⁶² This capacity for FcγRIIB to down-regulate IgE-dependent responses has been exploited through the development of a bispecific dimer of Fcε and Fcγ that down-regulated IgE-dependent basophil histamine release.⁶³ Administration of this dimeric molecule to mice transgenic for the human FcεRI alpha chain inhibited passive cutaneous anaphylactic responses.⁶³ The capacity of FcγRIIB to temper mast cell and basophil activation appears confined to counter-regulation of immunoglobulin-dependent adaptive immune responses elicited through FcγRIII and FcεRI. The possibility that LIR3 regulates both adaptive and innate immune responses is suggested by studies of mice with targeted disruption of the homologous mouse gp49B gene. Gp49B-null mice have exaggerated cutaneous mast cell responses to activation by IgE and hapten-specific antigen⁶⁴ and to stem cell factor.⁶⁵ Importantly, constitutive counter-regulation of innate immune responses by gp49B extends to the neutrophil, as the strain lacking gp49B is uniquely susceptible to the hemorrhagic Schwartzman-like microangiopathy mediated by intradermal lipopolysaccharide.⁶⁶ Further elucidation of the immunomodulatory role of LIR3 requires identification of its ligand(s), and any appreciation of an *in vivo* role would depend upon recognition of a deletion in a clinical setting.

Acknowledgments

The authors wish to gratefully acknowledge Bing Lam, PhD, for assistance with RP-HPLC analysis and Erin Rhode and Thomas Arm for excellent technical assistance.

References

1. Gurish MF, Tao H, Abonia JP, et al. Intestinal mast cell progenitors require CD49dβ7 (α4β7 integrin) for tissue-specific homing. *J Exp Med*. 2001;194:1243-1252.
2. Galli SJ. New concepts about the mast cell. *N Engl J Med*. 1993;328:257-265.
3. Denburg JA. Basophil and mast cell lineages *in vitro* and *in vivo*. *Blood*. 1992;79:846-860.
4. Denburg JA, Telizyn S, Messner H, et al. Heterogeneity of human peripheral blood eosinophil-type colonies: evidence for a common basophil-eosinophil progenitor. *Blood*. 1985;66:312-318.
5. Boyce JA, Friend D, Matsumoto R, Austen KF, Owen WF. Differentiation *in vitro* of hybrid eosinophil/basophil granulocytes: autocrine function of an eosinophil developmental intermediate. *J Exp Med*. 1995;182:49-57.
6. Juhlin L, Michaelsson G. A new syndrome characterized by absence of eosinophils and basophils. *Lancet*. 1977;1:1233-1235.
7. Kasugai T, Okada M, Morimoto M, et al. Infection of *Nippostrongylus brasiliensis* induces normal increase of basophils in mast cell-deficient Ws/Ws rats with a small deletion at the kinase domain of c-kit. *Blood*. 1993;81:2521-2529.
8. Schroeder JT. Regulation of the production and activation of basophils. In: Freedberg IM, Eisen AZ, Wolff K, Austen KF, Goldsmith LA, Katz SL, eds. *Dermatology in General Medicine*. New York: McGraw-Hill; 2003:324-329.
9. Galli SJ. Mast cells and basophils. *Curr Opin Hematol*. 2000;7:32-39.
10. Gauchat JF, Henchoz S, Mazzei G, et al. Induction of human IgE synthesis in B cells by mast cells and basophils. *Nature*. 1993;365:340-343.
11. MacGlashan DJ, White JM, Huang SK, et al. Secretion of IL-4 from human basophils: the relationship between IL-4 mRNA and protein in resting and stimulated basophils. *J Immunol*. 1994;152:3006-3016.
12. Brunner T, Heusser CH, Dahinden CA. Human peripheral blood basophils primed by interleukin 3 (IL-3) produce IL-4 in response to immunoglobulin E receptor stimulation. *J Exp Med*. 1993;177:605-611.
13. Kopley CL, McFeeley PJ, Oliver JM, Lipscomb MF. Immunohistochemical detection of human basophils in postmortem cases of fatal asthma. *Am J Respir Crit Care Med*. 2001;164:1053-1058.
14. Nouri-Aria KT, Irani A-M, Jacobson MR, et al. Basophil recruitment and IL-4 production during human allergen-induced late asthma. *J Allergy Clin Immunol*. 2001;108:205-211.
15. Naclerio RM, Proud D, Togias AG, et al. Inflammatory mediators in late antigen-induced rhinitis. *N Engl J Med*. 1985;313:65-70.
16. Bascom R, Wachs M, Naclerio RM, et al. Basophil influx occurs after nasal antigen challenge: effects of topical corticosteroid pretreatment. *J Allergy Clin Immunol*. 1988;81:580-589.
17. Dvorak AM, Mihm MC Jr, Dvorak HF. Degranulation of basophilic leukocytes in allergic contact dermatitis reactions in man. *J Immunol*. 1976;116:687-695.
18. Dvorak HF, Mihm MC Jr. Basophilic leukocytes in allergic contact dermatitis. *J Exp Med*. 1972;135:235-254.
19. Dvorak HF, Mihm MC Jr. Basophilic leukocytes in a case of poison ivy. *N Engl J Med*. 1971;285:54-55.
20. Borges L, Hsu ML, Fanger N, Kubin M, Cosman D. A family of human lymphoid and myeloid Ig-like receptors, some of which bind to MHC class I molecules. *J Immunol*. 1997;159:5192-5196.
21. Cosman D, Fanger N, Borges L, et al. A novel immunoglobulin superfamily receptor for cellular and viral MHC class I molecules. *Immunity*. 1997;7:273-282.
22. Cella M, Dohring C, Samaridis J, et al. A novel

- inhibitory receptor (ILT3) expressed on monocytes, macrophages, and dendritic cells involved in antigen processing. *J Exp Med*. 1997;185:1743-1751.
23. Colonna M, Navarro F, Bellón T, et al. A common inhibitory receptor for major histocompatibility complex class I molecules on human lymphoid and myelomonocytic cells. *J Exp Med*. 1997;186:1809-1818.
 24. Samaridis J, Colonna M. Cloning of novel immunoglobulin superfamily receptors expressed on human myeloid and lymphoid cells: structural evidence for new stimulatory and inhibitory pathways. *Eur J Immunol*. 1997;27:660-665.
 25. Banham AH, Colonna M, Cella M, et al. Identification of the CD85 antigen as ILT2, an inhibitory MHC class I receptor of the immunoglobulin superfamily. *J Leukocyte Biol*. 1999;65:841-845.
 26. Wilson MJ, Torkar M, Haude A, et al. Plasticity in the organization and sequences of human KIR/ILT gene families. *Proc Natl Acad Sci U S A*. 2000;97:4778-4783.
 27. Liu RW, Kim J, Nwankwo C, Ashworth LK, Arm JP. Genomic organization of the human leukocyte immunoglobulin-like receptors within the leukocyte receptor complex on chromosome 19q13.4. *Immunogenetics*. 2000;51:659-669.
 28. Trowsdale J, Barten R, Haude A, et al. The genomic context of natural killer receptor extended gene families. *Immunol Rev*. 2001;181:20-38.
 29. Saverino D, Fabbri M, Ghiotto F, et al. The CD85/LIR-1/ILT2 inhibitory receptor is expressed by all human T lymphocytes and down-regulates their functions. *J Immunol*. 2000;165:3742-3755.
 30. Nakajima H, Samaridis J, Angman L, Colonna M. Human myeloid cells express an activating ILT receptor (ILT1) that associates with Fc receptor gamma-chain. *J Immunol*. 1999;162:5-8.
 31. Tedla N, Gibson K, McNeil HP, et al. The co-expression of activating and inhibitory leukocyte immunoglobulin-like receptors in rheumatoid synovium. *Am J Pathol*. 2002;160:425-431.
 32. Riske F, Hakimi J, Mallamaci M, et al. High affinity human IgE receptor (FcεRI): analysis of functional domains of the alpha-subunit with monoclonal antibodies. *J Biol Chem*. 1991;266:11245-11251.
 33. Tedla N, Bandeira-Melo C, Tassinari P, et al. Activation of human eosinophils through leukocyte immunoglobulin-like receptor 7. *Proc Natl Acad Sci U S A*. 2003;100:1174-1179.
 34. Lam BK, Gagnon L, Austen KF, Soberman RJ. The mechanism of leukotriene B₄ export from human polymorphonuclear leukocytes. *J Biol Chem*. 1990;265:13438-13441.
 35. Sanchez Mejia RO, Lam BK, Arm JP. Matrix-associated TGF-β1 primes mouse bone marrow-derived mast cells for increased FcεRI-dependent eicosanoid biosynthesis. *Am J Respir Cell Mol Biol*. 2000;22:557-565.
 36. Okayama Y, Hagaman DD, Metcalfe DD. A comparison of mediators released or generated by IFN-γ-treated human mast cells following aggregation of FcγRI or FcεRI. *J Immunol*. 2001;166:4705-4712.
 37. Colonna M, Nakajima H, Cella M. A family of inhibitory and activating Ig-like receptors that modulate function of lymphoid and myeloid cells. *Semin Immunol*. 2000;12:121-127.
 38. Ochensberger B, Tassera L, Biffrare D, Rihs S, Dahinden CA. Regulation of cytokine expression and leukotriene formation in human basophils by growth factors, chemokines and chemotactic agonists. *Eur J Immunol*. 1999;29:11-22.
 39. Cella M, Jarrossay D, Facchetti F, et al. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat Med*. 1999;6:919-923.
 40. MacGlashan DW Jr, Peters SP, Warner J, Lichtenstein LM. Characteristics of human basophil sulfidopeptide leukotriene release: releasability defined as the ability of the basophil to respond to dimeric cross-links. *J Immunol*. 1986;136:2231-2239.
 41. Miura K, Saini SS, Gauvreau G, MacGlashan DWJ. Differences in functional consequences and signal transduction induced by IL-3, IL-5, and nerve growth factor in human basophils. *J Immunol*. 2001;167:2282-2291.
 42. Burgi B, Otten UH, Ochensberger B, et al. Basophil priming by neurotrophic factors: activation through the trk receptor. *J Immunol*. 1996;157:5582-5588.
 43. Ochensberger B, Rihs S, Brunner T, Dahinden CA. IgE-independent interleukin-4 expression and induction of a late phase of leukotriene C₄ formation in human blood basophils. *Blood*. 1995;86:4039-4049.
 44. Takafuji S, Bischoff SC, De Weck AL, Dahinden CA. IL-3 and IL-5 prime normal human eosinophils to produce leukotriene C₄ in response to soluble agonists. *J Immunol*. 1991;147:3855-3861.
 45. Weber M, Uguccioni M, Ochensberger B, et al. Monocyte chemotactic protein MCP-2 activates human basophil and eosinophil leukocytes similar to MCP-3. *J Immunol*. 1995;154:4166-4172.
 46. MacDonald SM, Lichtenstein LM, Proud D, et al. Studies of IgE-dependent histamine releasing factors: heterogeneity of IgE. *J Immunol*. 1987;139:506-512.
 47. MacDonald SM, Rafnar T, Langdon J, Lichtenstein LM. Molecular identification of an IgE-dependent histamine-releasing factor. *Science*. 1995;269:688-690.
 48. Schroeder JT, Lichtenstein LM, MacDonald SM. An immunoglobulin E-dependent recombinant histamine-releasing factor induces interleukin-4 secretion from human basophils. *J Exp Med*. 1996;183:1265-1270.
 49. Pearce N, Pekkanen J, Beasley R. How much asthma is really attributable to atopy? *Thorax*. 1999;54:268-272.
 50. Bleharski JR, Li H, Meinken C, et al. Use of genetic profiling in leprosy to discriminate clinical forms of the disease. *Science*. 2003;301:1527-1530.
 51. Bischoff SC, Dahinden CA. c-kit ligand: a unique potentiator of mediator release by human lung mast cells. *J Exp Med*. 1992;175:237-244.
 52. Alam R, Lett-Brown MA, Forsythe PA, et al. Monocyte chemotactic and activating factor is a potent histamine-releasing factor for basophils. *J Clin Invest*. 1992;89:723-728.
 53. Dahinden CA, Kurimoto J, Baggiolini M, Dewald B, Walz A. Histamine and sulfidoleukotriene release from human basophils: different effects of antigen, anti-IgE, C5a, f-Met-Leu-Phe and the novel neutrophil-activating peptide NAF. *Int Arch Allergy Appl Immunol*. 1989;90:113-118.
 54. Warner JA, MacGlashan DW Jr. Signal transduction events in human basophils: a comparative study of the role of protein kinase C in basophils activated by anti-IgE antibody and formyl-methionyl-leucyl-phenylalanine. *J Immunol*. 1990;145:1897-1905.
 55. Ravetch JV, Lanier LL. Immune inhibitory receptors. *Science*. 2000;290:84-89.
 56. Kubagawa H, Chen CC, Le Hong H, et al. Biochemical nature and cellular distribution of the paired immunoglobulin-like receptors, PIR-A and PIR-B. *J Exp Med*. 1999;189:309-318.
 57. Yokoyama WM. What goes up must come down: the emerging spectrum of inhibitory receptors. *J Exp Med*. 1997;186:1803-1808.
 58. Ravetch JV, Bolland S. IgG Fc receptors. *Annu Rev Immunol*. 2001;19:275-290.
 59. Muhlfeld AS, Segerer S, Hudkins K, et al. Deletion of the Fcγ receptor IIb in thymic stromal lymphopoietin transgenic mice aggravates membranoproliferative glomerulonephritis. *Am J Pathol*. 2003;163:1127-1136.
 60. Nakamura A, Yuasa T, Ujike A, et al. Fcγ receptor IIb-deficient mice develop Goodpasture's syndrome upon immunization with type IV collagen: a novel murine model for autoimmune glomerular basement membrane disease. *J Exp Med*. 2000;191:899-906.
 61. Ujike A, Ishikawa Y, Ono M, et al. Modulation of immunoglobulin (Ig)E-mediated systemic anaphylaxis by low-affinity Fc receptors for IgG. *J Exp Med*. 1999;189:1573-1579.
 62. Kopley CL, Cambier JC, Morel PA, et al. Negative regulation of FcεpsilonRI signaling by FcγRII costimulation in human blood basophils. *J Allergy Clin Immunol*. 2000;106:337-348.
 63. Zhu D, Kopley CL, Zhang M, Zhang K, Saxon A. A novel human immunoglobulin Fc gamma Fc epsilon bifunctional fusion protein inhibits Fc epsilon RI-mediated degranulation. *Nat Med*. 2002;8:518-521.
 64. Daheshia M, Friend DS, Grusby MJ, Austen KF, Katz HR. Increased severity of local and systemic anaphylactic reactions in gp49B1-deficient mice. *J Exp Med*. 2001;194:227-234.
 65. Feldweg AM, Friend DS, Zhou JS, et al. gp49B1 suppresses stem cell factor-induced mast cell activation-secretion and attendant inflammation in vivo. *Eur J Immunol*. 2003;33:2262-2268.
 66. Zhou JS, Friend DS, Feldweg AM, et al. Prevention of lipopolysaccharide-induced microangiopathy by gp49B1: evidence for an important role for gp49B1 expression on neutrophils. *J Exp Med*. 2003;198:1243-1251.