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IL-10 Inhibits FcεRI Expression in Mouse Mast Cells[1]

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FcεRI expression and function is a central aspect of allergic disease. Using bone marrow-derived mouse mast cell populations, we have previously shown that the Th2 cytokine IL-4 inhibits FcεRI expression and function. In the current study we show that the Th2 cytokine IL-10 has similar regulatory properties, and that it augments the inhibitory effects of IL-4. FcεRI down-regulation was functionally significant, as it diminished inflammatory cytokine production and IgE-mediated FcεRI up-regulation. IL-10 and IL-4 reduced FcεRI β protein expression without altering the α or γ subunits. The ability of IL-4 and IL-10 to alter FcεRI expression by targeting the β-chain, a critical receptor subunit known to modulate receptor expression and signaling, suggests the presence of a Th2 cytokine-mediated homeostatic network that could serve to both initiate and limit mast cell effector function. ***The Journal of Immunology*****, 2004, 172: 3181–3188.**

Mast cells are important effector cells in IgE-mediated allergic disease and innate immunity to parasites and bacteria (reviewed in Ref. 1). Recently a role for mast cells has also been shown in the pathology associated with multiple sclerosis, inflammatory arthritis, and cardiovascular disease (2–6). As such, understanding control of mast cell inflammatory activities is an area of increasing importance.

The best-characterized mediator of mast cell activation is the high affinity IgE receptor, FcεRI. In rodents, FcεRI is expressed as a tetramer consisting of one α-, one β-, and two γ-chains on mast cells and basophils (7, 8), while an FcεRI $\alpha\gamma_2$ trimer is expressed on monocytes, eosinophils, platelets, Langerhans cells, and dendritic cells (reviewed in Ref. 9). On mast cells and basophils, Ag-mediated FcεRI coaggregation through receptor-bound IgE induces secretion of histamine, proteases, inflammatory cytokines and chemokines, and lipid-derived mediators that collectively cause inflammation and tissue damage (reviewed in Refs. 1 and 9). This range of expression, coupled with critical functional roles, emphasizes the importance of proper FcεRI regulation.

The allergic responses in which mast cells are key players are orchestrated by Th2 lymphocytes. We have postulated that Th2 cytokines may serve as homeostatic regulators of mast cell function and survival. IL-4 is a mast cell growth factor (10), and has been shown to induce FcεRI expression on developing human mast cells (11–12). These activities, coupled with the linkage of IL-4 receptor polymorphisms to allergic disease (13, 14), argue for the proatopic nature of IL-4. However, our recent data demonstrating that IL-4 inhibits FcεRI and Kit expression (15, 16) supports a homeostatic role for IL-4 in allergy.

Our studies of IL-4 prompted us to investigate other Th2 cytokines for similar inhibitory effects. Although the anti-inflammatory effects of IL-4 were noted secondary to its proatopic role, IL-10 has long been viewed as a suppressive cytokine, in part due to its ability to inhibit monocyte cytokine production (reviewed in Refs. 17 and 18). Like IL-4, IL-10 is also produced by cultured mast cells (19). While IL-10 has been reported to inhibit mast cell cytokine production, it did so without affecting histamine, β-hexosaminidase, or leukotriene C4-like synthesis (20–22). IL-10 can also enhance mast cell protease expression (23). Moreover, several studies from the laboratories of Rennick and Thompson-Snipes (24–26) have demonstrated the growth-promoting activities of IL-10 on mouse mast cells and their progenitors. With the recent report of atopy-related polymorphisms in the human IL-10 promoter (27), the literature indicates that a full understanding of IL-10 effects on mast cell biology is clinically relevant but incomplete.

We recently found that IL-10 inhibits mast cell Kit expression and function (16), and that combined stimulation with IL-4 plus IL-10 induces mast cell apoptosis (28). In the current study, we assessed IL-10-mediated control over FcεRI expression and signaling using murine IL-3-dependent, bone marrow-derived mast cell (BMMC)[3] populations. We also assessed the mechanisms by which IL-4 and IL-10 exert their regulatory effects on FcεRI. Our findings indicate that IL-10 participates in Th2-mediated mast cell homeostasis.

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[3] Abbreviations used in this paper: BMMC, bone marrow-derived mast cell; WT, wild type; BFA, brefeldin A; MFI, mean fluorescence intensity; RPA, RNase protection assay; GFP, green fluorescence protein.

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Materials and Methods

BMMC cultures

BMMC were derived from C57BL/6, BALB/c, C3H, C57BL/6 × 129 wild type (WT), or C57BL/6 × 129 STAT6-deficient mice by culture in complete RPMI 1640 medium (cRPMI) and 25% WEHI-3 cell-conditioned medium as described previously (15). After 3–4 wk in culture, these populations were >99% mast cells, as judged by morphology and flow cytometry staining for expression of FcεRI, CD13, Kit, and T1/ST2 (data not shown). The resulting populations were maintained for <6 mo, and were generally used within the first 3 mo after their development.

Cytokines and reagents

DNP-conjugated BSA was the kind gift of D. Conrad (Virginia Commonwealth University, Richmond, VA). Brefeldin A (BFA) was purchased from Sigma-Aldrich (St. Louis. MO). Murine IL-3, IL-4, and IL-10 were purchased from R&D Systems (Minneapolis, MN). Rat anti-mouse FcγRII/RIII (2.4G2), mouse IgE, FITC-conjugated rat anti-mouse CD13, FITC-conjugated rat anti-mouse Kit, and FITC-conjugated rat IgG were purchased from BD PharMingen (San Diego, CA). FITC- or PE-conjugated rat anti-mouse IgE and goat $F(ab')_2$ anti-rat IgG were purchased from Southern Biotechnology Associates (Birmingham, AL). FITC-conjugated rat anti-mouse T1/ST2 was purchased from Morwell Diagnostics (Zurich, Switzerland).

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-4, IL-10, or mouse IgE. IL-4 and IL-10 were used at 10 ng/ml unless otherwise stated. Cultures were incubated for the indicated times. Every 4 days, half of the media and cytokines or IgE were replaced.

Flow cytometric analysis

Mast cell surface Ag expression was assessed by flow cytometry using standard methods, as described previously (15). To calculate percent inhibition of FcεRI expression, the mean fluorescence intensity (MFI) of a given culture was subtracted from that of the control IL-3 culture. This difference was divided by the MFI of the control culture, and the result was multiplied by 100%.

Retroviral infection

Stat6-deficient BMMC cultures were infected with retrovirus expressing a bicistronic construct consisting of green fluorescence protein (GFP) alone or GFP and the constitutively active Stat6 mutant termed Stat6VT as described previously (29). FcεRI surface expression was assessed on the GFP-positive population by flow cytometry analysis using PE-coupled anti-IgE staining.

RNase protection assay (RPA)

RPA assays were performed using the RiboQuant system (BD PharMingen) as per the manufacturer's instructions. Pixel intensities of individual bands were obtained using a Typhoon phosphorimager (Molecular Dynamics, Sunnyvale, CA). The ratio of the pixel intensity for each band of interest to the sum of the pixel intensities for the housekeeping genes (L32 plus GAPDH) in that lane were determined. Calculations of percent change in expression relative to control conditions were determined by comparing these ratios.

Western blot analysis

FcεRI β and γ subunits were detected with mouse anti-FcεRI β (the kind gift of J.-P. Kinet, Harvard University, Boston, MA) or rabbit anti-FcεRI γ subunit (Upstate Biotechnology, Lake Placid, NY). Western blotting was performed using 29 μg of total cellular protein as described previously (30). For immunoprecipitation and Western blotting of FcεRI α, cells (3×10^5 cells/ml) were cultured in the indicated culture conditions for 4 days, resuspended in cRPMI, pelleted, and solubilized in lysis buffer (0.5% Nonidet P-40, 10% glycerol; 5 M NaCl; 100 mM Tris; 100 mM $MgCl_2$; supplemented with an enhanced inhibitor mixture consisting of a 20× concentrate of Complete, with 80 mM benzamidine HCL, 50 mM ε-caproic acid, 16 mM iodoacetamide, 10 μg/ml leupeptin, 10 μg/ml pepstatin, and 100 μg/ml soybean trypsin inhibitor) for 1 h at 4°C. The lysates (3×10^7 cells/sample) were assayed (Bio-Rad, Hercules, CA) for protein concentration according to the manufacturer's recommended prodecure. A total of 760 μg per sample were immunoprecipitated using protein A-Sepharose beads (50 μl) that had been incubated overnight with 25 μg of rabbit anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) and either 5 μg of clone 5.14 anti-FcεRI α mAb, or Chrompure mouse IgG whole (Jackson Immunoresearch Laboratories) as a control. After 3 h of incubation at 4°C, the immunoprecipitates were analyzed by SDS-PAGE on a 10% Tris-glycine gel and transferred to a nitrocellulose membrane. Membranes were blocked and incubated overnight at 4°C with TW anti-FcεRI α mAb (1 μg/ml) followed by protein A-HRP (Zymed Laboratories, South San Francisco, CA). The reaction was developed with an ECL Western blotting detection kit (Amersham Life Science, Piscataway, NJ).

Mast cell activation assays

To assess β-hexosaminidase release or cytokine secretion, BMMC were cultured in IL-3 with or without IL-4 and/or IL-10 as described above, then activated with mouse IgE (10 μg/ml, 45 min, 4°C) plus rat anti-mouse IgE at the indicated concentrations for 1 h (β-hexosaminidase release) or 24 h (cytokine secretion). DNP-BSA was occasionally used in place of rat anti-mouse IgE with similar results. After activation, β-hexosaminidase release was assessed as described by Schwartz et al. (31). The percent β-hexosaminidase release was determined by dividing the amount of β-hexosaminidase in the supernatant by the total amount detected in the supernatant and cell pellet. TNF-α secretion was measured by standard ELISA (BD PharMingen).

Results

IL-10 inhibits FcεRI expression

To assess the effect of IL-10 on FcεRI expression, mouse BMMC were cultured in IL-3 alone or in IL-3 plus IL-10 for 4 days. IL-10 stimulation led to a marked reduction in FcεRI surface (Fig. 1*A*). These results were consistent in >80 independent experiments using at least 20 independent BMMC populations from various mouse strains, including C57BL/6, BALB/c, C3H, and C57BL/6 × 129 (data not shown). A concentration-response curve was generated by calculating the percent inhibition of FcεRI expression from the MFI of populations treated with or without IL-10 (Fig. 1*B*). Maximum inhibition required 5 ng/ml IL-10, with measurable effects occurring at concentrations as low as 0.1 ng/ml.

IL-4 enhances IL-10-mediated FcεRI down-regulation

The effects of IL-10 were strikingly similar to those we have reported for IL-4 (15). Given this, we assessed dual stimulation with IL-4 and IL-10. Combined stimulation with IL-4 plus IL-10 reduced FcεRI expression slightly more than did IL-4 or IL-10 alone (Fig. 2*A*). We initially assessed the kinetics of IL-4- and IL-10-mediated FcεRI repression through time course assays. As shown in Fig. 2*B*, the additive effect of IL-4 and IL-10 was apparent for 5 days, after which inhibition mirrored that of populations cultured with IL-3 plus IL-4 or with IL-3 plus IL-10. FcεRI repression was longstanding, detectable for at least 21 days in cultures receiving fresh cytokines every 4 days. It is interesting to note that the point at which IL-4 and IL-10 no longer exhibited additive effects immediately precedes the time at which we observe BMMC apoptosis induced by these conditions (day 6) (28). This could indicate that cells surviving in these conditions are less cytokine responsive.

We further investigated the stability of this FcεRI repression by removing IL-4 and IL-10 from BMMC cultures after 3 days of stimulation. Cells cultured for 3 days in media containing IL-4 and/or IL-10 were washed thoroughly and re-plated in IL-3 alone. Flow cytometric analysis revealed that FcεRI inhibition continued for ~5 days after the removal of IL-4 or IL-10 (Fig. 2*C*). These results contrast IL-4- or IL-10-mediated Kit inhibition, which is lost within 24 h of removing the inhibitory stimulus (16).

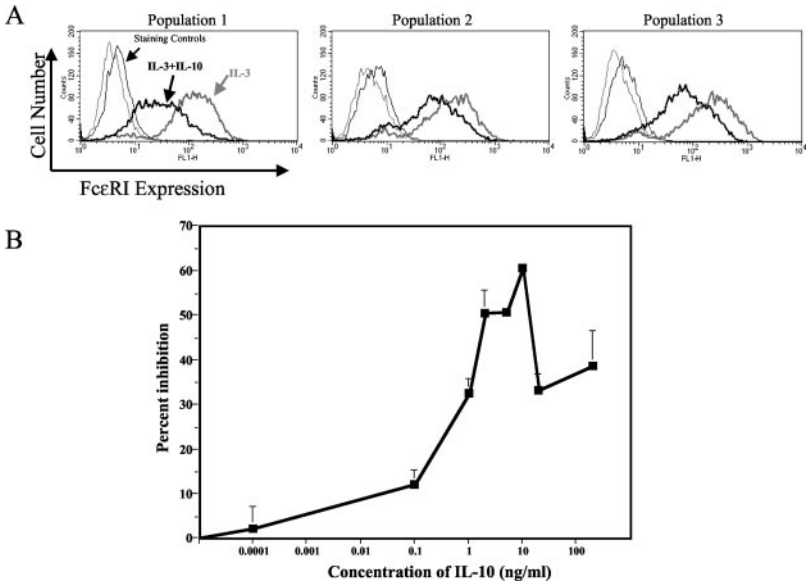

FIGURE 1. *A*, IL-10-mediated inhibition of FcεRI BMMC surface expression. BMMC were treated with IL-3 (5 ng/ml) alone or IL-3 plus IL-10 (10 ng/ml) for 4 days. FcεRI expression was assessed by flow cytometry. Results shown are from three independent BMMC populations that represent data from >80 experiments using >20 different BMMC populations. *B*, Effects of IL-10 dose on FcεRI expression. Percent inhibition and SE measurements were calculated by comparing MFI of IL-3 plus IL-10-treated BMMC to cells cultured in IL-3 alone as described in *Materials and Methods*.

Role of STAT6 in FcεRI down-regulation

We have previously shown that Stat6 expression is required for IL-4-mediated FcεRI down-regulation (15). Though necessary for this signaling event, we have not determined whether Stat6 activation alone is sufficient to decrease IgE receptor expression. To this end, we infected Stat6-deficient BMMC populations with a retrovirus expressing constitutively active Stat6 (Stat6VT) (32) and GFP or with a control retrovirus expressing GFP alone. As shown in Fig. 3*A*, BMMC cultures expressing Stat6 VT for 4 days exhibited FcεRI surface staining that was >50% lower than control cultures. It appears that Stat6 activation is necessary and sufficient to inhibit FcεRI expression.

Because IL-4 activates several signaling pathways, we also assessed the ability of IL-4 to augment IL-10-mediated FcεRI down-regulation in the absence of Stat6. This was accomplished by comparing FcεRI inhibition in cultures of WT and Stat6-deficient BMMC stimulated with IL-10 and/or IL-4. As expected, IL-4-mediated FcεRI repression was entirely Stat6-dependent, while IL-10 signaling, which is not known to involve this transcription factor, was unaltered by loss of Stat6 (Fig. 3*B*). However, Stat6-deficient BMMC were more responsive to IL-4 plus IL-10 than to IL-10 alone, indicating that a Stat6-independent IL-4 signaling pathway augments IL-10 effects.

Effects of IL-10 and IL-4 on FcεRI subunit mRNA and protein expression

RPA measurements of mRNA expression were used to assess transcriptional control of the three FcεRI subunits by IL-4 and IL-10. BMMC were stimulated for 1–7 days with IL-3 alone or with IL-3 plus IL-4 and/or IL-10. The data shown in Fig. 4*A* are from a 4-day stimulation. IL-4 caused a slight but reproducible decrease in FcεRI α and β mRNA expression, with no significant change in γ expression. By contrast, IL-10 conveyed no change in any of the subunits, and did not enhance the effects of IL-4. Similar results were obtained from 7-day cultures, and no inhibition occurred in 1-day cultures (data not shown).

To assess protein expression of the members of the FcεRI complex, we used immunoprecipitation and Western blotting to detect FcεRI α, or Western blotting of total cell lysates to detect the β and γ subunits. Because surface staining with IgE is a measure of FcεRI α expression, we expected to find that IL-4 and IL-10 reduced α subunit levels. However, total FcεRI α expression re-

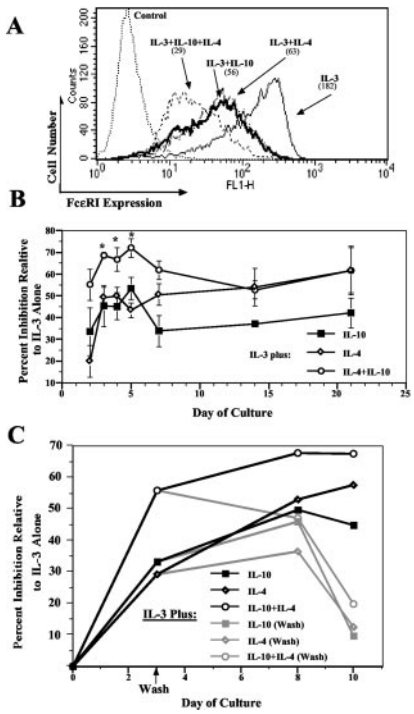

FIGURE 2. *A*, IL-10 enhances IL-4-mediated FcεRI down-regulation. BMMC were treated for 4 days with IL-3 alone, IL-3 plus IL-10, IL-3 plus IL-4, or with IL-3 plus IL-4 plus IL-10 as described in *Materials and Methods*. FcεRI expression was assessed by flow cytometry. Data shown are representative of more than 50 individual experiments. *B*, Time course of FcεRI inhibition by IL-4 and IL-10. BMMC were cultured for 2–21 days, with feeding every 4 days, as described in *Materials and Methods*. Percent inhibition was calculated by comparing MFI of each culture condition to control culture in IL-3 alone. Data shown are from a minimum of three individual BMMC populations. *, $p < 0.025$, when comparing BMMC treated with IL-3 plus IL-4 plus IL-10 to BMMC treated with IL-3 plus IL-4 or with IL-3 plus IL-10, using unpaired Student's *t* test. *C*, FcεRI inhibition persists after removal of IL-4 or IL-10. BMMC were treated with the indicated cytokines as previously described. One set of BMMC (gray lines) was washed on day 3 and replated in IL-3 alone, while a duplicate set was continuously cultured in the original conditions (black lines). Percent inhibition of FcεRI expression was calculated by comparing MFI of each culture condition to control culture in IL-3 alone. Data are means of three individual BMMC populations from one of two representative experiments.

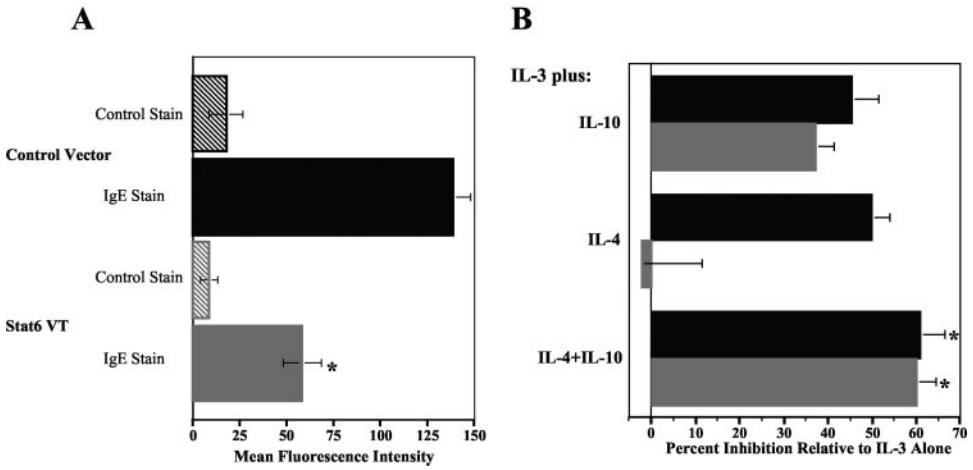

FIGURE 3. The role of Stat6 in FcεRI inhibition. *A*, Expression of constitutively active Stat6 reduces FcεRI expression. Stat6-deficient BMMC were infected with a bicistronic retrovirus expressing either GFP alone (control retrovirus) or coexpressing GFP and Stat6VT. After 4 days of culture in IL-3 (5 ng/ml), cells were stained for FcεRI expression. The MFI obtained from IgE staining of the GFP-positive population from each culture are shown. Data are means and SDs from three samples. ∗, $p < 0.05$ when comparing BMMC coexpressing Stat6VT and GFP to cells expressing GFP only, by unpaired Student's *t* test. *B*, Stat6 is required for IL-4-mediated FcεRI inhibition, but not for IL-4-mediated enhancement of IL-10-driven FcεRI down-regulation. WT (■) and Stat6-deficient (Stat6 knockout; □) BMMC were cultured in the indicated cytokine conditions for 4 days as described in *Materials and Methods*. FcεRI expression was assessed by flow cytometry, and percent inhibition relative to IL-3 alone was determined by comparing MFI. Data shown are means and SE measurements from at least eight individual BMMC populations. ∗, $p < 0.010$, when comparing WT or Stat6-deficient BMMC treated with IL-3 plus IL-4 plus IL-10 to the same cells treated with IL-3 plus IL-4 or with IL-3 plus IL-10, using unpaired Student's *t* test.

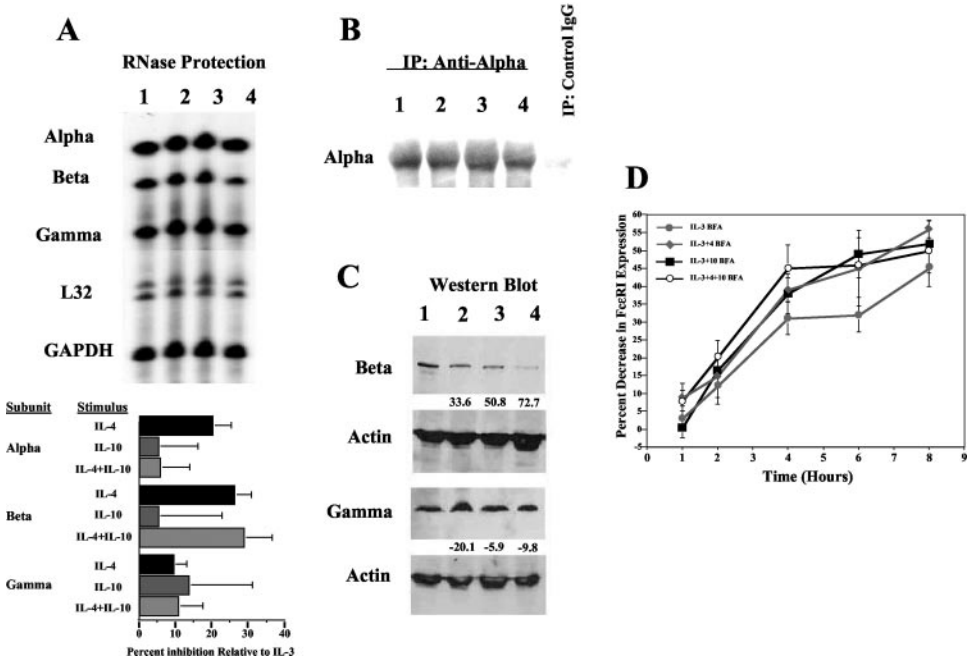

FIGURE 4. Regulation of FcεRI subunits by IL-4 and IL-10. *A*, *Top panel*, RPA was used to assess FcεRI α-, β- and γ-chain mRNA levels following 4 days of culture in: 1) IL-3 alone; 2) IL-3 plus IL-4; 3) IL-3 plus IL-10; or 4) IL-3 plus IL-4 plus IL-10. *Bottom panel*, Summary of IL-4- and IL-10-mediated effects on FcεRI subunit mRNAs. Data shown are the mean and SEM from a minimum of four samples, showing the percent inhibition induced by IL-4 or IL-10, relative to cells cultured in IL-3 alone. Percent inhibition was calculated after accounting for sample loading, as detailed in *Materials and Methods*. *B*, BMMC stimulated for 4 days as in *A* were lysed and immunoprecipitated with anti-FcεRI α-chain mAb or control IgG before Western blotting with anti-FcεRI α as described in *Materials and Methods*. Sample order is the same as *A*. *C*, BMMC were stimulated for 4 days as in *A*. Western blot analysis of whole cell lysates was performed using Abs specific for FcεRI β or γ subunits. These filters were then stripped and reprobed for actin to show sample loading. Data shown are from one of three representative experiments. Numbers below the β and γ blots represent the percent inhibition of expression compared with cells cultured in IL-3 alone. These were calculated by densitometry, using the ratio of β or γ expression to actin for each lane. Percent inhibition is the mean of two sample sets analyzed in the same experiment. *D*, BMMC were cultured in the indicated cytokines for 4 days, after which BFA (5 ng/ml final concentration) was used to inhibit vesicular transport for the periods indicated. FcεRI surface expression was measured by flow cytometry. Percent decrease in expression was determined by comparing the MFI of BFA-treated cells to untreated cells in the same cytokine conditions. Vehicle (ethanol) treatment reduced FcεRI expression by <5% at all time points, and is omitted for clarity. Data shown are from 10 BMMC populations assessed in two experiments.

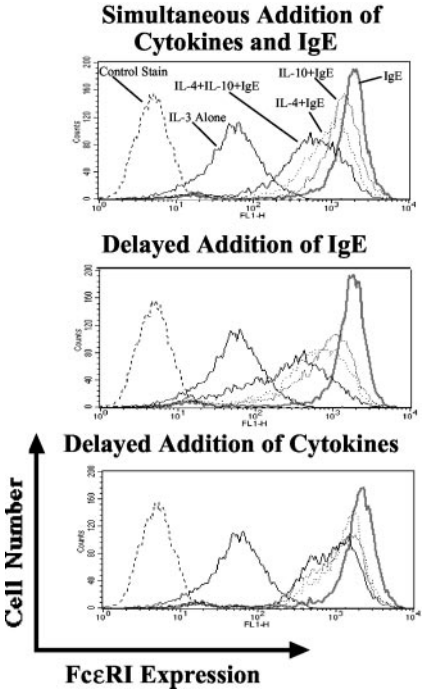

FIGURE 5. IL-4 and IL-10 inhibit IgE-mediated up-regulation of FcεRI. *Top*, BMMC were cultured in IL-3 plus IgE (1 μg/ml) and/or IL-4/IL-10 for 4 days, then assessed for FcεRI expression by flow cytometry. In the *middle* and *bottom panels*, either IgE or IL-4/IL-10 was not added until day 4 of culture, respectively. In these experiments, BMMC were then incubated for an additional 3 days, and assessed for FcεRI expression on culture day 7. Data shown are representative of five separate experiments.

mained constant in all samples (Fig. 4*B*). Similarly, total FcεRI γ expression did not decrease in BMMC stimulated for 4 days with IL-4 and/or IL-10 (Fig. 4*C*). In contrast, β subunit expression consistently decreased in samples stimulated with IL-4 or IL-10, with an additive effect from combined stimulation. Reduced FcεRI β expression was consistent in three separate experiments, indicating that IL-4 and IL-10 selectively target this subunit during FcεRI regulation.

It is possible that IL-4 and IL-10 diminish FcεRI expression through altering protein stability. To determine the effects of IL-4 and IL-10 on the stability of FcεRI surface expression, we assessed the half-life of this complex. Cells were cultured with IL-3 alone or IL-3 plus IL-4 and/or IL-10 for 4 days. On day 4 of culture, the vesicular transport inhibitor BFA or vehicle (ethanol) was added to these cells for an 8-h period, during which surface FcεRI expression was assessed by flow cytometry. Using untreated cells as a comparison, we calculated the percent decrease in FcεRI expression over time. In these assays we found that the cytokine conditions did not alter the rate at which the FcεRI complex was lost from the cell surface, indicating that IL-4 and IL-10 do not alter stability of the surface protein complex (Fig. 4*D*). Similarly, Western blotting experiments using the translation inhibitor cycloheximide showed no alterations in total β subunit protein stability (data not shown). Collectively these experiments indicate that IL-4 and IL-10 reduce FcεRI expression by directing a moderate decrease in α and β mRNAs, and a more profound loss of β-chain protein, with no alteration in protein stability.

IL-10 and IL-4 inhibit IgE-mediated enhancement of FcεRI expression

The ability of IgE to up-regulate FcεRI expression and signaling has been noted in vivo and in vitro (33–35). The time course of this response is similar to the timing of FcεRI inhibition by IL-4 and IL-10. Further, we have shown that IL-4 can diminish the effects of IgE in a dose- and time-dependent manner (15). To assess the functional effects of IL-10-mediated FcεRI down-regulation, we tested its ability to inhibit IgE-mediated FcεRI up-regulation.

BMMC populations were treated with 1 μg/ml IgE before, after, or simultaneously with IL-4 and/or IL-10 (Fig. 5). As expected, IgE dramatically up-regulated FcεRI expression. This >20-fold increase was attenuated by IL-4 or IL-10, with the most overt differences seen by combined stimulation with IL-4 plus IL-10. With this combination, IgE-mediated FcεRI up-regulation was limited to 6- to 12-fold, depending upon the timing of cytokine addition. This represented an approximate 50% inhibition of the IgE response, but under no conditions could IL-4 or IL-10 completely block the increase in FcεRI expression. Further, maximal inhibition of the IgE effect required that IL-4 and IL-10 be present before the addition of IgE. IgE appears to be a potent stimulus for FcεRI up-regulation, an effect that can be partially blocked by IL-4 and IL-10.

Effects of IL-10 and IL-4 on IgE-mediated mast cell activation

To further assess the functional consequence of FcεRI down-regulation, we examined the immediate and late phases of IgE-mediated mast cell activation. Mast cell degranulation, detected within minutes of IgE cross-linkage, can be assessed by measurements of β-hexosaminidase release (31). The late phase of mast cell activation is indicated in part by cytokine secretion. To this end, we cultured BMMC in IL-3 alone or IL-3 with IL-10 and/or IL-4 for 4 days, followed by IgE cross-linkage with Ag (DNP-BSA) or with anti-IgE Abs.

Measurements of β-hexosaminidase release, performed 60 min after cross-linkage, indicated that neither single nor combined stimulation with IL-10 or IL-4 greatly reduced mast cell degranulation (Fig. 6*A*). As we have previously published (15), IL-4 modestly decreased the sensitivity of the IgE response, but had no effect on maximal Ag signaling. Surprisingly, IL-10 consistently increased IgE-mediated degranulation, an effect directly at odds with its down-regulation of receptor expression. The mechanism of this modest but unexpected effect is unknown. These data support the claim that IL-4 and IL-10 reduce surface FcεRI expression but do not inhibit the immediate response characterized by degranulation.

IgE-mediated cytokine responses were measured by TNF-α secretion 24 h after IgE cross-linkage (Fig. 6*B*). We chose this cytokine as an assay tool because we have found BMMC IL-5 production to be extremely low, and because IL-4 was necessarily in the culture media during the experiment. Unlike β-hexosaminidase release, IL-4 and IL-10 reduced TNF-α secretion, with an additive effect of using both cytokines. It appears that IL-4 and IL-10 disrupt the FcεRI late phase response without decreasing the immediate response.

Discussion

Mast cells are critical effectors in Th2-driven allergic responses. Our interest in Th2 cytokines lies in how mast cell function and survival are regulated. Mirroring classic models, we have formed a hypothesis of Th2-cytokine-mediated mast cell homeostasis, in which the signaling molecules both elicit and limit mast cell effector function. In support of this theory, we have found that IL-4 inhibits expression of Kit, a mast cell survival receptor, as well as the FcεRI complex (15, 16). IL-10 appears to play a similar role, blocking Kit expression and combining with IL-4 to induce mast cell apoptosis (16, 28). Using primary mast cell populations, we now report that IL-10 can inhibit FcεRI expression and signaling.

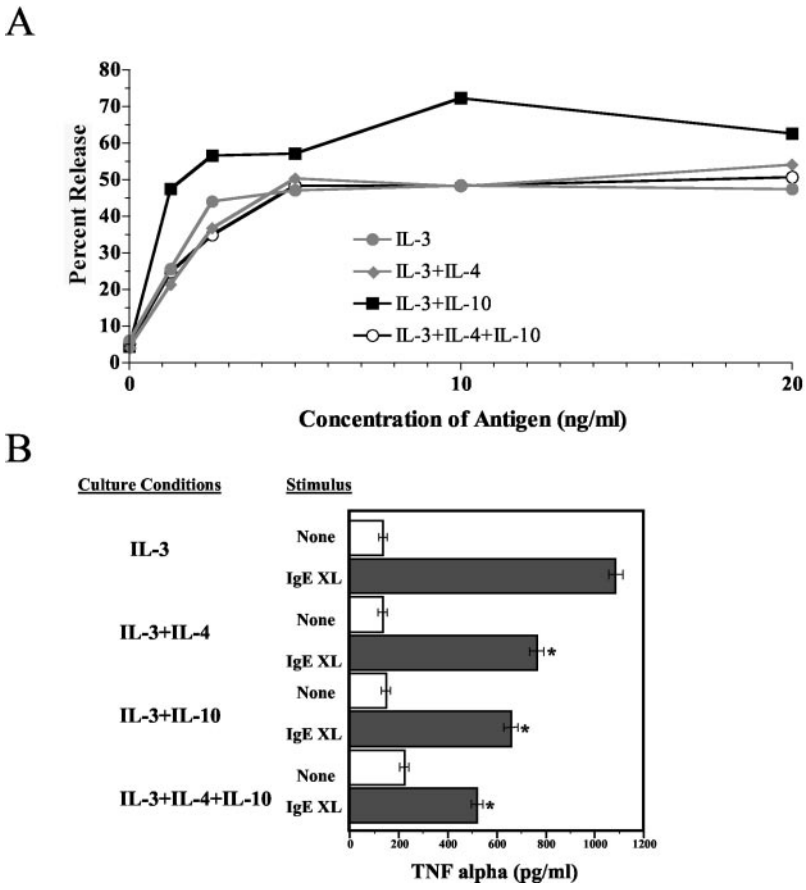

FIGURE 6. Effect of IL-4 and IL-10 on mast cell activation. BMMC were treated with cytokines as previously described for 4 days. *A*, Release of β-hexosaminidase was determined after 1 hr of IgE cross-linkage with IgE (10 μg/ml) and the indicated concentration of DNP-BSA (Ag). *B*, TNF-α secretion into culture supernatants was assessed by ELISA after 24 h of cross-linkage with IgE (10 μg/ml) and rat anti-mouse IgE (10 μg/ml). ∗, $p < 0.05$ using unpaired Student's *t* test.

This effect is not a broad capability of Th2 cytokines, as we have found that IL-5 and IL-13 do not inhibit FcεRI or Kit expression (data not shown). Thus IL-4 and IL-10 combine to limit mast cell function and survival, perhaps limiting the Th2-mast cell response in an attempt to avert pathology.

Central in our studies has been the element of timing: mast cells cultured in IL-4 or IL-10 show no inhibitory effects for 3 days, allowing what we believe is an "inflammatory window" wherein mast cells serve their protective effector roles in immunity. This is followed by an "inhibitory window" defined by reduced mast cell proliferation, limited effector responses, and finally death by apoptosis, noted on day 6 of culture.

Loss of homeostatic constraints lies at the root of many chronic diseases, and atopic diseases are likely no exception. Loss of mast cell homeostasis may be involved in atopic diseases such as allergic asthma, a condition in which mast cells are chronically activated (39, 40). If Th2 cytokines do control mast cell responses, our data would predict that chronic Th2 cytokine production in allergic asthma should prevent the mast cell response. The fact that this is not apparent in asthmatic patients could be used to argue that Th2 cytokines do not inhibit mast cell survival and function in vivo. However, one could also argue that this pathological condition is caused partly by loss of mast cell homeostasis. Support for altered homeostasis in Th2 diseases finds credence in studies of mutations associated with these conditions. Allergic disease-linked polymorphisms of the IL-10 promoter, the IL-4 promoter, and the IL-4R α-chain have been noted, as well as polymorphisms of FcεRI β (27, 13, 14, 41–46). It is possible that such genetic changes may prevent Th2-mediated regulation of mast cell survival and/or FcεRI regulation, though such effects are unknown. It is interesting to note that two recent papers find that IL-4 can have potent inflammatory or inhibitory effects on allergic inflammation in vivo (61, 62). Though much remains to be understood, it is becoming clear that Th2 cytokines can positively or negatively modulate the mast cell response.

IL-10 fits the description of a homeostatic cytokine more aptly than IL-4. First described as cytokine synthesis inhibitory factor, IL-10 is widely known to modulate many immune effects so as to hamper immune pathology, particularly with regard to macrophage function (reviewed in Refs. 17 and 18). IL-10 has been shown to regulate mast cell function, proliferation, survival, development, and protease gene expression (23–26). Our observation that IL-10 mimicked the IL-4-mediated inhibition of mast cell Kit expression (15), combined with IL-4 plus IL-10-induced mast cell apoptosis (28) made FcεRI studies logical.

We show that IL-4 and IL-10 regulate FcεRI expression largely at the level of β-chain protein production. The α subunit has been shown to exist as a cellular "pool" in some cells (54) explaining its continued expression even as IL-4 and IL-10 decrease surface FcεRI levels by 70%. Also, FcεRI γ can pair with FcγRIII (55), preventing its degradation. The effects of IL-4 and IL-10 on FcεRI β mRNA were more modest, and no change in protein stability was found. Our current work is focused on the hypothesis that IL-4 and IL-10 alter the expression or activation of mRNA binding proteins that regulate β-chain translation, resulting in reduced protein synthesis.

Targeting of FcεRI β is unique, but has correlates and predictable outcomes. For example, loss of FcεRI β should reduce competition for the γ subunit (55–58). Accordingly, we have observed increased FcγRIII expression and signaling coincidental with FcεRI down-regulation (63). Kinet's laboratory convincingly showed that FcεRI β amplifies both the expression and signaling of the IgE receptor (55–58). Given the novel role of the β-chain as an FcεRI amplifier and the presence of β-chain polymorphisms in

atopic families (41–43), the idea that Th2 cytokines may be selectively targeting this subunit for regulation is very intriguing and warrants further study.

While searching for the mechanism of FcεRI regulation, Stat6 was a logical starting place. It is interesting that Stat6 activation was found to be both necessary and sufficient to inhibit FcεRI expression in this study. However, the IL-4 signaling pathway that augments IL-10-mediated FcεRI inhibition did not require Stat6 expression. The identity of this IL-4 signaling pathway is under investigation. Further, we have not been able to assess the levels of Stat6VT expression in the small numbers of transduced mast cells. Therefore the Stat6VT results need to be viewed with the caveat that signals in addition to endogenous Stat6 may be essential for FcεRI regulation under normal conditions.

Generally protein-level regulation is thought to make a system more nimble, able to vary expression levels quickly under changing conditions. However, FcεRI regulation by IL-4 and IL-10 defies this norm: loss of FcεRI expression requires 3–4 days of stimulation, and this effect persists several days after IL-4 and IL-10 removal. Together these data offer a view of FcεRI regulation in which longstanding alterations in expression are accomplished through methods generally reserved for rapidly changing systems.

The functional consequences of FcεRI down-regulation were mixed. Although IL-4 plus IL-10 decreased FcεRI expression as much as 80%, we found no inhibition of IgE-mediated mast cell degranulation. In fact, IL-10 offered a modest but consistent increase in this response. IL-4 and IL-10 did decrease TNF-α production. Although TNF-α is reportedly stored in mast cell granules (60), it is unlikely that the reduction in TNF production is related to defective degranulation. First, the aforementioned β-hexosaminidase release assays revealed no such defect, and second, we have found these BMMC to secrete very little TNF-α during the first hour of IgE stimulation in any cytokine combination (data not shown). Thus TNF-α appears to be predominantly newly synthesized in these BMMC populations. These data collectively argue that IL-4 and IL-10 alter the late phase mast cell response without affecting the early response. These results fit well with a recent report by Gonzalez-Espinosa and colleagues (64), demonstrating that mast cell degranulation requires greater FcεRI signaling than does cytokine secretion. Following this theory, reduced FcεRI expression after culture in IL-4 or IL-10 may force IgE receptor levels below the threshold needed for cytokine production without altering degranulation.

In conclusion, we demonstrate that IL-10 is a potent inhibitor of FcεRI expression and function, effects that are amplified by IL-4. Together these Th2 cytokines can limit mast cell function and survival. These data support our on-going study of mast cell homeostasis and offer a new means by which Th2 cytokines may control this critical effector cell in the allergic response.

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References

1. Ryan J. J., and T. F. Huff. Biology of mast cells. In *Middleton's Allergy: Principles and Practice.* N. Franklin Adkinson, ed. Mosby, St. Louis. *In press.*
2. Brenner, T., D. Soffer, M. Shalit, and F. Levi-Schaffer. 1994. Mast cells in experimental allergic encephalomyelitis: characterization, distribution in the CNS, and in vitro activation by myelin basic protein and neuropeptides. *J. Neurosci. 122:210.*
3. Secor, V. H., W. E. Secor, C. A. Gutekunst, and M. A. Brown. 2000. Mast cells are essential for early onset and severe disease in a murine model of multiple sclerosis. *J. Exp. Med. 191:813.*
4. Lee, D. M., D. S. Friend, M. F. Gurish, C. Benoist, D. Mathis, and M. B. Brenner. 2002. Mast cells: a cellular link between autoantibodies and inflammatory arthritis. *Science 297:1689.*
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.*
6. Hara, M., K. Ono, M. Hwang, A. Iwasaki, M. Okada, K. Nakatani, S. Sasayama, and A. Matsumori. 2002. Evidence for a role of mast cells in the evolution of congestive heart failure. *J. Exp. Med. 195:375.*
7. Blank, U., C. Ra, L. Miller, K. White, H. Metzger, and J. P. Kinet. 1989. Complete structure and expression in transfected cells of high affinity IgE receptor. *Nature 337:187.*
8. Ra, C., M. H. Jouvin, and J. P. Kinet. 1989. Complete structure of the mouse mast cell receptor for IgE (FcεRI) and surface expression of chimeric receptors (rat-mouse-human) on transfected cells. *J. Biol. Chem. 264:15323.*
9. Kinet, Jean-Pierre, S. C. Garman, and T. S. Jardetzky. 1999. On the high-affinity IgE receptor (FcεRI): from physiology to pathology. *Annu. Rev. Immunol. 17: 931.*
10. Mosmann, T. R., M. W. Bond, R. L. Coffman, J. Ohara, and W. E. Paul. 1986. T-cell and mast cell lines respond to B-cell stimulatory factor-1. *Proc. Natl. Acad. Sci. USA 83:5654.*
11. Toru. H., C. Ra, S. Nonoyama, K. Suzuke, J. Yata, and T. Nakahata. 1996. Induction of the high affinity IgE receptor (FcεRI) on human mast cells by IL-4. *Int. Immunol. 8:1367.*
12. Hu, H. Z., Z. Du, S. Craig, G. Klisch, N. Noben-Trauth, J. P. Kochan, T. F. Huff, A. M. Irani, and L. B. Schwartz. 1997. Effect of recombinant IL-4 on tryptase, chymase, and Fcε receptor type I expression in recombinant human stem cell-factor dependent fetal liver-derived human mast cells. *J. Immunol. 150:2911.*
13. Khurana-Hershey, G., M. F. Friedrich, L. A. Esswein, M. L. Thomas, and T. A. Chatila. 1997. The association of atopy with a gain-of-function mutation in the human interleukin-4 receptor. *N. Engl. J. Med. 337:1720.*
14. Mitsuyasu, H., K. Izuhara, X. Q. Mao, P. S. Gao, Y. Arinobu, T. Enomoto, M. Kawai, S. Sasaki, Y. Dake, N. Hamasaki, et al. 1998. Ile50Val variant of IL4R α upregulates IgE synthesis and associates with atopic asthma. *Nat. Genet. 19: 119.*
15. 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.*
16. Mirmonsef, P., C. P. Shelburne, C. F. Yeatman II, 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.*
17. Moore K. W., A. O'Garra, R. de Waal Malefyt, P. Vieira, and T. Mosmann. 1993. Interleukin-10. *Annu. Rev. Immunol. 11:165.*
18. Donnelly, R. P., H. Dickensheets, and D. S. Finbloom. 1999. The interleukin-10 signal transduction pathway and regulation of gene expression in mononuclear phagocytes. *J. Interferon Cytokine Res. 19:563.*
19. Marietta, E. V., Y. Chen, and J. H. Weis. 1996. Modulation of expression of the anti-inflammatory cytokines interleukin-13 and interleukin-10 by interleukin-3. *Eur. J. Immunol. 26:49.*
20. 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.*
21. Marshall, J. S., I. Leal-Berumen, L. Nielsen, M. Gilbetic, and M. Jordana. 1996. IL-10 inhibits long-term IL-6 production but not preformed mediator release from rat peritoneal mast cells. *J. Clin. Invest. 97:1122.*
22. Arock, M., C. Zuany-Amorim, M. Singer, M. Benhamou, and M. Pretolani. 1996. IL-10 inhibits cytokine generation from mast cells. *Eur. J. Immunol. 26:166.*
23. Ghildyal, N., H. P. McNeil, M. F. Gurish, K. F. Austen, and R. L. Stevens. 1992. Transcriptional regulation of the mucosal mast cell-specific protease gene, MMCP-2, by interleukin-10 and interleukin-3. *J. Biol. Chem. 267:8473.*
24. 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.*
25. Rennick, D., B. Hunte, W. Dang, L. Thompson-Snipes, and S. Hudak. 1994. Interleukin-10 promotes the growth of megakaryocyte, mast cell, and multilineage colonies: analysis with committed progenitors and Thy1^{low}Sca1^{+} stem cells. *Exp. Hematol. 22:136.*
26. Rennick, D., B. Hunte, G. Holland, and L. Thompson-Snipes. 1995. Cofactors are essential for stem cell factor-dependent growth and maturation of mast cell progenitors: comparative effects of interleukin-1 (IL-3), IL-4, IL-10, and fibroblasts. *Blood 85:57.*
27. 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.*
28. Yeatman II, C. F., 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.*
29. Zhu, J., L. Guo, C. J. Watson, J. Hu-Li, and W. E. Paul. 2001. Stat6 is necessary and sufficient for IL-4's role in Th2 differentiation and cell expansion. *J. Immunol. 166:7276.*
30. Shelburne, C. P., R. P. Piekorz, L. A. Bouton, H. J. Chong, and J. J. Ryan. 2002. Mast cell-restricted p70 Stat6 isoform is a product of selective proteolysis. *Cytokine 19:218.*
31. Schwartz L. B., K. Austen, S. I. Wasserman. 1979. Immunological release of β-hexosminidase and β-glucuronidase from purified rat serosal mast cells. *J. Immunol. 123:1445.*
32. Daniel, C., A. Salvekar, and U. Schindler. 2000. A gain-of-function mutation in STAT6. *J. Biol. Chem. 275:14255.*

33. Malveaux, F. J., M. C. Conroy, N. F. Adkinson Jr., and L. M. Lichtenstein. 1978. IgE receptors on human basophils: relationship to serum IgE concentration. *J. Clin. Invest. 62:176.*
34. Furuichi, K., J. Rivera, and C. Isersky. 1985. The receptor for immunoglobulin E on rat basophilic leukemia cells: effect of ligand binding on receptor expression. *Proc. Natl. Acad. Sci. USA 82:1522.*
35. Yamaguchi, M., C. S. Lantz, H. C. Oettgen, I. M. Katona, T. Fleming, I. Miyajima, J. P. Kinet, and S. J. Galli. 1997. IgE enhances mouse mast cell FcεRI expression in vitro and in vivo: evidence for a novel amplification mechanism in IgE-dependent reactions. *J. Exp. Med. 185:663.*
36. Friend, D. S., M. F. Gurish, K. F. Austen, J. Hunt, and R. Stevens. 2000. Senescent jejunal mast cells and eosinophils in the mouse preferentially translocate to the spleen and draining lymph node, respectively, during the recovery phase of helminth infection. *J. Immunol. 165:344.*
37. Urban, J. E., N. Noben-Trauth, D. D. Donaldson, K. B. Madden, S. C. Morris, M. Collins, and F. D. Finkelman. 1998. IL-13, IL-4Rα, and STAT6 are required for the expulsion of the gastrointestinal nematode parasite *Nippostrongylus brasiliensis. Immunity 8:255.*
38. Chen H. J., N. Lycke, and L. Enerback. 1999. Surface and gene expression of immunoglobulin E receptors on mast cells and mast-cell numbers in interleukin-4-gene knockout mice. *Immunology 96:544.*
39. Broide, D. H., G. J. Gleich, A. J. Cuomo, D. A. Coburn, E. C. Federman, L. B. Schwartz, and S. I. Wasserman. 1991. Evidence of ongoing mast cell and eosinophil degranulation in symptomatic asthma airway. *J. Allergy Clin. Immunol. 88:637.*
40. Jarjour, N. N., W. J. Calhoun, L. B. Schwartz, and W. W. Busse. 1991. Elevated bronchoalveolar lavage fluid histamine levels in allergic asthmatics are associated with increased airway obstruction. *Am. Rev. Respir. Dis. 144:83.*
41. Hill, M. R., and W. O. Cookson. 1996. A new variant of the β-subunit of the high affinity receptor for immunoglobulin E (FcεRI-β E237G): associations with measures of atopy and bronchial hyper-responsiveness. *Hum. Mol. Genet. 5:959.*
42. Hill M. R., A. James, J. Faux, G. Ryan, J. Hopkin, P. le Souef, A. Musk, and W. O. Cookson. 1995. FcεRI-β polymorphism and risk of atopy in a general population samples. *Br. Med. J. 311:776.*
43. Shirakawa T., A. Li, M. Dubowitz, J. Dekker, A. Shaw, J. Faux, C. Ra, W. Cookson, and J. Hopkin. 1994. Association between atopy and variants of the β subunit of the high affinity immunoglobulin E receptor. *Nat. Genet. 7:125.*
44. Suzuki, I., N. Hizawa, E. Yamaguchi, and Y. Kawakami. 2000. Association between a C + 33T polymorphism in the IL-4 promoter region and total serum IgE levels. *Clin. Exp. Allergy 30:1746.*
45. Takabayashi, A., K. Ihara, Y. Sasaki, Y. Suzuki, S. Nishima, K. Izuhara, N. Hamasaki, and T. Hara. 2000. Childhood atopic asthma: positive association with a polymorphism of IL-4 receptor α gene but not with that of IL-4 promoter or FcεRI β gene. *Exp. Clin. Immunogenet. 17:63.*
46. Kawashima, T., E. Noguchi, T. Arinami, K. Yamakawa-Kobayashi, H. Nakagawa, F. Otsuka, and H. Hamaguchi. 1998. Linkage and association of an interleukin 4 gene polymorphism with atopic dermatitis in Japanese families. *J. Med. Genet. 35:502.*
47. Garriga, M. M., M. M. Friedman, and D. D. Metcalfe. 1988. A survey of the number and distribution of mast cells in the skin of patients with mast cell disorders. *J. Allergy Clin. Immunol. 82:425.*
48. Nagata, H., A. S. Worobec, C. Oh, B. A. Chowdhury, S. Tannebaum, Y. Suzuki, and D. D. Metcalfe. 1995. Identification of a point mutation in the catalytic domain of the protooncogene *Kit* in peripheral blood mononuclear cells of patients who have mastocytosis with associated hematologic disorder. *Proc. Natl. Acad. Sci. USA 92:10560.*
49. Tsujimura, T., T. Furitsu, M. Morimoto, K. Isozaki, S. Nomura, Y. Matsuzawa, Y. Kitamura, and Y. Kanakura. 1994. Ligand independent activation of Kit receptor tyrosine kinase in a murine mastocytoma cell line P815 generated by a point mutation. *Blood 83:2619.*
50. Vieira P., R. de Waal-Malefyt, M. N. Dang, K. E. Johnson, R. Jastelein, D. F. Fiorentino, J. E. deVries, M. G. Roncarolo, T. R. Mosmann, and K. W. Moore. 1991. Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones: homology to Epstein-Barr virus open reading frame BCRFI. *Proc. Natl. Acad. Sci. USA 88:1172.*
51. Schlaak, J. F., E. Schmitt, C. Huls, K. H. Meyer zum Buschenfelde, and B. Fleischer. 1994. A sensitive and specific bioassay for the detection of human interleukin-10. *J. Immunol. Methods 168:49.*
52. Ho, A. S., Y. Liu, T. A. Khan, D. H. Hsu, J. F. Bazan, and K. W. Moore. 1993. A receptor for interleukin 10 is related to interferon receptors. *Proc. Natl. Acad. Sci. USA 90:11267.*
53. Tan, J. C., S. R. Indelicato, S. K. Narula, P. J. Zavodny, and C. C. Chou. 1993. Characterization of interleukin-10 receptors on human and mouse mast cells. *J. Biol. Chem. 268:21053.*
54. Kraft S., J. H. Wessendorf, D. Hanau, and T. Bieber. 1998. Regulation of the high affinity recepotr for IgE on human epidermal Langerhans cells. *J. Immunol. 161: 1000.*
55. Takai, R., M. Li, D. Sylvestre, R. Clynes, and J. V. Ravetch. 1994. FcR γ chain deletion results in pleiotrophic effector cell defects. *Cell 76:519.*
56. Lin, S., C. Cicala, A. M. Scharenberg, and J. P. Kinet. 1996. The FcεRI β subunit functions as an amplifier of FcεRI mediated cell activation signals. *Cell 85:22076.*
57. Dombrowicz, D., S. Lin, V. Flamand, A. Brini, B. Koller, and J. P. Kinet. 1998. Allergy-associated FcR β is a molecular amplifier of IgE- and IgG-mediated in vivo responses. *Immunity 8:517.*
58. Donnadieu, D., M. H. Jouvin, and J. P. Kinet. 2000. A second amplifier function for the allergy-associated *FcεRI-β* subunit. *Immunity 12:515.*
59. Ryan, J. J., C. A. Kinzer, and W. E. Paul. 1995. Mast cells lacking the high affinity immunoglobulin E receptor are deficient in FcεRI γ messenger RNA. *J. Exp. Med. 182:567.*
60. Gordon, J. R., and S. J. Galli. 1990. Mast cells as a source of both preformed and immunologically inducible TNF-α/cachectin. *Nature 346:274.*
61. Strait, R. T., S. C. Morris, K. Smiley, J. F. Urban, and F. D. Finkelman. 2003. IL-4 exacerbates anaphylaxis. *J. Immunol. 170:3835.*
62. Suzuki, K., H. Nakajima, K. Ikeda, Y. Maezawa, A. Suto, H. Takatori, Y. Saito, and I. Iwamoto. 2003. IL-4-Stat6 signaling induces tristetraprolin expression and inhibits TNFα production in mast cells. *J. Exp. Med. 198:171.*
63. Chong, H. J., L. A. Bouton, D. P. Bailey, H. V. Wright, C. D. Ramirez, A. Gharse, C. Oskeritzian, H. Z. Xia, J. Zhu, W. E. Paul, et al. 2003. IL-4 selectively enhances FcγRIII expression and signaling on mouse mast cells. *Cell Immunol. 224:65.*
64. 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.*