The Journal of Immunology

RESEARCH ARTICLE | MARCH 01 2008

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Sarah Kennedy Norton; ... et. al

J Immunol (2008) 180 (5): 2848–2854.
https://doi.org/10.4049/jimmunol.180.5.2848

IL-10 Suppresses Mast Cell IgE Receptor Expression and Signaling In Vitro and In Vivo[1]

Sarah Kennedy Norton,* Brian Barnstein,* Jennifer Brenzovich,* Daniel P. Bailey,* Mohit Kashyap,* Kelly Speiran,* Jill Ford,† Daniel Conrad,† Stephanie Watowich,‡ Matthew R. Moralle,§ Christopher L. Kepley,§ Peter J. Murray,¶ and John J. Ryan[2]*

Mast cells are known for their roles in allergy, asthma, systemic anaphylaxis, and inflammatory disease. IL-10 can regulate inflammatory responses and may serve as a natural regulator of mast cell function. We examined the effects of IL-10 on in vitro-cultured mouse and human mast cells, and evaluated the effects of IL-10 on FcεRI in vivo using mouse models. IgE receptor signaling events were also assessed in the presence or absence of IL-10. IL-10 inhibited mouse mast cell FcεRI expression in vitro through a Stat3-dependent process. This down-regulation was consistent in mice tested in vivo, and also on cultured human mast cells. IL-10 diminished expression of the signaling molecules Syk, Fyn, Akt, and Stat5, which could explain its ability to inhibit IgE-mediated activation. Studies of passive systemic anaphylaxis in IL-10-transgenic mice showed that IL-10 overexpression reduced the IgE-mediated anaphylactic response. These data suggest an important regulatory role for IL-10 in dampening mast cell FcεRI expression and function. IL-10 may hence serve as a mediator of mast cell homeostasis, preventing excessive activation and the development of chronic inflammation. *The Journal of Immunology,* **2008, 180: 2848–2854.**

Mast cells are important in protection against parasitic and bacterial infections (1), and have recently been shown to play a role in resistance to snake and honeybee venom (2). However, when inappropriately activated, mast cells have the ability to induce allergy, asthma, and systemic anaphylaxis (1). A role for these cells is also suspected in inflammatory diseases such as multiple sclerosis, arthritis, cardiovascular disease, and colitis (3–7). Thus, mechanisms that regulate mast cell function are of increasing clinical importance.

In allergic disease, mast cells are most commonly activated by the cell surface high-affinity IgE receptor, FcεRI. FcεRI is expressed on mast cells as a tetramer, consisting of an α-chain that binds IgE, a β-chain that amplifies receptor signaling (8), and two γ-chains that are responsible for signal initiation (9). FcεRI aggregation by multivalent Ag leads to degranulation, production of arachidonic acid metabolites, and cytokine secretion, which collectively elicit inflammation. Recently, the signaling cascades linking IgE-Ag interactions with cellular activation have been viewed as two major branches. The best characterized is activation of the tyrosine kinases Lyn and Syk, leading to phosphorylation of the adapter protein linker for activation of T cells. This pathway principally leads to activation of the Ras-MAPK and phospholipase Cγ-protein kinase C cascades. A second branch of the FcεRI-signaling cascade is triggered by Fyn phosphorylation, which activates the PI3K pathway (9, 10). In addition to these major cascades, we recently found that activation of the transcription factor STAT5 is necessary for normal IgE signaling (11). Although these pathways have some overlapping functions, loss of a single component (e.g., Syk, Fyn, STAT5) can have potent deleterious effects on mast cell activation. Hence, these signaling proteins are a potential means by which mast cell function may be regulated.

Among the possible natural regulators of FcεRI signaling is IL-10, which belongs to a family of related cytokines that includes IL-19, IL-20, IL-22, IL-24, and IL-26 (12). IL-10 can be produced by cells involved in the allergic response, including T cells and mast cells. Its well-documented ability to inhibit macrophage activation and Ag-presenting function suggested an important role in regulating the immune response. This was clearly confirmed by the phenotype of IL-10-deficient mice, which develop severe autoimmune disease (13). IL-10 has been shown to regulate inflammatory responses to pathogen infection (14, 15), to protect from endotoxic shock (16, 17), and to control both acute and chronic inflammatory responses (18). Clinically, IL-10 inhibits progression of rheumatoid arthritis when delivered directly to the site of inflammation (19). As such, IL-10 and its methods of suppression have become increasingly important areas of study, with the hope of eventually developing a more effective inflammatory therapeutic (20).

We and others have shown that IL-10 can inhibit FcεRI expression and IgE-mediated cytokine production in murine mast cells (21–25). In the present study, we have examined the mechanisms by which IL-10 inhibits mast cell activation, and extend our observations to include primary human mast cells and an in vivo mouse model of anaphylactic shock. Our findings indicate that IL-10 suppresses FcεRI expression and function both in vitro and in vivo and suggest that this cytokine may be a natural regulator of the mast cell response.

*Department of Biology and †Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA 23284; ‡Department of Immunology, M. D. Anderson Cancer Center, Houston, TX 77030; §Department of Internal Medicine, Virginia Commonwealth University, Richmond, VA 23284; and ¶Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN 38105

Received for publication August 6, 2007. Accepted for publication December 17, 2007.

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[1] This work was supported by grants from the National Institutes of Health (1R01 AI59638) and The Jeffress Trust Foundation (J-833) (to J.J.R.).

[2] Address correspondence and reprint requests to Dr. John J. Ryan, Biology Department, Virginia Commonwealth University, Box 842012, Richmond, VA 23284-2012. E-mail address: jjryan@vcu.edu

Materials and Methods

Animals

C57BL/6 mice were purchased from The Jackson Laboratory. CD68-IL-10 transgenic (Tg)[3] mice have been described previously (26). Passive systemic anaphylaxis (PSA) was performed using unanesthetized mice at 12 wk of age or older, with approval from the university animal care and use committee. STAT3 fl and Tie2-Cre mice were provided by Drs. Takeda (Osaka University, Osaka, Japan) and Koni (Yale University, New Haven, CT) (27, 28) and were bred to yield fl/Δ Cre^+ and Cre^- littermates as described (29).

Bone marrow-derived mast cell (BMMC) cultures

BMMC were derived from C57BL/6 and C57BL/6 × 129 mice by culture in complete RPMI (cRPMI) 1640 medium (Invitrogen Life Technologies) (containing 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg streptomycin, 1 mM sodium pyruvate, and 1 mM HEPES; Biofluids), supplemented with 30% WEHI-3 cell-conditioned medium as described previously (30).

Tissue-culture conditions for human mast cells

All study protocols involving human tissues were approved by the Human Studies Internal Review Board at Virginia Commonwealth University (Richmond, VA). Surgical skin samples were obtained from Virginia Commonwealth University Medical Center, the Cooperative Human Tissue Network of the National Cancer Institute, or the National Disease Research Interchange. After removing s.c. fat by blunt dissection, residual tissue was cut into 1- to 2-mm fragments and digested with type 2 collagenase (1.5 mg/ml), hyaluronidase (0.7 mg/ml), and type 1 DNase (0.3 mg/ml) in HBSS for 2 h at 37°C. The dispersed cells were collected by filtering through a no. 80 mesh sieve and resuspended in HBSS containing 1% FCS and 10 mM HEPES. Cells were resuspended in HBSS, layered over a Percoll cushion, and centrifuged at 700 × *g* at room temperature for 20 min. Nucleated cells were collected from the buffer/Percoll interface, while erythrocytes sediment to the bottom of the tube. Cells enriched by Percoll density-dependent sedimentation were resuspended at a concentration of 1×10^6 cells/ml in serum-free AIM-V medium (Invitrogen Life Technologies) containing 100 ng/ml recombinant human stem cell factor (SCF; a gift from Amgen). Skin mast cells were split into separate wells every 4–5 days. Total cell numbers and viabilities were assessed by trypan blue staining. Cultures of skin-derived mast cells were maintained for up to 3 mo and were ~100% mast cells. Alternatively, freshly dispersed, Percoll-enriched mast cells were labeled with anti-FcεRI-α and anti-CD117 mAbs (5 μg/ml), and then with FITC-labeled anti-mouse $F(ab')_2$ at 4°C. Labeled cells were purified to ≥95% by sorting in a MoFlo high-performance cell sorter (Cytomation). Lung mast cells were cultured in similar conditions, and were used in some experiments.

Cytokines and reagents

DNP-specific mouse IgE was purified as described previously (31). Murine IL-3 and IL-10 were purchased from R&D Systems. Murine SCF was purchased from PeproTech. Cell Permeable STAT3 inhibitor peptide (STAT3i) was purchased from Calbiochem. Akt, p-Akt, STAT5, p-STAT5, Lyn, Syk Abs were purchased from Cell Signaling. Phospho-p38 was purchased from New England Biolabs. Anti-β-actin was purchased from Sigma-Aldrich. Rat anti-mouse FcγRII/RIII (2.4G2), purified mouse IgE, purified anti-mouse IgE, FITC-conjugated rat IgG isotype control, and FITC-conjugated anti-mouse CD117 (c-*kit*) were purchased from BD Pharmingen. PE-conjugated rat IgG2b isotype control and PE-conjugated anti-mouse IgE were purchased from eBioscience.

Tissue-culture conditions for inhibition of BMMC FcεRI expression

BMMC were washed to remove WEHI-3 cell-conditioned medium, and incubated at 37°C for 4–6 h in cRPMI without cytokines. Cells were then plated at 3×10^5 cells/ml, 200 μl/well in 96-well flat-bottom plates. IL-3 was added to 5 ng/ml, followed by IL-10 and/or STAT3i. We have previously shown that IL-10 at concentrations of 1–10 ng/ml suppresses mouse mast cell FcεRI expression (25). IL-10 was used at 10 ng/ml. STAT3i was added daily at 5 μM final concentration. Cultures were incubated for the indicated times. Every 4 days, half of the medium and cytokines were replaced. FcεRI expression was observed by flow cytometry. Human mast cells were cultured using the same protocol, with the exception of using Aim V-based medium.

Flow cytometric analysis

To detect FcεRI expression on BMMC, cell pellets were incubated with 2.4G2 rat anti-mouse FcγRII/III culture supernatant for 10 min at 4°C, followed by 10 μg/ml IgE for 45 min at 4°C in PBS/3% FCS/0.1% sodium azide (FACS buffer). Cells were then washed twice and stained with secondary Abs for 30 min at 4°C, washed twice, and analyzed with a FACScan (BD Biosciences). Control samples were stained with PE-IgG in place of anti-IgE.

Western blot analysis

Western blotting was performed using 50 μg of total cellular protein per sample. Protein was loaded and separated over a 10% polyacrylamide gel (Bio-Rad). Proteins were transferred to nitrocellulose (Pall Corporation), and blocked for 30 min in 5% nonfat dry milk (NFDM) in TBS plus 0.1% Tween 20 (TBST). Blots were incubated in 5% NFDM/TBST with a 1/1,000 dilution of Akt, p-Akt, Lyn, Syk, STAT5, p-STAT5, or p38, or with a 1/5,000 dilution of β-actin overnight at 4°C with gentle rocking. Blots were washed five times for 10 min each in TBST, followed by incubation in 5% NFDM/TBST containing a 1/2,000 dilution of HRP-linked anti-rabbit IgG (Cell Signaling), or HRP-linked rabbit anti-mouse IgG at 1/2,000 (p38) or 1/10,000 (β-actin; Jackson ImmunoResearch Laboratories). Size estimates for proteins were obtained using m.w. standards from Bio-Rad.

ELISA

IL-10, TNF-α, MIP-1α, and histamine were detected by standard ELISA kit as described by the manufacturer. IL-10 and TNF-α ELISA kits were purchased from BD Biosciences. A MIP-1α kit was purchased from R&D Systems. A Histamine kit was purchased from Neogen. Serum IgE levels were measured by ELISA, as described previously (32).

IL-10 injection and ex vivo mast cell activation assay

C57BL/6 × 129 mice were injected i.p. twice daily for 5 days with 2 μg of IL-10/injection (PeproTech) in 100 μl of sterile PBS. Injections were 8 h apart, with the last injection 3 h before sacrificing animals. Peritoneal cells were harvested, resuspended at 2×10^6 cells/ml in cRPMI, and either analyzed for FcεRI expression (as indicated above) or activated ex vivo. Peritoneal cells activated ex vivo were plated at 200 μl/well and incubated with IgE (10 μg/ml) for 45 min at 4°C in cRPMI, washed, and resuspended in cRPMI supplemented with IL-3 (5 ng/ml) and SCF (50 ng/ml). Cells were activated with rat anti-mouse IgE (10 μg/ml) for 16–24 h. Cytokines were measured by ELISA.

Passive systemic anaphylaxis

C57BL/6 mice were injected i.p. with 50 μg of DNP-specific mouse IgE. Mice were injected i.p. 16 h later with 100 μg of DNP-albumin in PBS (Sigma-Aldrich). Body temperature was measured every 10 min for 30 min by rectal probe. After 30 min, mice were sacrificed, and blood was collected by cardiac puncture for serum analysis. Control mice were injected with PBS in place of IgE. These mice showed no significant change in body temperature (±1°C), and had blood histamine levels near the limit of detection, ~50 ng/ml.

Results

IL-10-mediated FcεRI suppression requires STAT3 expression

IL-10 effects have been shown to be largely dependent on STAT3 expression in macrophages (33–35). To determine the importance of STAT3 in IL-10 effects on mast cells, we first used a population of STAT3-deficient mouse mast cells. We noted that these STAT3-deficient bone marrow cells did not develop into a uniform mast cell population, retaining some FcεRI-negative cells unlike the wild-type control population. However, we did find that the FcεRI-positive STAT3-deficient population showed no change in FcεRI expression when cultured ±IL-10. By comparison, mast cells derived from littermate animals showed the expected decrease in FcεRI expression when treated with IL-10 (Fig. 1*A*), suggesting that STAT3 expression is required for IL-10-mediated FcεRI suppression. The importance of STAT3 was further supported by

[3] Abbreviations used in this paper: Tg, transgenic; BMMC, bone marrow-derived mast cell; SCF, stem cell factor; NFDM, nonfat dry milk; PSA, passive systemic anaphylaxis; cRPMI, complete RPMI.

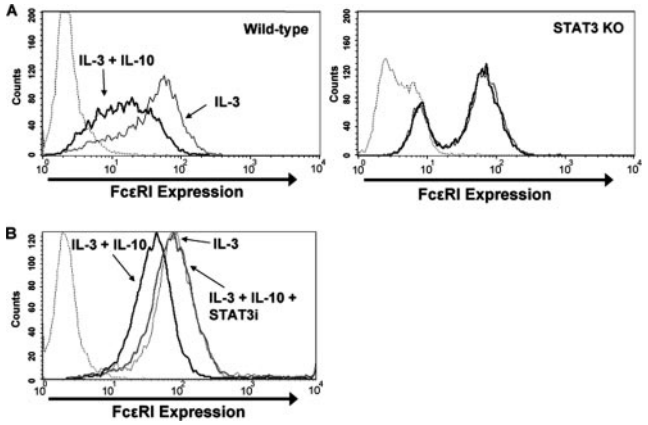

FIGURE 1. IL-10-mediated FcεRI inhibition is STAT-3 dependent. *A*, Littermate or STAT3-deficient (knockout (KO)) BMMC were cultured in IL-3 ± IL-10 for 4 days. FcεRI expression was determined by flow cytometry. Dotted line is isotype control; thin line is anti-FcεRI staining of cells cultured in IL-3; heavy line is anti-FcεRI staining of cells cultured in IL-3 + IL-10. *B*, BMMC were cultured as in *A* in the presence or absence of 5 μM STAT3i, which was added daily. FcεRI expression was determined by flow cytometry. Results are representative of two independent experiments using four to five populations each.

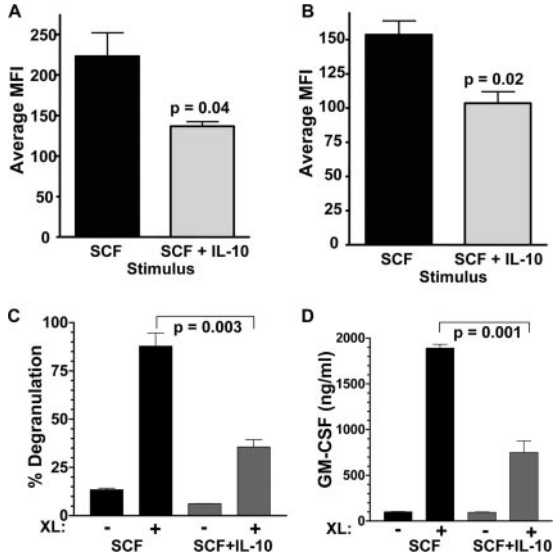

FIGURE 2. IL-10 inhibits FcεRI expression and function on human mast cells. Human lung (*A*) or skin (*B*) mast cells were cultured for 4 days in medium containing SCF ± IL-10 as described in *Materials and Methods*. FcεRI expression was determined by flow cytometry. Results are the average mean fluorescence intensity (MFI) value of three samples that are representative of four independent experiments using three populations of lung mast cells and four populations of skin mast cells. In *C* and *D*, skin mast cells were cultured for 4 days in SCF ± IL-10, then activated with IgE +Ag for 1 h (*C*) or 24 h (*D*), and analyzed for β-hexosaminidase release (*C*) or GM-CSF secretion (*D*) as described in *Materials and Methods*. Data shown are means and SEs of three samples per group analyzed in one experiment that is representative of two to three total experiments.

treating wild-type mouse BMMC with a cell-permeable STAT3 peptide inhibitor (STAT3i). The addition of STAT3i to BMMC prevented IL-10-mediated FcεRI suppression, corroborating our results with STAT3-deficient BMMC (Fig. 1*B*). Collectively, these data demonstrated the importance of STAT3 in IL-10-mediated IgE receptor inhibition.

IL-10 inhibits FcεRI expression and function on human mast cells

Although we have previously found that IL-10 suppressed murine mast cell FcεRI expression, its effects on human mast cell IgE receptor expression were not examined. As shown in Fig. 2, the addition of IL-10 to human mast cell cultures derived from either lung (Fig. 2*A*) or skin (Fig. 2*B*) suppressed FcεRI expression. These results indicated that IL-10 can inhibit FcεRI expression in both mouse and human mast cells.

Because the effect of IL-10 on human FcεRI expression was somewhat less than the 40–50% inhibition we have observed with mouse mast cells (25), we tested skin mast cells for IgE-mediated degranulation and cytokine production in the presence and absence of IL-10. As shown in Fig. 2, human skin mast cells cultured for 4 days in SCF plus IL-10 exhibited reduced β-hexosaminidase release and GM-CSF secretion. These suppressive effects were similar to previous reports showing IL-10-mediated inhibition of degranulation or cytokine production in cord blood-derived mast cells (23, 24). Our data with skin-derived mast cells supports that contention that IL-10 suppresses IgE responses in human mast cells.

IL-10 decreases Syk, Fyn, Akt, and STAT5 expression

In addition to its effects on FcεRI expression, we reasoned that IL-10 may alter the expression or activation of signaling molecules activated by the IgE receptor. Because signal transduction downstream of the IgE receptor proceeds via a central Lyn-Syk pathway and a complementary Fyn-Akt pathway (9), we assessed expression of these key signaling intermediates. We recently showed that STAT5 expression is critical for FcεRI function (11), and hence we also determined the effects of IL-10 on STAT5.

We assessed protein expression after culture for 4 days in IL-3 ± IL-10, using Western blot analysis. Because Lyn is known to be a negative regulator of mast cell function (36–38), it was interesting to note that IL-10 treatment had no effect on Lyn expression (Fig. 3*A*). In contrast, IL-10 significantly suppressed expression of the activating kinases Syk and Fyn (Fig. 3*A*). Densitometry

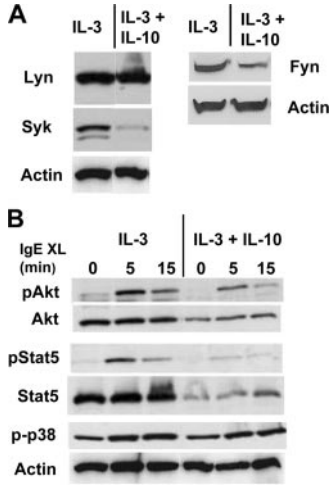

FIGURE 3. IL-10 selectively inhibits expression of FcεRI-related signaling proteins. Cells were cultured for 4 days in IL-3 ± IL-10. On day 4, cells were lysed and blotted to detect Lyn, Syk, and Fyn (*A*), or cells were activated with IgE +Ag for 0, 5, or 15 min and blotted to detect tyrosine-phosphorylated STAT5 or p38, and serine-phosphorylated Akt (*B*). The same membranes were stripped and reprobed to detect total proteins. Data shown are representative of three separate populations.

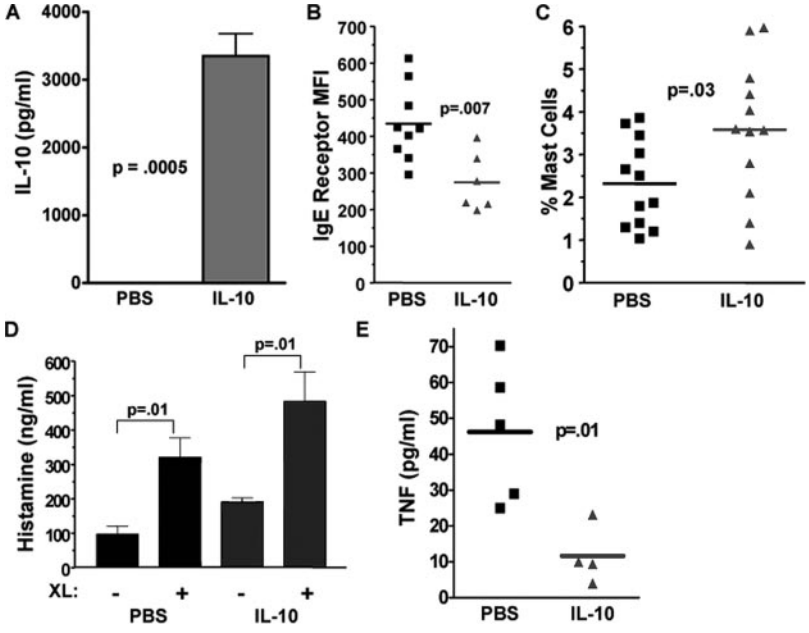

FIGURE 4. IL-10 injection inhibits FcεRI expression and IgE-mediated cytokine production. Mice were injected twice daily for 4 days with IL-10 as described in *Materials and Methods*. *A*, Serum was collected and analyzed for the presence of IL-10 by ELISA. *B* and *C*, Peritoneal cells were collected, and used to determine FcεRI expression (*B*) and percent mast cells (*C*) by flow cytometry. *D* and *E*, Peritoneal cells were incubated with purified mouse IgE and cross-linked with rat anti-mouse IgE, after which supernatants were examined for histamine release at 60 min (*D*) or TNF secretion at 24 h (*E*) by ELISA. Data shown in *D* are from four to five mice per group analyzed in one experiment. Data shown in *E* are from one of two representative experiments using a total of 15 mice per treatment.

showed an average 49.22% inhibition of Syk expression and 42.54% inhibition of Fyn expression in three BMMC separate populations. Thus, IL-10 had potent but selective inhibitory effects on downstream signaling proteins.

Because Syk and Fyn are involved in signal initiation, we expected IL-10 to inhibit the activation of downstream signaling molecules. To study this, mouse BMMC were cultured for 4 days in IL-3 ± IL-10, then stimulated with IgE + Ag for 5–15 min. Total and phosphoprotein expression was measured by Western blotting. As expected, FcεRI-mediated phosphorylation of both STAT5 and Akt was inhibited by IL-10. In contrast, p38 activation was unchanged (Fig. 3*B*). A striking aspect of this selective inhibition was that the reduction in STAT5 and Akt phosphorylation was matched by a loss of overall protein expression. Stat5 activation was the most affected, with densitometry showing an average 79.26% inhibition of STAT5 expression in three experiments. Thus, IL-10 appeared to suppress FcεRI signaling largely by blocking the expression of key signaling proteins, rather than by preventing their activation.

IL-10 injection decreases FcεRI expression and IgE-mediated cytokine production

To assess the in vivo effects of IL-10 on FcεRI, mice were injected with IL-10. To confirm the functionality of this model, IL-10 serum levels were quantified from IL-10- and PBS-injected mice. After 4 days of injections, IL-10-injected mice had significantly higher serum IL-10 levels than PBS-injected mice (Fig. 4*A*). Peritoneal mast cells harvested from IL-10-injected mice had lower FcεRI expression than PBS-injected littermates (Fig. 4*B*), demonstrating that IL-10 consistently suppressed FcεRI expression in vitro and in vivo.

Importantly, the changes in IgE receptor expression were not caused by altered IgE levels. Mean IgE levels in PBS-injected mice were 74.1 ± 7.3 ng/ml, which was not different from IL-10-injected mice with mean IgE levels of 70.1 ± 12.4 ng/ml ($p = 0.78$). In addition to its effects on FcεRI, IL-10 has been shown to inhibit Kit expression (39). We observed a slight but significant inhibition of Kit receptor expression, between 18 and 26% (data not shown). IL-10 injection also increased peritoneal cell numbers from 8.0×10^6 to 14.7×10^6 ($n \geq 4$, $p = 0.002$), and also increased the percentage of mast cells in the peritoneum (Fig. 4*C*). These effects are in keeping with the ability of IL-10 to induce mast cell proliferation (40).

To assess the effects of decreased FcεRI expression on mast cell function, peritoneal cells were harvested from PBS- or IL-10-injected mice, and activated with anti-IgE ex vivo. In contrast to the effect of IL-10 on human mast cells (Fig. 2), mast cells harvested from IL-10-injected mice did not demonstrate reduced IgE-mediated degranulation (Fig. 4*D*; $p = 0.18$ when comparing activated cells harvested from PBS- or IL-10-injected mice). However, peritoneal cells from IL-10-injected mice produced significantly less TNF than their PBS-injected littermates, indicating that IL-10 inhibited the late stage of FcεRI function (Fig. 4*E*). These results are in keeping with previous in vitro studies performed by our group and others with rodent cells (21, 22, 25), which demonstrated IL-10 inhibitory effects on cytokine production but not degranulation.

Chronic overexpression of IL-10 inhibits mast cell FcεRI expression and suppresses anaphylaxis

Although IL-10 injection demonstrated the consistent effects of IL-10 in vivo, we sought a model that could be used for long-term studies. To observe the effects of chronic IL-10 overexpression, we used CD68-IL-10-transgenic mice. Transgenic mice expressed an average of 1626 pg/ml IL-10 in serum samples, while their wild-type littermates had undetectable IL-10 levels (Fig. 5*A*). This concentration of IL-10 is similar to the amount required for FcεRI suppression in vitro (25). We noted a trend toward higher IgE levels in naive IL-10-transgenic mice compared with their littermates, although no significant difference was noted. Littermates had mean IgE levels of 236.7 ± 47.28 ng/ml, while IL-10 transgenics had mean IgE levels of 856.1 ± 401.9 ng/ml. This is consistent with the stimulatory effects of IL-10 on B cells (41, 42). In contrast to IL-10 injection, transgene expression did not affect mast cell numbers (data not shown).

Despite the fact that elevated IgE levels are known to enhance FcεRI expression (43), CD68-IL-10Tg mice demonstrated lower FcεRI expression on peritoneal mast cells compared with littermates (Fig. 5*B*), consistent with our in vitro and IL-10 injection

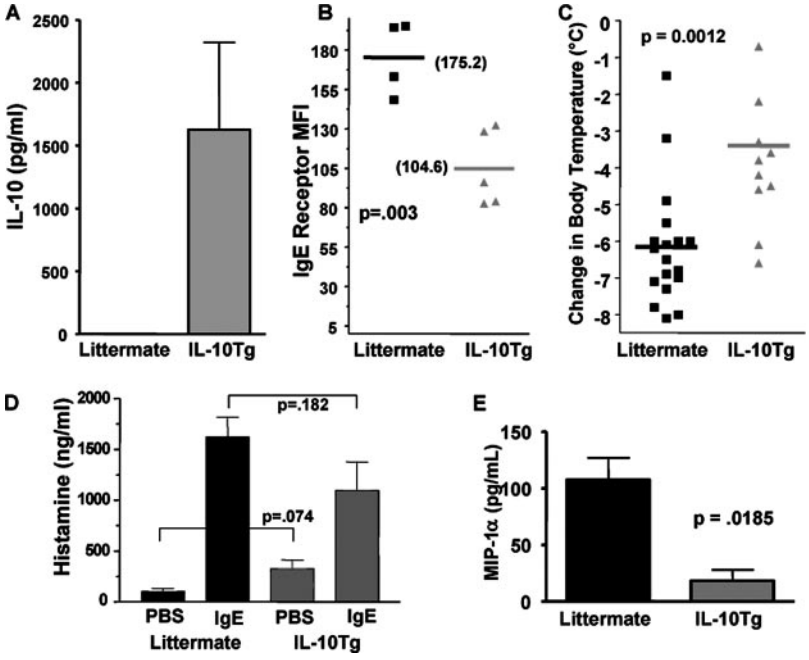

FIGURE 5. Chronic IL-10 overexpression suppresses FcεRI expression and function in vivo. Serum and peritoneal cells were collected from CD68-IL-10Tg and littermate mice. *A*, Serum IL-10 levels were quantified by ELISA. *B*, FcεRI expression on peritoneal cells was determined by flow cytometry. *C–E*, Body temperature change, serum histamine, and MIP-1α were determined 30 min after PSA challenge. Data shown in *C–E* are from one of three representative experiments using a total of 18 littermates and 11 IL-10Tg mice.

studies. To further assess the effects of IL-10 overexpression on mast cell function in vivo, we used IgE-mediated PSA. CD68-IL-10Tg mice demonstrated a reduced anaphylactic response compared with littermates. This was noted by a mitigated drop in body temperature 30 min after Ag challenge (Fig. 5*C*). Surprisingly, this improvement was not matched by changes in serum histamine levels, which were elevated in both littermate and transgenic mice. IL-10 transgene expression did suppress IgE-mediated cytokine production, as CD68IL-10Tg mice had much lower serum MIP-1α levels post-Ag challenge. These data support the contention that IL-10 can suppress mast cell activation and related anaphylactic shock.

Discussion

Mast cells are best known for their role in allergy and asthma, where they serve as an initiator of inflammation (1). This pathological role has been expanded by the recent demonstration that mast cells function in mouse models of multiple sclerosis, inflammatory arthritis, cardiovascular disease, and colitis (3–7). Mast cells have even been suspected to play a role in sudden infant death syndrome, due to elevated postmortem β-tryptase levels in some sudden infant death syndrome victims (44). Therefore, our understanding of how mast cell function is regulated is important for developing treatment strategies for a variety of illnesses.

In contrast to their pathological functions, mast cells have a protective role in bacterial and parasitic infections. In response to infection with parasites, mast cell hyperplasia correlates with parasite elimination (1). Even more dramatically, mast cell-deficient mice succumb to infection in bacterial peritonitis and pneumonia models (45, 46). This protective function stems from rapid release of TNF after contact with bacterial ligands, and subsequent neutrophil recruitment (45, 46). Collectively, mast cell activation appears poised to rapidly respond to infection, but is also capable of significant and broad pathology when aberrantly activated. Understanding mast cell signaling is therefore a critical aspect of developing new clinical interventions and predicting disease progression.

Allergy and allergic asthma may be caused in part by a loss of mast cell homeostasis, leading to chronic inflammation. We have postulated that in a normal response, mast cells can proliferate and induce inflammation for 3–6 days, at which point cytokines such as IL-4, IL-10, and TGF-β1 inhibit mast cell function (47). Our previous data showed that these cytokines suppress FcεRI expression and function in vitro, and subsequently induce mast cell apoptosis (25, 30, 48–50). Of these cytokines, IL-10 and TGFβ1 are well-documented for their immunosuppressive capabilities. Loss of IL-10 function has been shown to cause colitis (13), while TGF-β1 deficiency results in widespread autoimmunity (51, 52).

Our previous work with IL-10 used mouse mast cells and in vitro assays. In the current study, we demonstrate the consistency of IL-10-mediated suppression, showing IL-10-mediated suppression of FcεRI expression and function on human skin and lung mast cells. This consistency is important, because some cytokine effects vary between species. For example, IL-4 suppresses FcεRI expression on mouse mast cells (30), but has the reverse effect on terminally differentiated human mast cells (53). There are many variables that differ between mouse and human mast cells, particularly culture systems and growth factors. Therefore, it is compelling that despite these inherent differences, IL-10 has similar effects on mouse and human mast cells.

We also found that IL-10 can act in vivo. IL-10 injection suppressed mast cell FcεRI expression in vivo, despite the presence of IgE as an opposing force. The down-regulation we observed in vivo may be at best a partial explanation for suppressing IgE-mediated activation. We detected a 30% decrease in surface FcεRI after IL-10 injection, which would likely leave many more receptors on the cell surface than are required for activation. In contrast, omalizumab therapy, a clinically effective monoclonal anti-IgE capable of preventing IgE binding to FcεRI, reduces surface IgE receptor expression >90% (54, 55). Because this partial inhibitory effect on surface receptor expression did not appear to fully explain the ability of IL-10 to repress FcεRI signaling, we further investigated IL-10 effects on FcεRI signal transduction downstream.

IL-10 treatment of mouse BMMC revealed an interestingly selective suppressive effect on FcεRI signaling. We found it particularly striking that IL-10 suppressed expression of Fyn, Syk, Akt, and STAT5—molecules with critical functions in mast cell activation—without altering Lyn expression. Lyn is argued to be a

negative regulator of FcεRI signaling, so its continued expression may serve to inhibit IgE-mediated activation (37, 38). In contrast, Fyn and Syk represent the apical end of IgE signaling, while Akt and STAT5 are linked to mast cell cytokine production (11, 38). Like its repression of FcεRI levels, IL-10 partially reduced expression of these signaling molecules, typically in the range of 50% suppression (Fig. 3). The combined effect of fewer surface receptors and reduced signaling resulted in reduced cytokine production, but did not affect degranulation in the mouse system. This is in contrast to human mast cells, which showed reduced degranulation and cytokine production. These data offer two areas for future studies. It will be interesting to dissect the suppressive effects of IL-10 on mouse vs human mast cells, to determine the mechanism providing species-restricted inhibition of degranulation, a clinically important event in mast cell activation. Related to this issue, Gonzalez-Espinosa et al. (56) recently showed that weak IgE-mediated signals could elicit cytokine production without degranulation. Their study indicates that the signaling threshold for cytokine production is lower than that for degranulation—hence, we would have expected IL-10 to exert its more prominent effect on histamine release. Yet, we and others have consistently found the reverse effect of IL-10 both in vitro and in vivo (21, 22, 25). How this threshold effect is attained and altered by IL-10 is a focus of our current studies.

The consistent ability of IL-10 to suppress FcεRI expression and function both in vivo and across species supports our focus on the molecular mechanisms of IL-10 function. We are currently investigating how IL-10 suppresses protein expression and the role of STAT3 in this process. Our current work includes studies of how IL-10 inhibits expression of FcεRI-signaling molecules, and the role of STAT3 in this process. If these effects occur at the protein level, they could be partly explained by induction of suppressor of cytokine signaling family proteins and subsequent ubiquitination (57). Although Stat3 appears to be critical for the effects of IL-10, we found that Stat3-deficient mast cells differentiate incompletely and hence may be a difficult model in which to study the effects of IL-10.

Because IL-10 polymorphisms correlate with increased incidence of atopic disease (58), it is tempting to speculate that loss of IL-10-mediated suppression is part of the disease etiology, and that IL-10 normally functions to limit mast cell activation. Our in vivo experiments showed that raising IL-10 levels via injection or transgene expression suppressed mast cell function. These experiments also revealed an interesting effect on mast cell numbers. Although short-term (4-day) injection with IL-10 raised mast cell numbers, we found no such effect in the IL-10Tg mice with chronically high IL-10 levels (Figs. 4 and 5, and data not shown). IL-10 has been shown to both increase and decrease mast cell proliferation (40, 59). Interestingly, the suppressive effects on proliferation were found in an in vivo model in which IL-10 levels were elevated for >17 days (59), suggesting that chronic IL-10 may suppress mast cell proliferation. Our own in vitro work supports this contention. We have found that IL-10 in combination with IL-4 initially promotes mast cell proliferation, but by day 6 of culture this effect has reverted to cell cycle arrest and apoptosis (50). One wonders if the linkage of IL-10 promoter polymorphisms to atopic disease is related to loss of this late suppressive effect on mast cell numbers and function.

Consistent with our mouse in vitro studies (25), IL-10 had no effect on histamine release in IgE-challenged CD68-IL-10Tg mice. However, chronic IL-10 still improved the drop in body temperature noted during the immediate vasodilatory phase of anaphylactic shock. This appears to reflect some other function of IL-10, possibly on the release of mast cell mediators other than histamine, or on smooth muscle cell responsiveness. A striking and consistent effect of IL-10 was its blockade of IgE-mediated cytokine production in vivo. We chose to measure MIP-1 α because it is a representative inflammatory cytokine/chemokine (60). The role of mast cells in atopy, and especially in nonallergic inflammatory diseases, may hinge on production of cytokines and subsequent inflammatory cell recruitment (61). If this is the case, IL-10 or small molecules mimicking IL-10 signaling may prove to be efficacious clinical interventions.

Acknowledgments

We are grateful for the technical assistance of Hoainam Nguyen-Jackson.

Disclosures

The authors have no financial conflict of interest.

References

1. Ryan, J. J., and T. F. Huff. 2003. Biology of mast cells. In *Middleton's Allergy: Principles and Practice, Vol. 1*. N. F. Adkinson, and E. Middleton, eds. Mosby, St. Louis, pp. 333–346.
2. Metz, M., A. M. Piliponsky, C. C. Chen, V. Lammel, M. Abrink, G. Pejler, M. Tsai, and S. J. Galli. 2006. Mast cells can enhance resistance to snake and honeybee venoms. *Science* 313: 526–530.
3. Gregory, G. D., M. Robbie-Ryan, V. H. Secor, J. J. Sabatino, Jr., and M. A. Brown. 2005. Mast cells are required for optimal autoreactive T cell responses in a murine model of multiple sclerosis. *Eur. J. Immunol.* 35: 3478–3486.
4. Nigrovic, P. A., B. A. Binstadt, P. A. Monach, A. Johnsen, M. Gurish, Y. Iwakura, C. Benoist, D. Mathis, and D. M. Lee. 2007. Mast cells contribute to initiation of autoantibody-mediated arthritis via IL-1. *Proc. Natl. Acad. Sci. USA* 2325–2330.
5. Kelley, J. L., D. S. Chi, W. Abou-Auda, J. K. Smith, and G. Krishnaswamy. 2000. The molecular role of mast cells in atherosclerotic cardiovascular disease. *Mol. Med. Today* 6: 304–308.
6. Hara, M., K. Ono, M. W. Hwang, A. Iwasaki, M. Okada, K. Nakatani, S. Sasayama, and A. Matsumori. 2002. Evidence for a role of mast cells in the evolution to congestive heart failure. *J. Exp. Med.* 195: 375–381.
7. Rijnierse, A., A. S. Koster, F. P. Nijkamp, and A. D. Kraneveld. 2006. TNF-α is crucial for the development of mast cell-dependent colitis in mice. *Am. J. Physiol.* 291: G969–G976.
8. Lin, S., C. Cicala, A. M. Scharenberg, and J. P. Kinet. 1996. The FcεRIβ subunit functions as an amplifier of FcεRIγ-mediated cell activation signals. *Cell* 85: 985–995.
9. Rivera, J., and A. M. Gilfillan. 2006. Molecular regulation of mast cell activation. *J. Allergy Clin. Immunol.* 117: 1214–1225.
10. Gilfillan, A. M., and C. Tkaczyk. 2006. Integrated signalling pathways for mast-cell activation. *Nat. Rev. Immunol.* 6: 218–230.
11. Barnstein, B. O., G. Li, Z. Wang, S. Kennedy, C. Chalfant, H. Nakajima, K. D. Bunting, and J. J. Ryan. 2006. Stat5 expression is required for IgE-mediated mast cell function. *J. Immunol.* 177: 3421–3426.
12. Kotenko, S. V. 2002. The family of IL-10-related cytokines and their receptors: related, but to what extent? *Cytokine Growth Factor Rev.* 13: 223–240.
13. Kuhn, R., J. Lohler, D. Rennick, K. Rajewsky, and W. Muller. 1993. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 75: 263–274.
14. Gazzinelli, R. T., M. Wysocka, S. Hieny, T. Scharton-Kersten, A. Cheever, R. Kuhn, W. Muller, G. Trinchieri, and A. Sher. 1996. In the absence of endogenous IL-10, mice acutely infected with *Toxoplasma gondii* succumb to a lethal immune response dependent on $CD4^+$ T cells and accompanied by overproduction of IL-12, IFN-γ, and TNF-α. *J. Immunol.* 157: 798–805.
15. Hunter, C. A., L. A. Ellis-Neyes, T. Slifer, S. Kanaly, G. Grunig, M. Fort, D. Rennick, and F. G. Araujo. 1997. IL-10 is required to prevent immune hyperactivity during infection with *Trypanosoma cruzi*. *J. Immunol.* 158: 3311–3316.
16. Berg, D. J., R. Kuhn, K. Rajewsky, W. Muller, S. Menon, N. Davidson, G. Grunig, and D. Rennick. 1995. Interleukin-10 is a central regulator of the response to LPS in murine models of endotoxic shock and the Shwartzman reaction but not endotoxin tolerance. *J. Clin. Invest.* 96: 2339–2347.
17. Howard, M., T. Muchamuel, S. Andrade, and S. Menon. 1993. Interleukin 10 protects mice from lethal endotoxemia. *J. Exp. Med.* 177: 1205–1208.
18. Roers, A., L. Siewe, E. Strittmatter, M. Deckert, D. Schluter, W. Stenzel, A. D. Gruber, T. Krieg, K. Rajewsky, and W. Muller. 2004. T cell-specific inactivation of the interleukin 10 gene in mice results in enhanced T cell responses but normal innate responses to lipopolysaccharide or skin irritation. *J. Exp. Med.* 200: 1289–1297.
19. Trachsel, E., F. Bootz, M. Silacci, M. Kaspar, H. Kosmehl, and D. Neri. 2007. Antibody-mediated delivery of interleukin-10 inhibits the progression of established collagen-induced arthritis. *Arthritis Res. Ther.* 9: R9.
20. Murray, P. J. 2006. Understanding and exploiting the endogenous interleukin-10/STAT3-mediated anti-inflammatory response. *Curr. Opin. Pharmacol.* 6: 379–386.
21. Lin, T. J., and A. D. Befus. 1997. Differential regulation of mast cell function by IL-10 and stem cell factor. *J. Immunol.* 159: 4015–4023.

22. Marshall, J. S., I. Leal-Berumen, L. Nielsen, M. Glibetic, and M. Jordana. 1996. Interleukin (IL)-10 inhibits long-term IL-6 production but not preformed mediator release from rat peritoneal mast cells. *J. Clin. Invest.* 97: 1122–1128.
23. Royer, B., S. Varadaradjalou, P. Saas, A. C. Gabiot, B. Kantelip, F. Feger, J. J. Guillosson, J. P. Kantelip, and M. Arock. 2001. Autocrine regulation of cord blood-derived human mast cell activation by IL-10. *J. Allergy Clin. Immunol.* 108: 80–86.
24. Royer, B., S. Varadaradjalou, P. Saas, J. J. Guillosson, J. P. Kantelip, and M. Arock. 2001. Inhibition of IgE-induced activation of human mast cells by IL-10. *Clin. Exp. Allergy* 31: 694–704.
25. Gillespie, S. R., R. R. DeMartino, J. Zhu, H. J. Chong, C. Ramirez, C. P. Shelburne, L. A. Bouton, D. P. Bailey, A. Gharse, P. Mirmonsef, et al. 2004. IL-10 inhibits FcεRI expression in mouse mast cells. *J. Immunol.* 172: 3181–3188.
26. Lang, R., R. L. Rutschman, D. R. Greaves, and P. J. Murray. 2002. Autocrine deactivation of macrophages in transgenic mice constitutively overexpressing IL-10 under control of the human CD68 promoter. *J. Immunol.* 168: 3402–3411.
27. Koni, P. A., S. K. Joshi, U. A. Temann, D. Olson, L. Burkly, and R. A. Flavell. 2001. Conditional vascular cell adhesion molecule 1 deletion in mice: impaired lymphocyte migration to bone marrow. *J. Exp. Med.* 193: 741–754.
28. Takeda, K., T. Kaisho, N. Yoshida, J. Takeda, T. Kishimoto, and S. Akira. 1998. Stat3 activation is responsible for IL-6-dependent T cell proliferation through preventing apoptosis: generation and characterization of T cell-specific Stat3-deficient mice. *J. Immunol.* 161: 4652–4660.
29. Panopoulos, A. D., L. Zhang, J. W. Snow, D. M. Jones, A. M. Smith, K. C. El Kasmi, F. Liu, M. A. Goldsmith, D. C. Link, P. J. Murray, and S. S. Watowich. 2006. STAT3 governs distinct pathways in emergency granulopoiesis and mature neutrophils. *Blood* 108: 3682–3690.
30. Ryan, J. J., S. DeSimone, G. Klisch, C. Shelburne, L. J. McReynolds, K. Han, R. Kovacs, P. Mirmonsef, and T. F. Huff. 1998. IL-4 inhibits mouse mast cell FcεRI expression through a STAT6-dependent mechanism. *J. Immunol.* 161: 6915–6923.
31. Schwartz, W., J. Jiao, J. Ford, D. Conrad, J. F. Hamel, P. Santanbien, L. Bradbury, and T. Robin. 2004. Application of chemically-stable immunoglobulin-selective sorbents: harvest and purification of antibodies with resolution of aggregate. *BioProcessing J.* 3: 53–62.
32. Ford, J. W., M. A. Kilmon, K. M. Haas, A. E. Shelburne, Y. Chan-Li, and D. H. Conrad. 2006. In vivo murine CD23 destabilization enhances CD23 shedding and IgE synthesis. *Cell. Immunol.* 243: 107–117.
33. Williams, L. M., U. Sarma, K. Willets, T. Smallie, F. Brennan, and B. M. Foxwell. 2007. Expression of constitutively active STAT3 can replicate the cytokine-suppressive activity of interleukin-10 in human primary macrophages. *J. Biol. Chem.* 282: 6965–6975.
34. El Kasmi, K. C., J. Holst, M. Coffre, L. Mielke, A. de Pauw, N. Lhocine, A. M. Smith, R. Rutschman, D. Kaushal, Y. Shen, et al. 2006. General nature of the STAT3-activated anti-inflammatory response. *J. Immunol.* 177: 7880–7888.
35. Lang, R., D. Patel, J. J. Morris, R. L. Rutschman, and P. J. Murray. 2002. Shaping gene expression in activated and resting primary macrophages by IL-10. *J. Immunol.* 169: 2253–2263.
36. Hernandez-Hansen, V., A. J. Smith, Z. Surviladze, A. Chigaev, T. Mazel, J. Kalesnikoff, C. A. Lowell, G. Krystal, L. A. Sklar, B. S. Wilson, and J. M. Oliver. 2004. Dysregulated FcεRI signaling and altered Fyn and SHIP activities in Lyn-deficient mast cells. *J. Immunol.* 173: 100–112.
37. Odom, S., G. Gomez, M. Kovarova, Y. Furumoto, J. J. Ryan, H. V. Wright, C. Gonzalez-Espinosa, M. L. Hibbs, K. W. Harder, and J. Rivera. 2004. Negative regulation of immunoglobulin E-dependent allergic responses by Lyn kinase. *J. Exp. Med.* 199: 1491–1502.
38. Hernandez-Hansen, V., G. A. Mackay, C. A. Lowell, B. S. Wilson, and J. M. Oliver. 2004. The Src kinase Lyn is a negative regulator of mast cell proliferation. *J. Leukocyte Biol.* 75: 143–151.
39. Mirmonsef, P., C. P. Shelburne, C. Fitzhugh Yeatman, 2nd, H. J. Chong, and J. J. Ryan. 1999. Inhibition of Kit expression by IL-4 and IL-10 in murine mast cells: role of STAT6 and phosphatidylinositol 3′-kinase. *J. Immunol.* 163: 2530–2539.
40. Thompson-Snipes, L., V. Dhar, M. W. Bond, T. R. Mosmann, K. W. Moore, and D. M. Rennick. 1991. Interleukin 10: a novel stimulatory factor for mast cells and their progenitors. *J. Exp. Med.* 173: 507–510.
41. Defrance, T., B. Vanbervliet, F. Briere, I. Durand, F. Rousset, and J. Banchereau. 1992. Interleukin 10 and transforming growth factor β cooperate to induce anti-CD40-activated naive human B cells to secrete immunoglobulin A. *J. Exp. Med.* 175: 671–682.
42. Rousset, F., E. Garcia, T. Defrance, C. Peronne, N. Vezzio, D. H. Hsu, R. Kastelein, K. W. Moore, and J. Banchereau. 1992. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. *Proc. Natl. Acad. Sci. USA* 89: 1890–1893.
43. Saini, S. S., and D. MacGlashan. 2002. How IgE upregulates the allergic response. *Curr. Opin. Immunol.* 14: 694–697.
44. Nishio, H., and K. Suzuki. 2004. Serum tryptase levels in sudden infant death syndrome in forensic autopsy cases. *Forensic Sci. Int.* 139: 57–60.
45. Echtenacher, B., D. N. Mannel, and L. Hultner. 1996. Critical protective role of mast cells in a model of acute septic peritonitis. *Nature* 381: 75–77.
46. Malaviya, R., T. Ikeda, E. Ross, and S. N. Abraham. 1996. Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-α. *Nature* 381: 77–80.
47. Ryan, J. J., M. Kashyap, D. Bailey, S. Kennedy, K. Speiran, J. Brenzovich, B. Barnstein, C. Oskeritzian, and G. Gomez. 2007. Mast cell homeostasis: a fundamental aspect of allergic disease. *Crit. Rev. Immunol.* 27: 15–32.
48. Gomez, G., C. D. Ramirez, J. Rivera, M. Patel, F. Norozian, H. V. Wright, M. V. Kashyap, B. O. Barnstein, K. Fischer-Stenger, L. B. Schwartz, et al. 2005. TGF-β1 inhibits mast cell FcεRI expression. *J. Immunol.* 174: 5987–5993.
49. Bouton, L. A., C. D. Ramirez, D. P. Bailey, C. F. Yeatman, J. Yue, H. V. Wright, J. Domen, R. R. Rosato, S. Grant, K. Fischer-Stenger, and J. J. Ryan. 2004. Costimulation with interleukin-4 and interleukin-10 induces mast cell apoptosis and cell-cycle arrest: the role of p53 and the mitochondrion. *Exp. Hematol.* 32: 1137–1145.
50. Yeatman, C. F., 2nd, S. M. Jacobs-Helber, P. Mirmonsef, S. R. Gillespie, L. A. Bouton, H. A. Collins, S. T. Sawyer, C. P. Shelburne, and J. J. Ryan. 2000. Combined stimulation with the T helper cell type 2 cytokines interleukin (IL)-4 and IL-10 induces mouse mast cell apoptosis. *J. Exp. Med.* 192: 1093–1103.
51. Shull, M. M., I. Ormsby, A. B. Kier, S. Pawlowski, R. J. Diebold, M. Yin, R. Allen, C. Sidman, G. Proetzel, D. Calvin, et al. 1992. Targeted disruption of the mouse transforming growth factor-β1 gene results in multifocal inflammatory disease. *Nature* 359: 693–699.
52. Kulkarni, A. B., C. G. Huh, D. Becker, A. Geiser, M. Lyght, K. C. Flanders, A. B. Roberts, M. B. Sporn, J. M. Ward, and S. Karlsson. 1993. Transforming growth factor β1 null mutation in mice causes excessive inflammatory response and early death. *Proc. Natl. Acad. Sci. USA* 90: 770–774.
53. Thienemann, F., B. M. Henz, and M. Babina. 2004. Regulation of mast cell characteristics by cytokines: divergent effects of interleukin-4 on immature mast cell lines versus mature human skin mast cells. *Arch. Dermatol. Res.* 296: 134–138.
54. Beck, L. A., G. V. Marcotte, D. MacGlashan, A. Togias, and S. Saini. 2004. Omalizumab-induced reductions in mast cell FcεRI expression and function. *J. Allergy Clin. Immunol.* 114: 527–530.
55. MacGlashan, D. W., Jr., B. S. Bochner, D. C. Adelman, P. M. Jardieu, A. Togias, J. McKenzie-White, S. A. Sterbinsky, R. G. Hamilton, and L. M. Lichtenstein. 1997. Down-regulation of FcεRI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J. Immunol.* 158: 1438–1445.
56. Gonzalez-Espinosa, C., S. Odom, A. Olivera, J. P. Hobson, M. E. Martinez, A. Oliveira-Dos-Santos, L. Barra, S. Spiegel, J. M. Penninger, and J. Rivera. 2003. Preferential signaling and induction of allergy-promoting lymphokines upon weak stimulation of the high affinity IgE receptor on mast cells. *J. Exp. Med.* 197: 1453–1465.
57. Ilangumaran, S., and R. Rottapel. 2003. Regulation of cytokine receptor signaling by SOCS1. *Immunol. Rev.* 192: 196–211.
58. Hobbs, K., J. Negri, M. Klinnert, L. J. Rosenwasser, and L. Borish. 1998. Interleukin-10 and transforming growth factor-β promoter polymorphisms in allergies and asthma. *Am. J. Respir. Crit. Care Med.* 158: 1958–1962.
59. Palaniyandi, S. S., K. Watanabe, M. Ma, H. Tachikawa, M. Kodama, and Y. Aizawa. 2004. Inhibition of mast cells by interleukin-10 gene transfer contributes to protection against acute myocarditis in rats. *Eur. J. Immunol.* 34: 3508–3515.
60. Kehrl, J. H. 2006. Chemoattractant receptor signaling and the control of lymphocyte migration. *Immunol. Res.* 34: 211–227.
61. Boyce, J. A. 2003. Mast cells: beyond IgE. *J. Allergy Clin. Immunol.* 111: 24–32.