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# Interleukin-10 Suppresses Mast Cell IgE Receptor Expression And Signaling In Vitro And In Vivo

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# INTERLEUKIN-10 SUPPRESSES MAST CELL IgE RECEPTOR EXPRESSION AND SIGNALING *IN VITRO* AND *IN VIVO*

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

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# ABSTRACT


INTERLEUKIN-10 SUPPRESSES MAST CELL IgE RECEPTOR EXPRESSION AND SIGNALING IN VITRO AND IN VIVO

By Sarah Kennedy, B.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

Virginia Commonwealth University, 2007

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**Background:** Mast cells are known for their role in allergy, asthma, and systemic anaphylaxis, and have been shown to play a role in inflammatory disease. Interleukin-10 can regulate inflammatory responses both in vitro and in vivo, and may be a natural regulator of mast cell activation.

**Objective:** To examine Interleukin-10 mediated regulation of FcεRI expression and related downstream signaling molecules, and to determine how this affects mast cell function in vitro and in vivo.

**Methods:** Mast cell FcεRI expression was evaluated with and without IL-10 treatment in human lung and skin mast cells, and on peritoneal mast cells from mice overexpressing IL-10 via injection or a transgenic model. Mast cell function was evaluated by observing responses of IL-10 treated mice to passive systemic anaphylaxis.

**Results:** Interleukin-10 inhibited FcεRI expression on mouse and human mast cells, both in vitro and in vivo. IL-10 also suppressed expression of the key signaling molecules Syk, Fyn, Akt and Stat5. Mice chronically overexpressing IL-10 had a reduced response to passive systemic anaphylaxis, indicating impaired mast cell activation.

**Conclusion:** Interleukin-10 suppresses mast cell FcεRI expression in vitro and in vivo, and reduces IgE-mediated activation. The anti-inflammatory effects of IL-10 may relate to its suppression of critical signaling molecules.

**Clinical Implications:** Interleukin-10 polymorphism is associated with increased IgE levels and incidence of atopic disease; hence IL-10 dysregulation may affect atopic etiology. Further, IL-10 therapy is a possible treatment for atopic allergy and asthma.

# Interleukin-10 Suppresses Mast Cell IgE Receptor Expression and Signaling *in vitro* and *in vivo*

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## INTRODUCTION

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.

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 alpha chain that binds IgE, a beta chain that amplifies receptor signaling,[8] and two gamma chains that are responsible for signal initiation.[9] FcεRI aggregation by multivalent antigen leads to degranulation, production of arachidonic acid metabolites, and cytokine secretion, which collectively elicit inflammation. Recently the signaling cascades linking

IgE-antigen interactions with cellular activation have been characterized as two major branches. The best characterized is activation of the tyrosine kinases Lyn and Syk, leading to phosphorylation of the adapter protein LAT. This pathway principally leads to activation of the Ras-MAPK and PLC gamma-PKC cascades. A second branch of the FcεRI signaling cascade is triggered by Fyn phosphorylation, which activates the PI-3 kinase 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] While 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 Interleukin-10 (IL-10), which belongs to a family of related cytokines, including IL-19, IL-20, IL-22, IL-24, and IL-26.[12] IL-10 is produced by cells involved in the allergic response, including T cells and mast cells. Its well-documented ability to inhibit macrophage activation and antigen 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 anaphylaxis. Our findings indicate that IL-10 suppresses FcεRI expression and function both in vitro and in vivo and suggest that this cytokine may function as a natural regulator of the mast cell response.

## MATERIALS AND METHODS

*BMMC Cultures*

BMMC were derived from C57BL/6 and C57BL/6x129 mice by culture in complete RPMI (cRPMI) 1640 medium (Invitrogen Life Techonologies) (10% FBS, 2mM L-glutamine, 100 U/ml penicillin, 100 ug streptomycin, 1mM sodium pyruvate, and 1MM HEPES; Biofluids), supplemented with 30% WEHI-3 cell conditioned medium as described previously.[26] Stat3 fl and Tie2-Cre mice were generously provided by Drs. Takeda and Koni[27, 28] and were bred to yield fl/Δ Cre+ and Cre–littermates as described.[29]

*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. Skin-derived mast cells were prepared as described.[30] After removing s.c. fat by blunt dissection, residual tissue is 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 x *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 x $10^6$ cells/ml in serum-free AIM-V medium (Life Technologies) containing 100 ng/ml of 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 months and were >95% 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).

*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 (Minneapolis, MN). Murine SCF was purchased from PeproTech (Rocky Hill, NJ). Cell Permeable Stat3 inhibitor peptide (Stat3i) was purchased from Calbiochem (San Diego, CA). Akt, p-Akt, Stat5, p-Stat5, Lyn, Syk antibodies were purchased from Cell Signaling (Danvers, MA). Phospho-p38 was purchased from New England Biolabs (Beverly, MA). β-actin was purchased from Sigma (St. Louis, MO). 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 (San Diego, CA). PE-conjugated Rat IgG2b isotype control and PE-conjugated anti-mouse IgE were purchased from eBioscience (San Diego, CA).

*Cell 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 hours in cRPMI without cytokines. Cells were then plated at $3x10^5$ cells/ml, 200µl/well in 96-well flat-bottom plates. IL-3 was added to 5ng/ml, and SCF to 50 ng/ml, followed by IL-10 and/or Stat3i. IL-10 was used at 10 ng/ml, Stat3i was used at 5µM final concentration. Cultures were incubated for the indicated times. Every 4 days, half of the media 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 the Aim V-based media described above.

*Flow Cytometric Analysis*

To detect expression of FcεRI on BMMC, cell pellets were incubated with 10µl 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 antibodies for 30 min at 4°C, washed twice, and analyzed with a Becton Dickinson FACScan (Becton Dickinson, San Jose, CA). Control samples were stained with PE-IgG in place of anti-IgE.

*Western blot analysis*

Western blotting was performed with 50 µg of total cellular protein per sample. Protein was loaded and separated over a 10% polyacrylamide gel (Biorad, Hercules, CA, USA). Proteins were transferred to nitrocellulose (Pall Corporation, Ann Arbor, MI, USA), and blocked for 30 minutes in 5% Non-Fat Dry Milk (NFDM) in Tris Buffered Saline plus .1% Tween (TBST) (1.21 g Tris Base, 9.0 g NaCl, pH = 7.4, QS to 1L). Blots were incubated in 5% NFDM/TBST with a 1:1000 dilution of Akt, p-Akt, Lyn, Syk, Stat5, p-Stat5 or p38; or with a 1:5000 dilution of β-actin overnight at 4° with gentle rocking. Blots were washed five times for 10 min each in TBST, followed by incubation in 5% NFDM/TBST containing a 1:2000 dilution of HRP-linked anti-rabbit IgG (Cell signaling, Danvers, MA), or HRP-linked rabbit anti-mouse IgG at 1:2000 (p38) or 1:10,000 (β-actin) (Jackson ImmunoResearch, West Grove, PA). Size estimates for proteins were obtained with molecular weight standards from Bio-Rad (Hercules, CA).

*Enzyme-linked immunosorbant assay (ELISA)*

IL-10, TNF-α, IL-13, 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 (San Diego, CA). MIP-1α and IL-13 kits were purchased from R&D Systems (Minneapolis, MN). The histamine kit was purchased from Neogen Corporation (Lexington, KY).

*IL-10 injection and ex-vivo mast cell activation assay*

C57Bl/6 X129 mice were injected intraperitoneally twice daily for 5 days with 2 µg IL-10/injection (Peprotech, Rocky Hill, NJ) in 100µl sterile PBS. Injections were 8 hours apart, with the last injection 3 hours prior to sacrificing animals. Peritoneal cells were

harvested, re-suspended at $2x10^6$ 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 purified mouse IgE (10µg/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 purified anti-mouse IgE (10 µg/ml) for 16 – 24 h. Cytokines were measured by ELISA.

*Passive Systemic Anaphylaxis*

C57BL/6 retired breeders were injected intraperitoneally (i.p.) with 50 µg DNP-specific mouse IgE. Mice were injected i.p. 16 h later with 100 µg DNP-Albumin in PBS (Sigma, St. Louis, MO). Body temperature was measured every 10 minutes for 30 minutes total by rectal probe. Mice were sacrificed, and blood was collected by cardiac puncture for serum analysis.

## RESULTS

*Interleukin-10 inhibited FcεRI expression on murine and human mast cells*

Because the IgE receptor can transduce signals leading to mast cell degranulation, we have previously investigated the effects of IL-10 on FcεRI expression.[25] While we found that IL-10 suppressed murine mast cell FcεRI expression, we have not assessed its effects on human mast cells. As shown in Fig. 1A, addition of IL-10 to murine mast cell cultures decreased FcεRI expression approximately 50%, as we have previously reported.[25] We next examined whether this result could be reproduced with human mast cells. Addition of IL-10 to human cell cultures significantly decreased FcεRI expression in

human mast cells isolated from either lung (Fig.1B) or skin (Fig. 1C). These results indicated that IL-10 is consistently able to suppress mast cell FcεRI expression.

*IL-10-mediated FcεRI suppression requires STAT3 expression*

In macrophages, IL-10 effects are largely dependent on signal transducer and activator of transcription (STAT) 3.[32] To determine the importance of Stat3 in IL-10 effects on mast cells, we first investigated a population of STAT3-deficient mouse mast cells. We noted that these STAT3-deficient cells did not develop into a uniform mast cell population, retaining some FcεRI-negative cells unlike the wild type control population. Despite this, we found that FcεRI–positive STAT3-deficient mast cells cultured ±IL-10 showed no change in FcεRI expression. By comparison, mast cells derived from littermates showed the expected decrease in FcεRI expression when treated with IL-10 (Fig. 2A). The importance of Stat3 was further supported by treating wild type mouse BMMC with a cell-permeable STAT3 inhibitor (Stat3i). The addition of STAT3i to BMMC prevented IL-10-mediated FcεRI suppression, corroborating our results with Stat3-deficient BMMC. Collectively these data demonstrate the importance of Stat3 in IL-10-mediated IgE receptor inhibition.

*IL-10 decreased Syk, Fyn, Akt, and Stat5 expression.*

In addition to its effects on FcεRI expression, we reasoned that IL-10 may affect the expression or activation of signaling molecules activated by the IgE receptor. Since 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 tested for the effects of IL-10 on Stat5.

After culture for 4 four days in IL-3±IL-10, we assessed protein expression by western blot analysis. Since Lyn is known to be a negative regulator of mast cell function,[33-35] it was interesting to note that IL-10 treatment had no effect on Lyn expression (Fig. 3A). In contrast, IL-10 significantly suppressed expression of the activating kinases Syk and Fyn (Fig. 3A). 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 stiumulated with IgE+antigen for 5-15 minutes. Total and phosphorylated protein expression were measured by Western blotting. As expected, FcεRI-mediated Stat5 and Akt phosphorylation were clearly inhibited by IL-10. In contrast, p38 activation was unchanged (Fig. 3B). 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. 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 mast cell function, 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. 4A). Peritoneal mast cells taken

from IL-10-injected mice demonstrated lower FcεRI expression compared to PBS-injected littermates (Fig. 4B), 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=.78).

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. Peritoneal mast cells from IL-10-injected mice produced significantly less IL-13 and TNFα than their PBS-injected littermates, indicating that IL-10 inhibited FcεRI function (Fig. 4C and 4D). We also assessed the effects of IL-10 using an in vivo model of anaphylaxis. Mice were subjected to passive systemic anaphylaxis after four days of PBS or IL-10 injections. Consistent with our earlier findings that IL-10 does not alter BMMC degranulation,[25] IL-10 injection had no effect on body temperature loss or histamine release (Figure 4E, and data not shown). In contrast, IL-10 significantly decreased serum MIP-1α levels (Fig.4F). This data indicate that IL-10 may have little effect on the early phase of mast cell activation, but is more effective at inhibiting the late phase response.

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

While 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 employed 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. 5A). We noted a trend toward higher IgE levels in naïve IL-10 transgenic mice compared to 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.[36, 37] Despite the fact that elevated IgE levels are known to enhance FcεRI expression,[38] CD68-IL-10Tg mice demonstrated a consistent reduction in FcεRI expression on peritoneal mast cells compared to littermates (Fig. 5B). To further assess the effects of IL-10 overexpression on mast cell function *in vivo*, we employed passive systemic anaphylaxis (PSA). CD68-IL-10Tg mice demonstrated a reduced anaphylactic response compared to littermates, as measured by change in body temperature 30 minutes after antigen challenge (Fig. 5C). Like IL-10 injection, IL-10 transgene expression appeared to suppress the late phase of mast cell activation. CD68IL-10Tg mice showed no difference in the elevation of serum histamine levels following antigen stimulation compared to littermates, but had much lower serum MIP-1α levels.

## 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 (SIDS), due to elevated postmortem β-tryptase levels in some SIDS victims.[39]

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.[40, 41] This protective function stems from rapid release of TNFα after contact with bacterial ligands, and subsequent neutrophil recruitment. [40, 41] 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 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.[42] Our previous data show that these cytokines suppress FcεRI expression and function in vitro, and subsequently induce mast cell apoptosis. [25, 26, 43-45] 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.[46, 47]

Our previous work with IL-10 utilized mouse mast cells and in vitro assays. In the current study we demonstrate the consistency of IL-10-mediated suppression, showing IL-10-mediated FcεRI downregulation on human skin and lung mast cells. Moreover, IL-10 injection suppressed mast cell FcεRI expression in vivo, despite the presence of IgE

as an opposing force. This consistency is important, since some cytokine effects vary between species. For example, IL-4 suppresses FcεRI expression on mouse mast cells,[26] but has the reverse effect on terminally differentiated human mast cells.[48] 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.

It should be made clear that the FcεRI suppression we observed in vivo may be at best a partial explanation for suppression of 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 more than 90%.[49, 50] 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.

In contrast to its partial effects on FcεRI expression, IL-10 potently and selectively suppressed signal transduction pathways linked to FcεRI. 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.[34, 35] In contrast, Fyn and Syk represent the apical end of IgE signaling, while Akt and Stat5 are tightly linked to mast cell cytokine production.[11, 35] The consistent ability of IL-10 to act on human mast cells and in vivo justifies our current 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.

Since IL-10 polymorphisms correlate with increased incidence of atopic disease,[51] it is tempting to speculate that loss of IL-10-mediated suppression is part of disease etiology, and that IL-10 normally functions to dampen mast cell activation. Our in vivo experiments offered a complement to this theory, showing that raising IL-10 levels via injection or transgene expression suppressed mast cell function. Consistent with our previous in vitro studies,[25] IL-10 had no effect on histamine release, but suppressed MIP-1$\alpha$ release in response to antigen challenge. We chose to measure MIP-1alpha because it is a potent chemoattractant and a representative inflammatory cytokine/chemokine.[52] The role of mast cells in atopy, and especially in non-allergic inflammatory diseases, may hinge on its production of cytokines and subsequent inflammatory cell recruitment.[53] If this is the case, IL-10 or small molecules mimicking aspects of IL-10 signaling may prove to be efficacious clinical interventions.

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**Figure 1.** IL-10 inhibits FcεRI expression on mouse and human mast cells. (A) Mouse BMMC were cultured for 4 days in medium containing IL-3±IL-10 as described in Materials and Methods. FcεRI expression was determined by flow cytometry. Human lung (B) or skin (C) mast cells were cultured for 4 days in media containing SCF±IL-10 as described in Materials and Methods. FcεRI expression was determined by flow cytometry. Results are representative of 2 independent experiments.

**Figure 2.** Inhibition of FcεRI expression by IL-10 is Stat-3 dependent. (A) Littermate or Stat3-deficient (KO) BMMC were cultured in IL-3±IL-10 for 4 days. FcεRI expression was determined by flow cytometry. (B) BMMC were cultured as in part (A) in the presence or absence of 5μM Stat3i, which was added daily. FcεRI expression was determined by flow cytometry. Results are representative of 2 independent experiments using 4-5 populations each.

**Figure 3**. IL-10 selectively inhibits expression of FcεRI-related signaling proteins. Cells were cultured for four days in IL-3±IL-10. On day 4, cells were activated with IgE+antigen for 0, 5 or 15 minutes as described in Materials and Methods. Total cell lysates were used to detect Lyn, Syk, and Fyn by western blotting in (A). In (B), tyrosine-phosphorylated Stat5 or p38, and serine-phosphorylated Akt were measured by western blotting. The same membranes were stripped and re-probed to detect total proteins.

**Figure 4**. IL-10 injection inhibits FcεRI expression and IgE-mediated cytokine production. Mice were injected twice daily for four days IL-10 as described in Materials and Methods.

(A) Serum was collected and analyzed for the presence of IL-10. (B) Peritoneal cells were collected and FcεRI expression was analyzed by flow cytometry. (C, D) Peritoneal cells were incubated with purified mouse IgE and crosslinked with purified anti-mouse IgE, after which supernatants were examined for IL-13 and TNFα by ELISA. (E, F) Mice were injected with IL-10 or saline for four days as in (A). On day 3, mice were injected with 50μg anti-DNP IgE, and challenged with DNP on day 4, as described in Materials and Methods. Serum was analyzed for histamine (E) and MIP-1α (F) content by ELISA.

**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 (C), and serum histamine (D) and MIP-1α (E) were determined 30 minutes after PSA challenge.

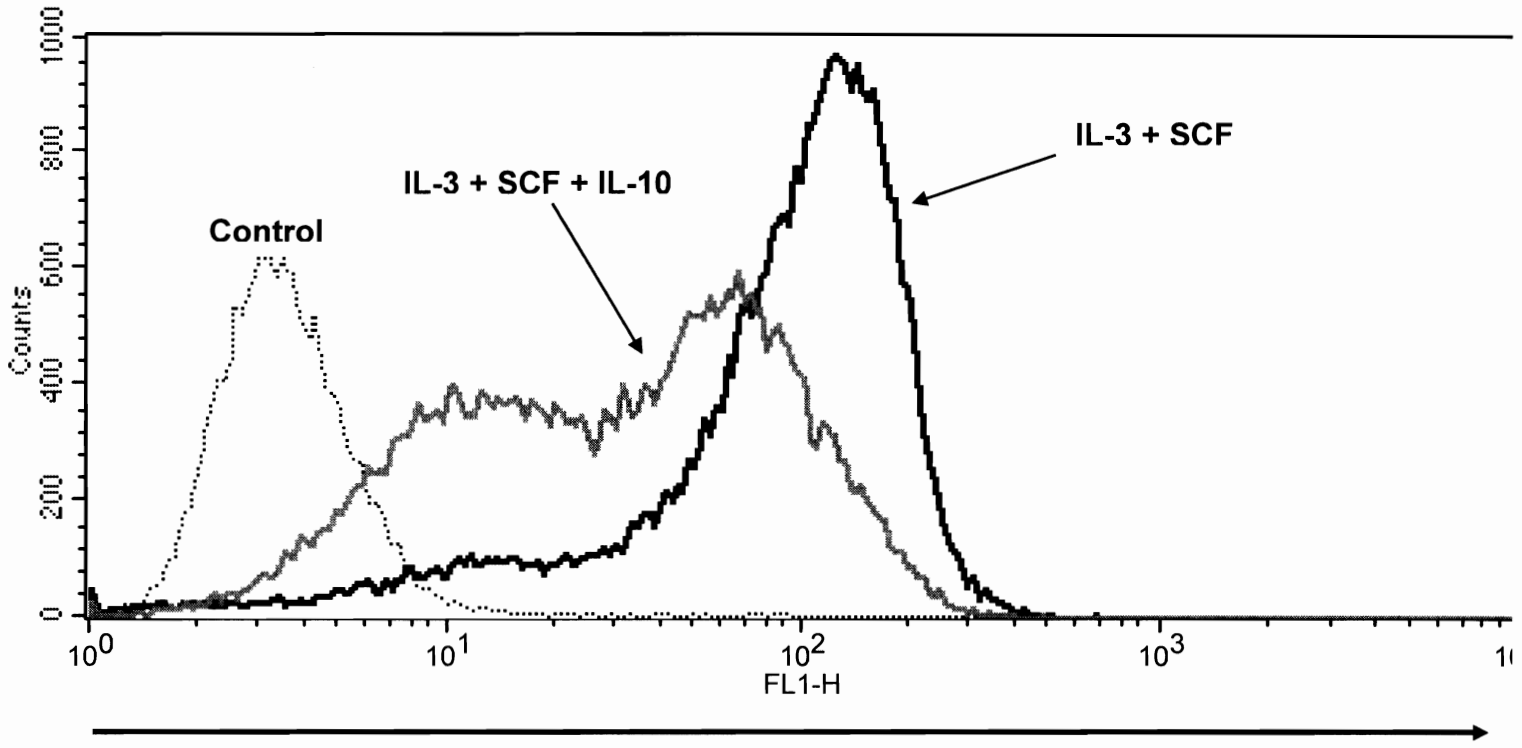
Counts
0
200
400
600
800
1000
Control
IL-3 + SCF + IL-10
IL-3 + SCF
$10^0$
$10^1$
$10^2$
$10^3$
FL1-H
FcεRI Expression

**Figure 1A**

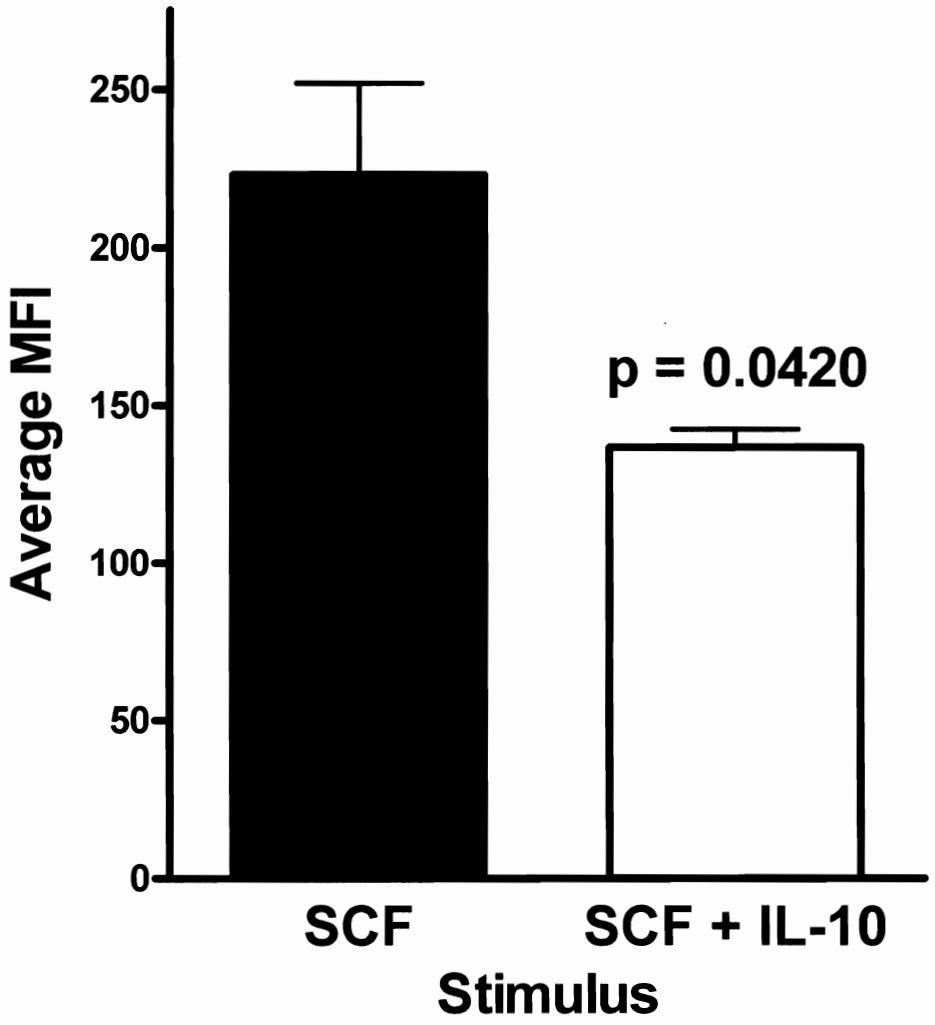


Figure 1B

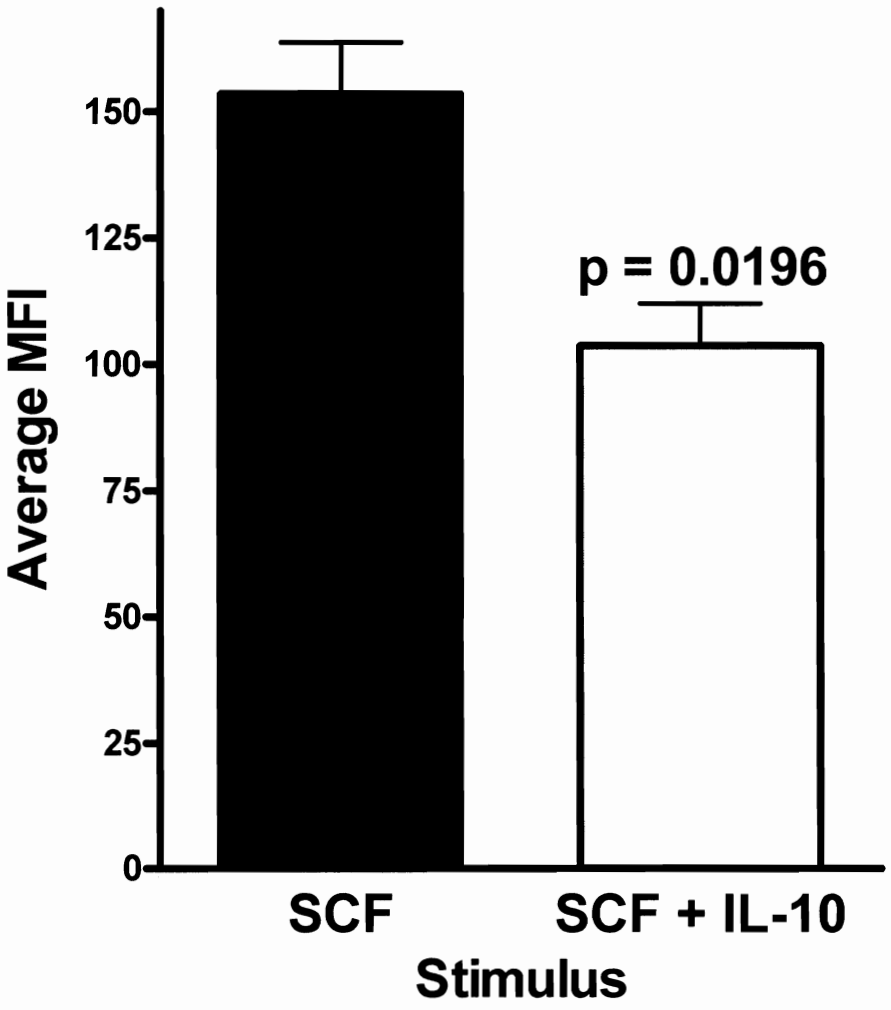
Average MFI
150
125
100
75
50
25
0
p = 0.0196
SCF
SCF + IL-10
Stimulus

Figure 1C

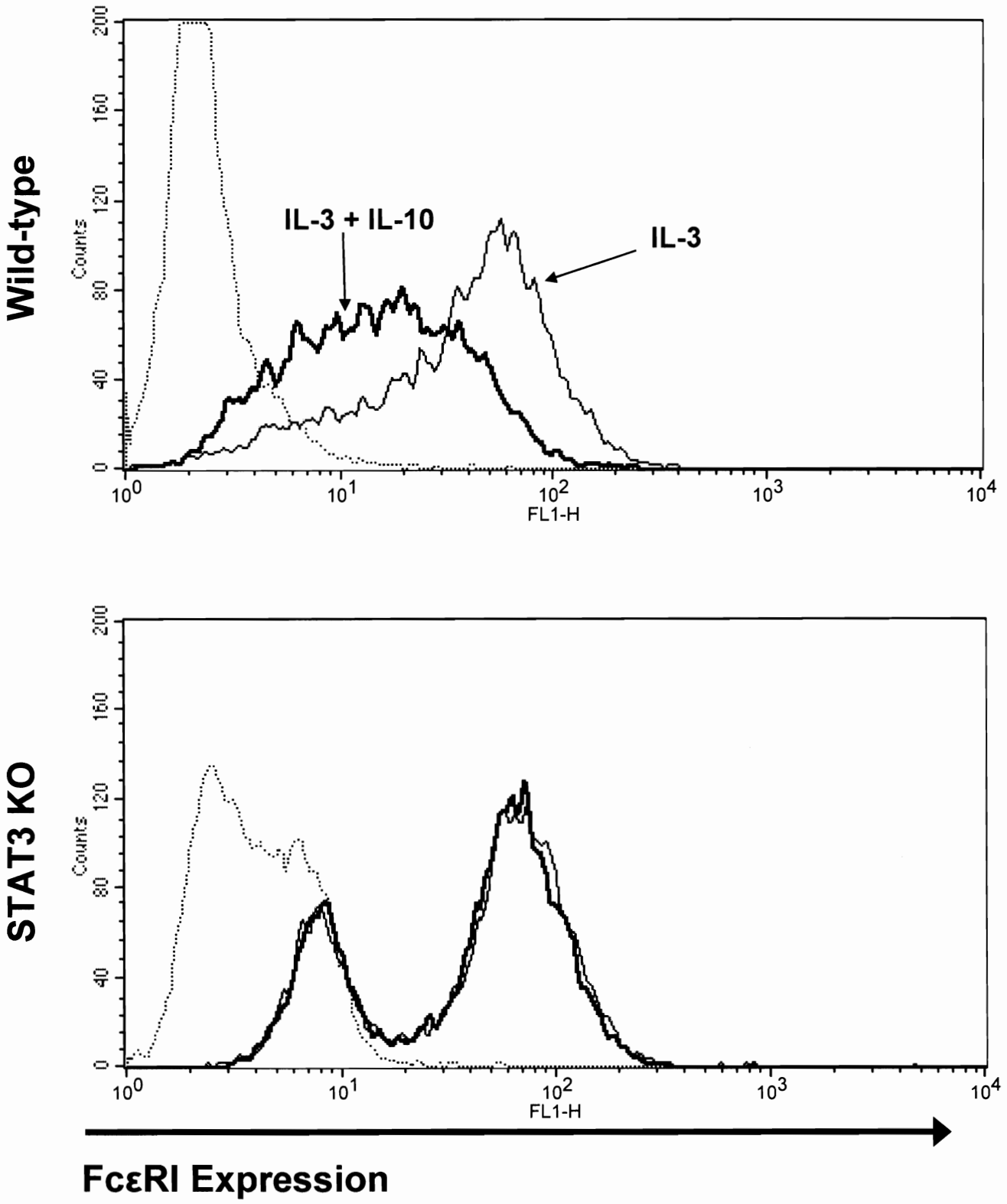
Wild-type
IL-3 + IL-10
IL-3
Counts
FL1-H
STAT3 KO
Counts
FL1-H
FcεRI Expression

**Figure 2A**

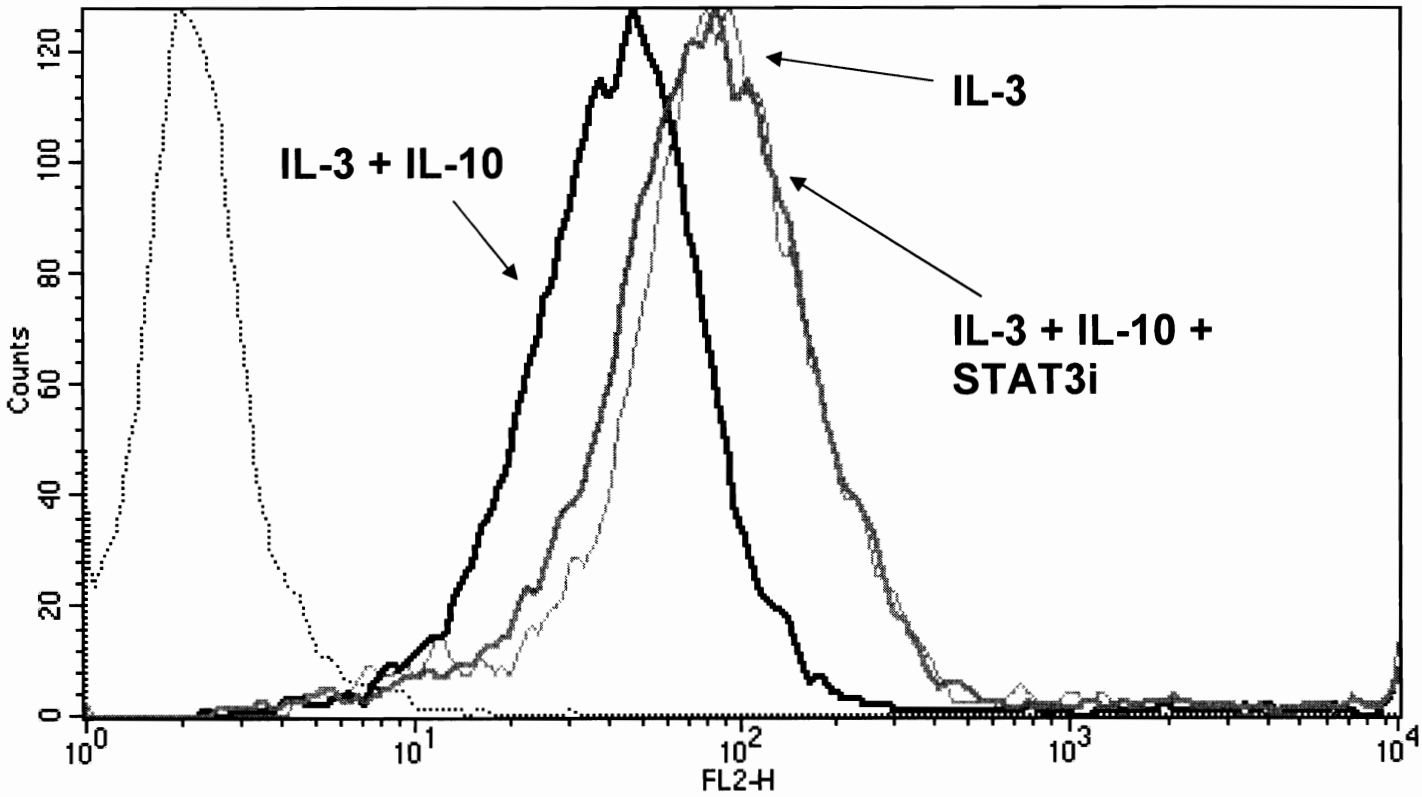
IL-3
IL-3 + IL-10
IL-3 + IL-10 +
STAT3i
Counts
FL2-H
0
20
40
60
80
100
120
$10^0$
$10^1$
$10^2$
$10^3$
$10^4$

**Figure 2B**

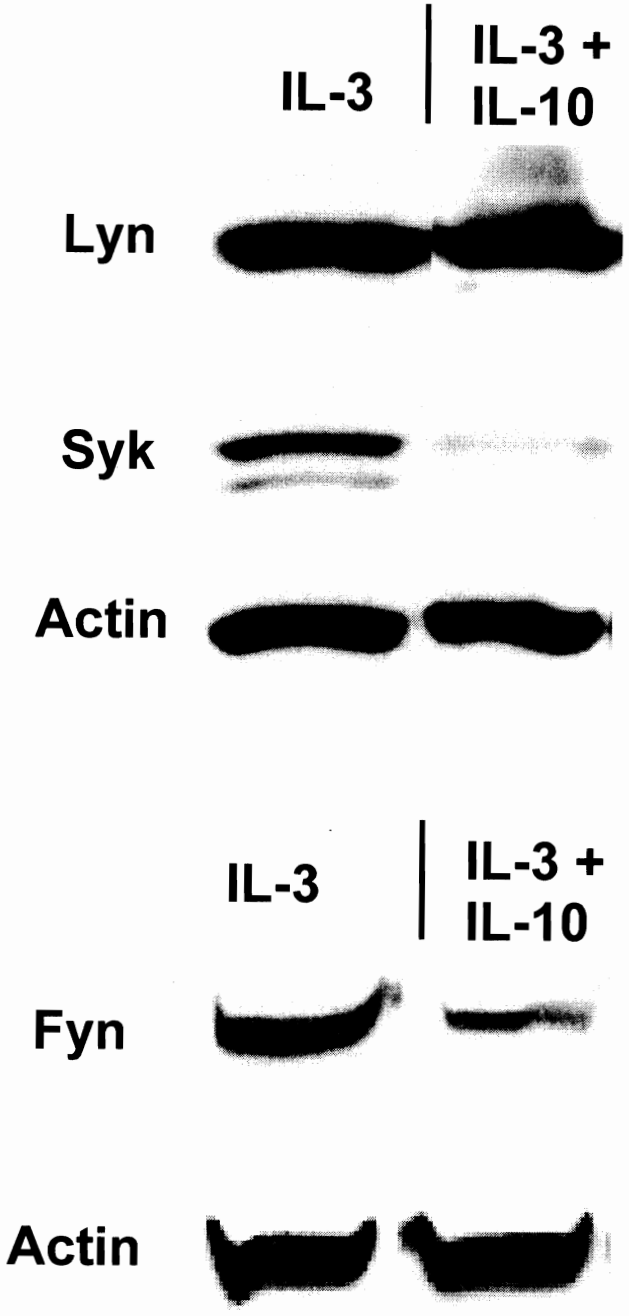


Figure 3A

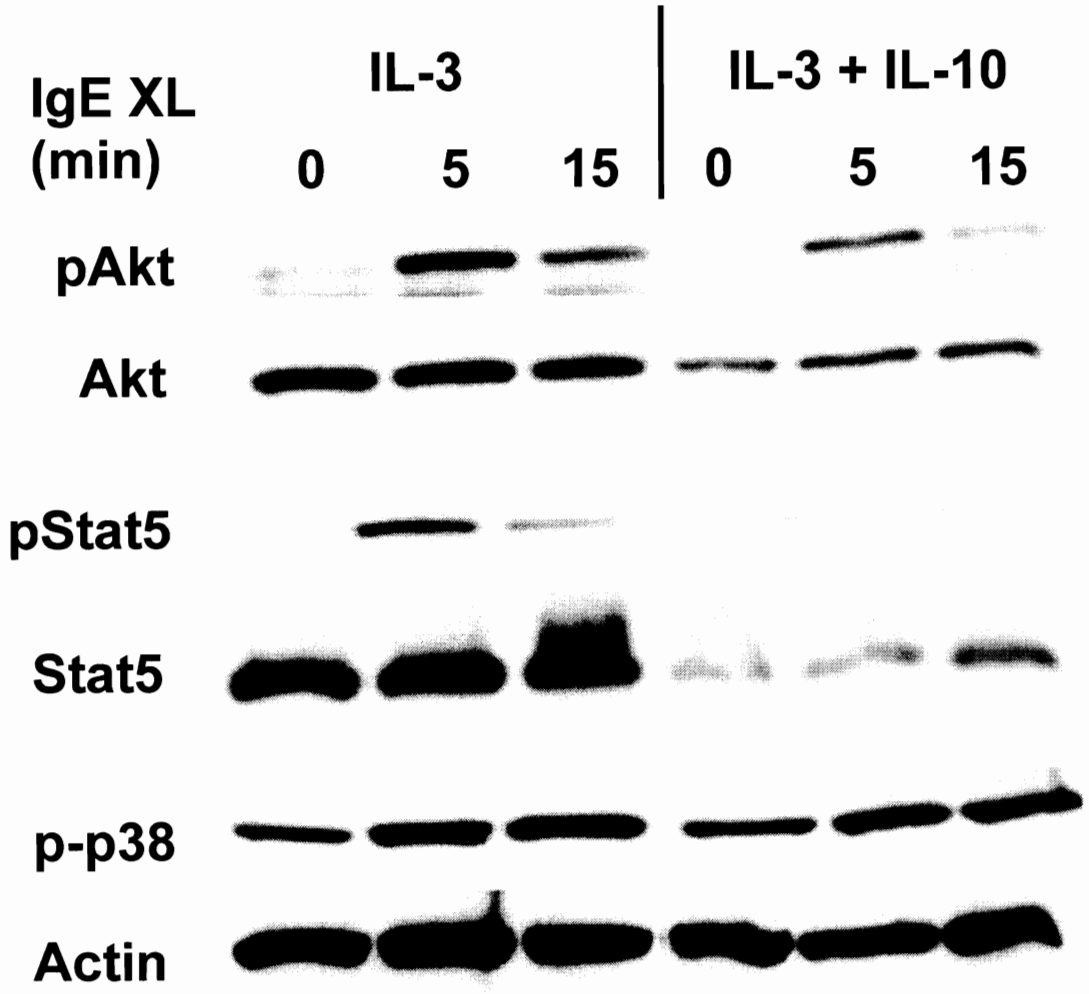
IgE XL
(min)
IL-3
0
5
15
IL-3 + IL-10
0
5
15
pAkt
Akt
pStat5
Stat5
p-p38
Actin

Figure 3B

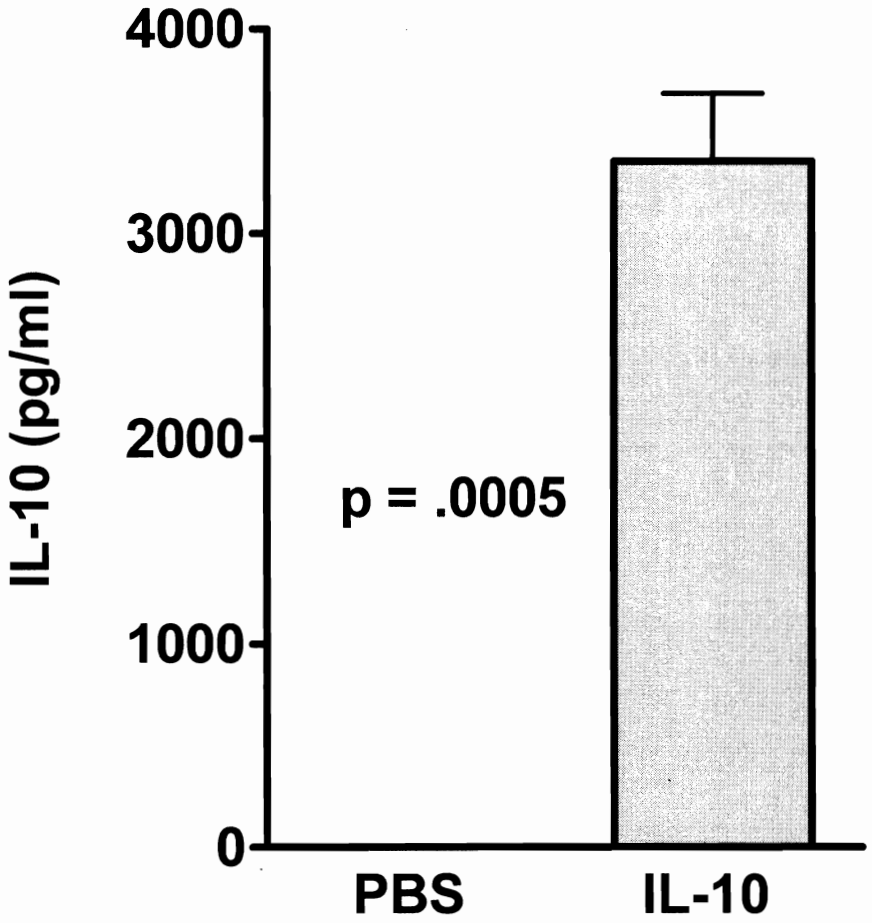


**Figure 4A**

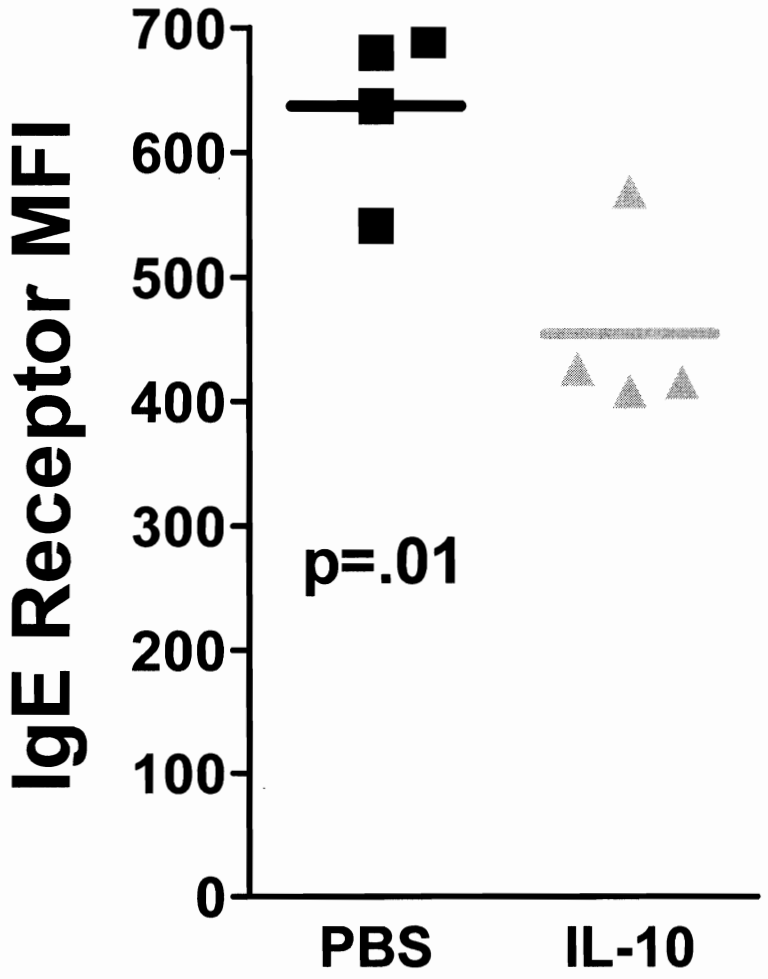


Figure 4B

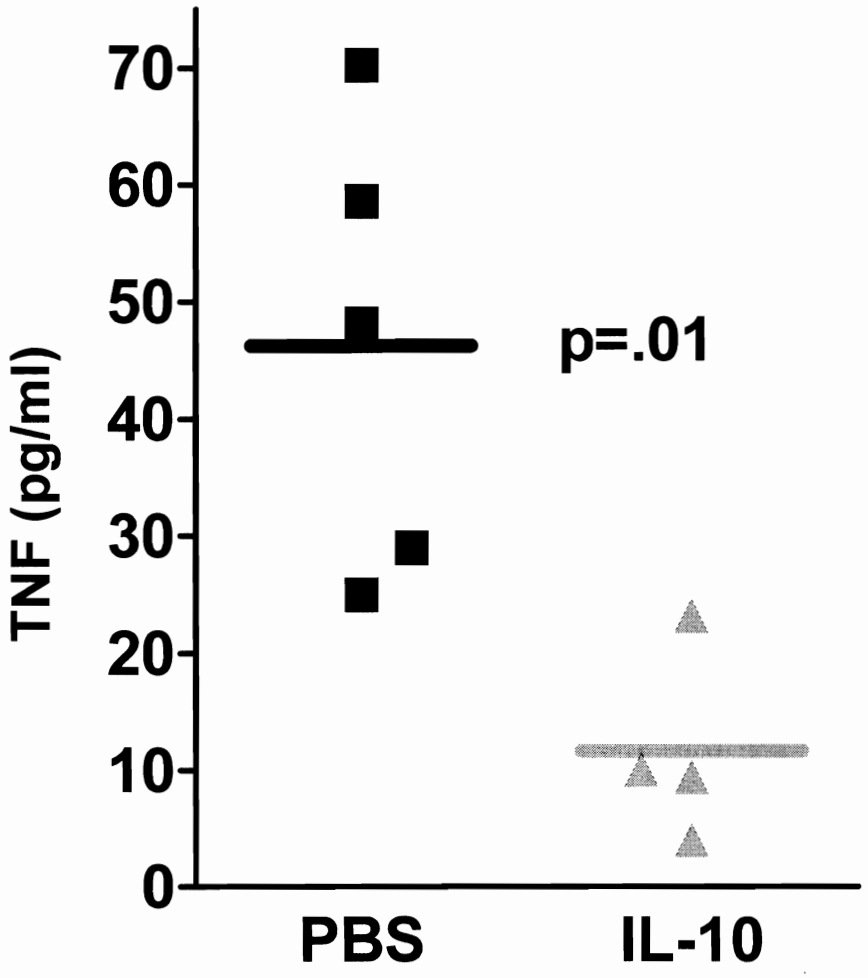


Figure 4C

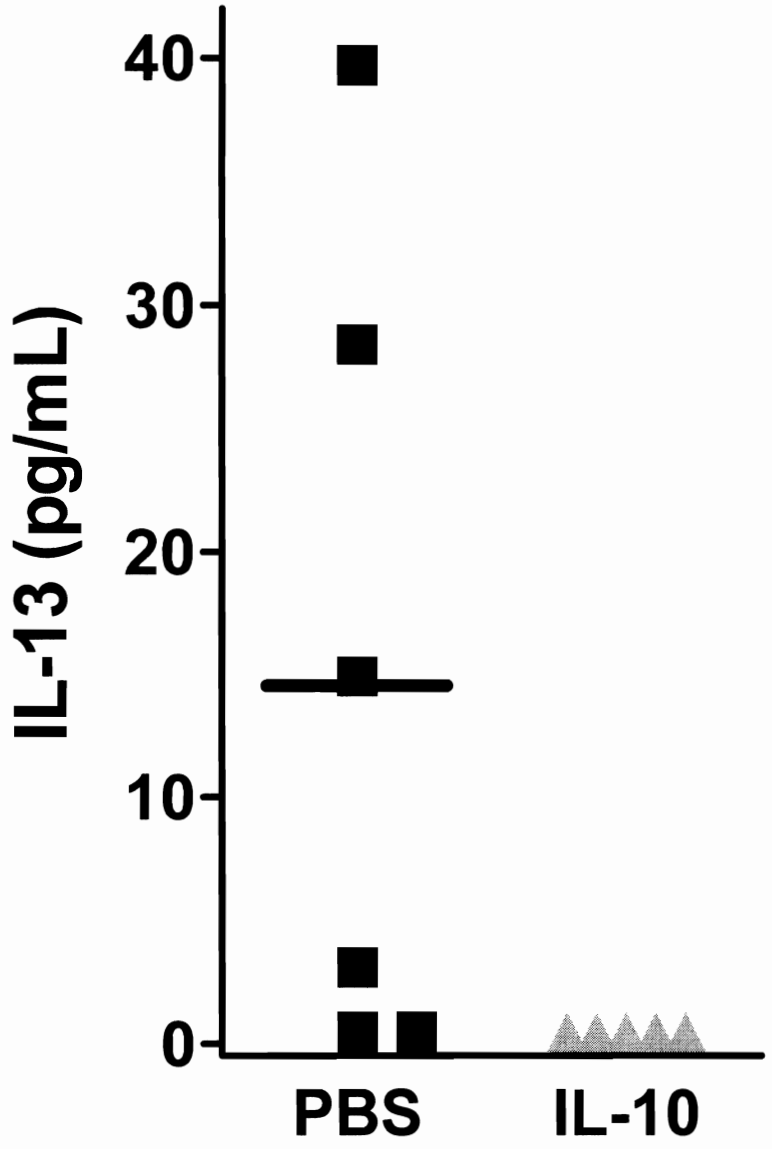


Figure 4D

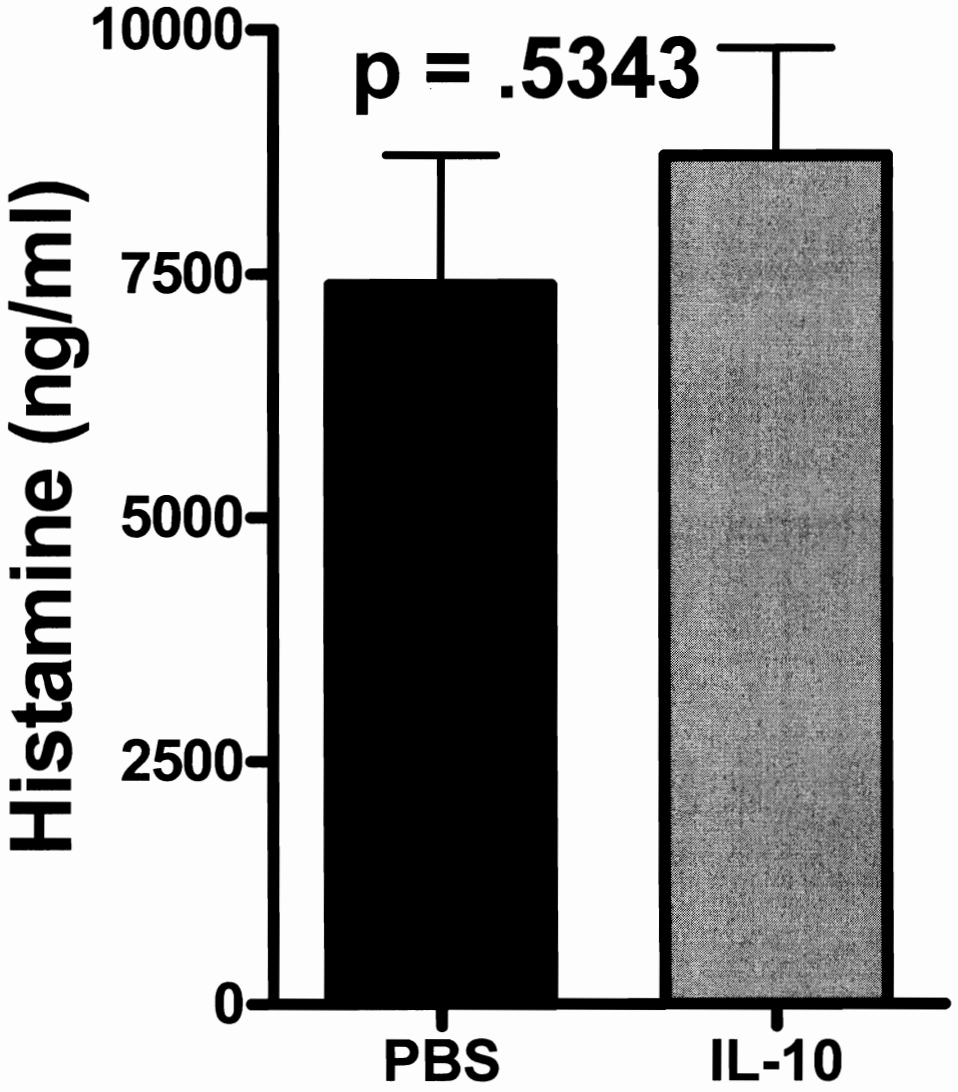


Figure 4E

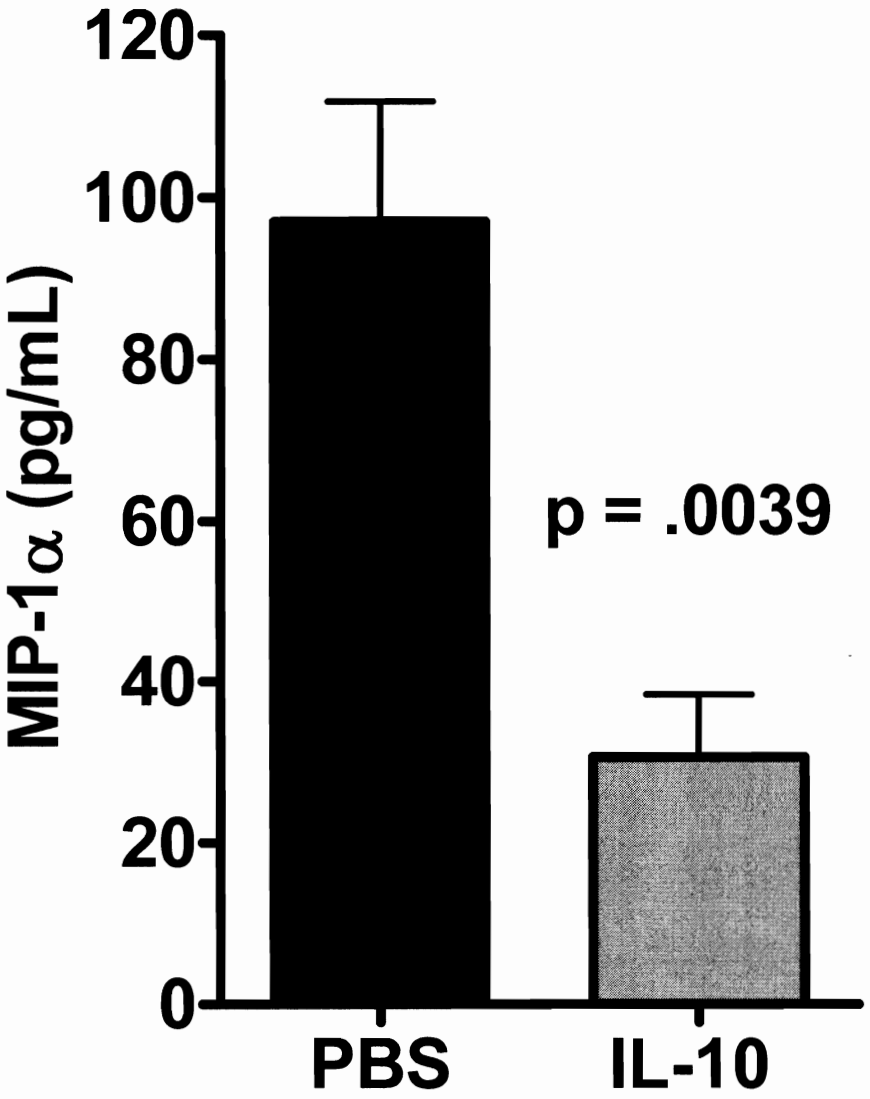


Figure 4F

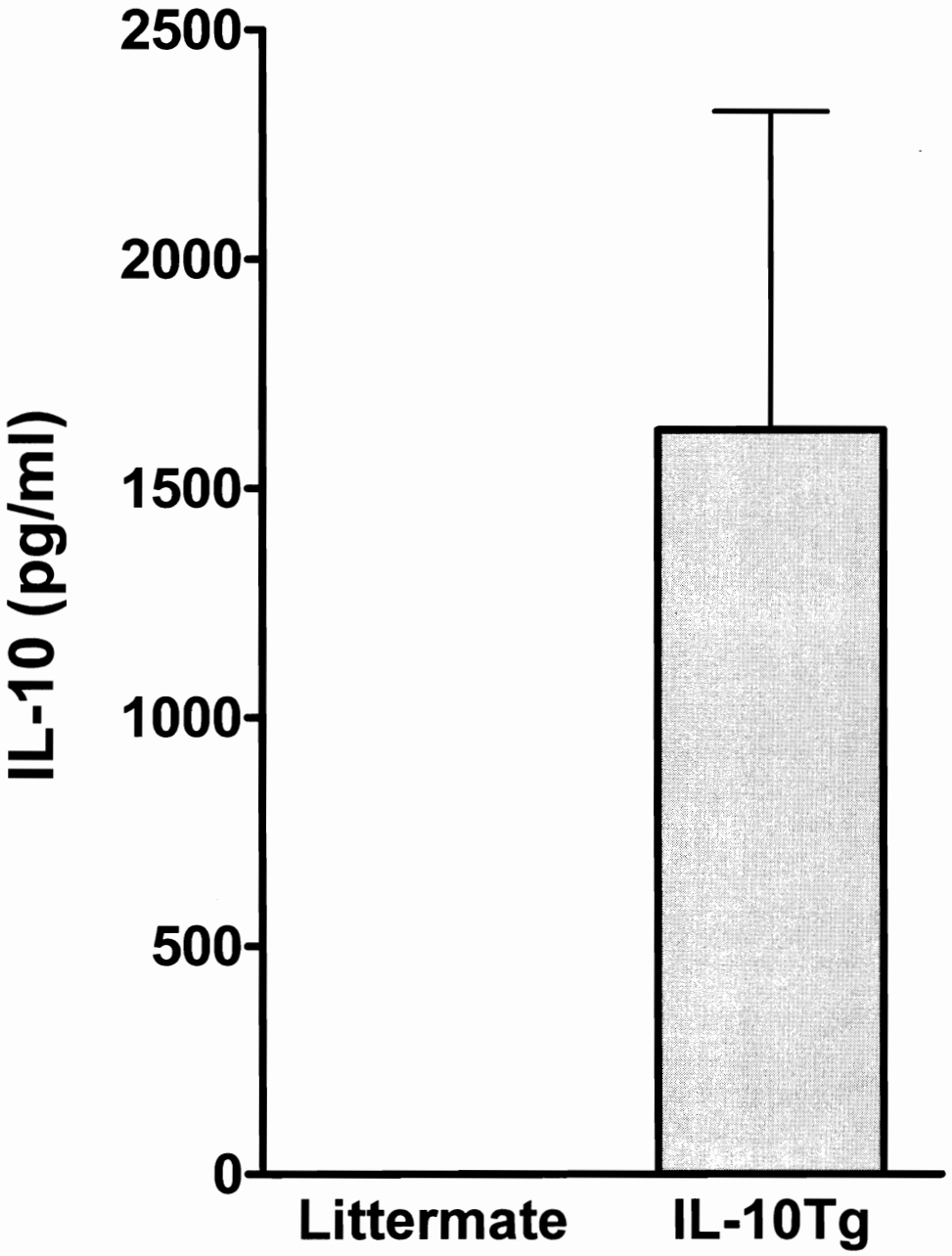


Figure 5A

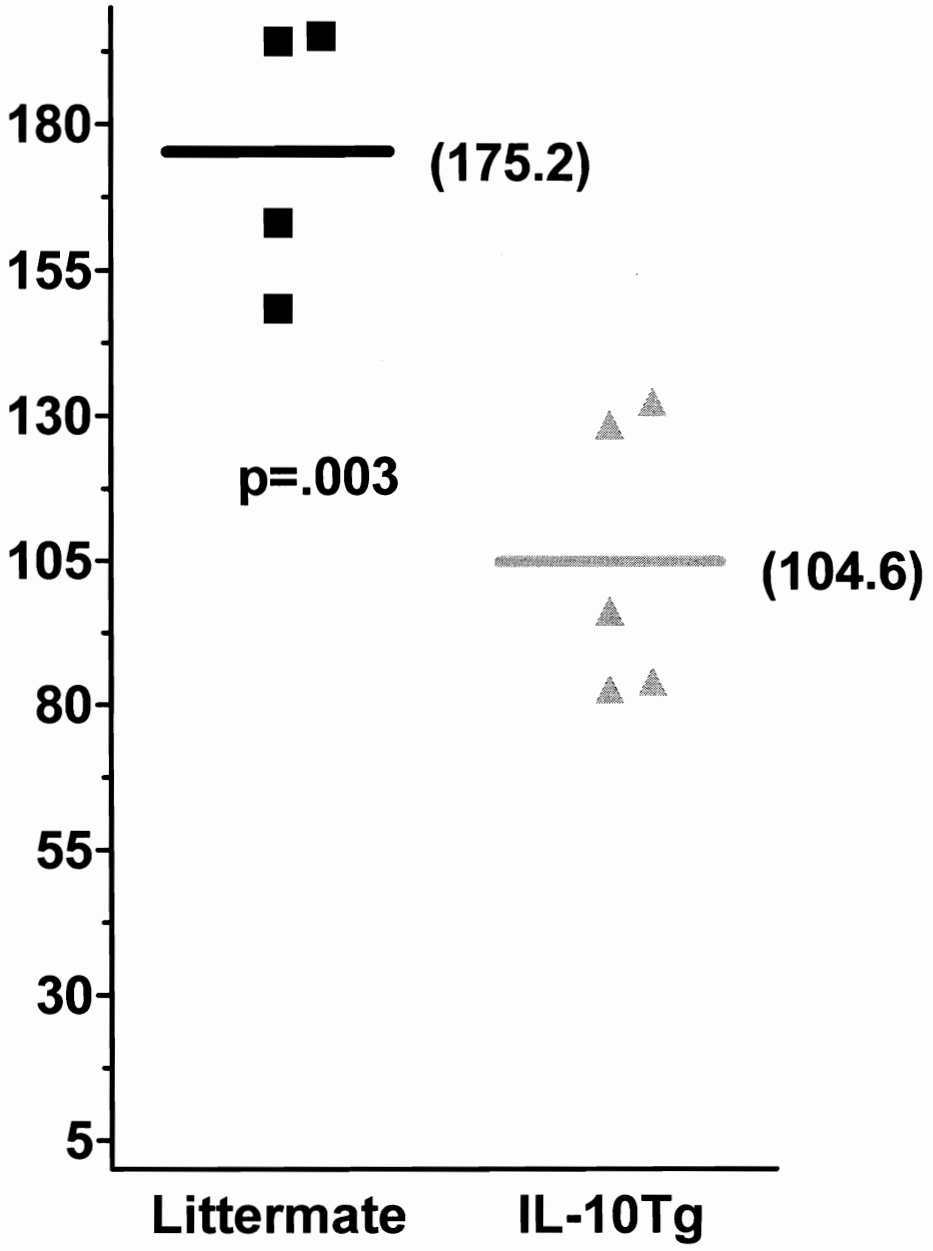


Figure 5B

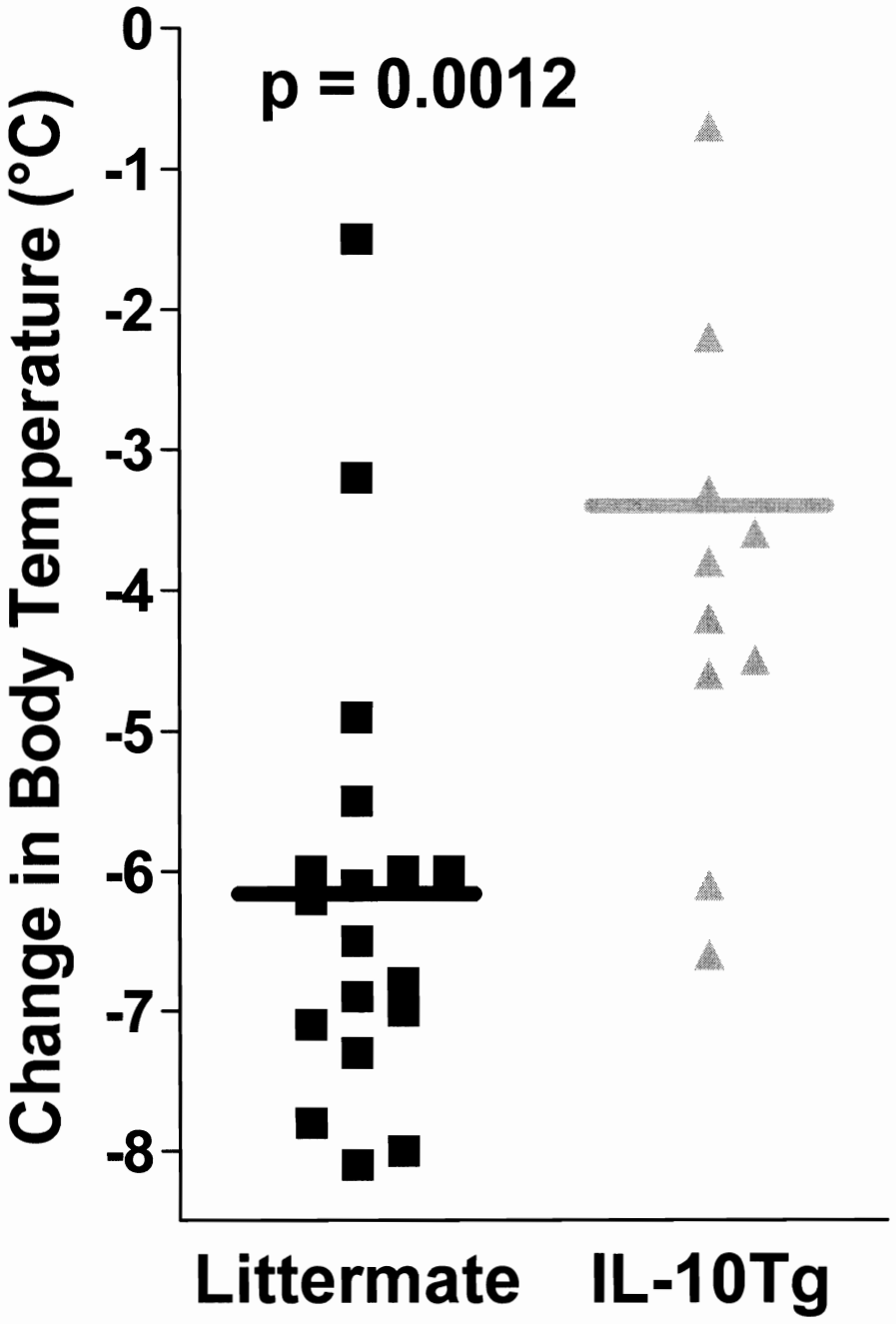
p = 0.0012
Change in Body Temperature (°C)
0
-1
-2
-3
-4
-5
-6
-7
-8
Littermate
IL-10Tg

Figure 5C

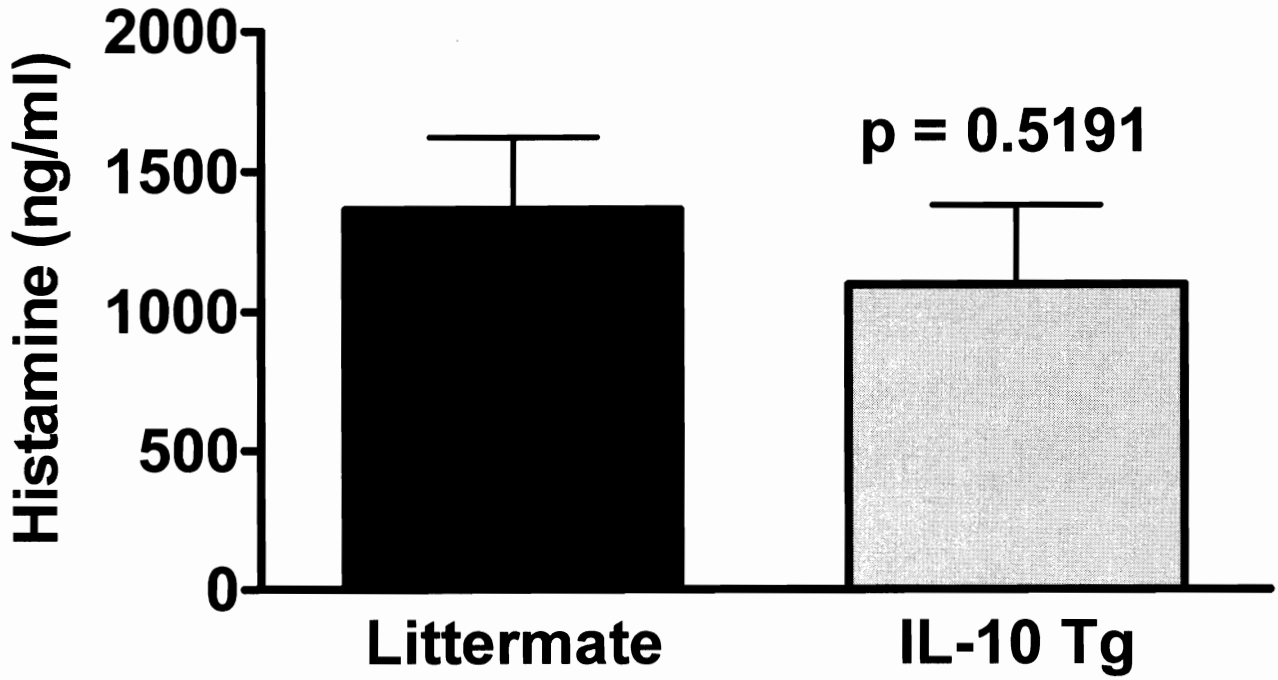
Histamine (ng/ml)
2000
1500
1000
500
0
p = 0.5191
Littermate
IL-10 Tg

Figure 5D

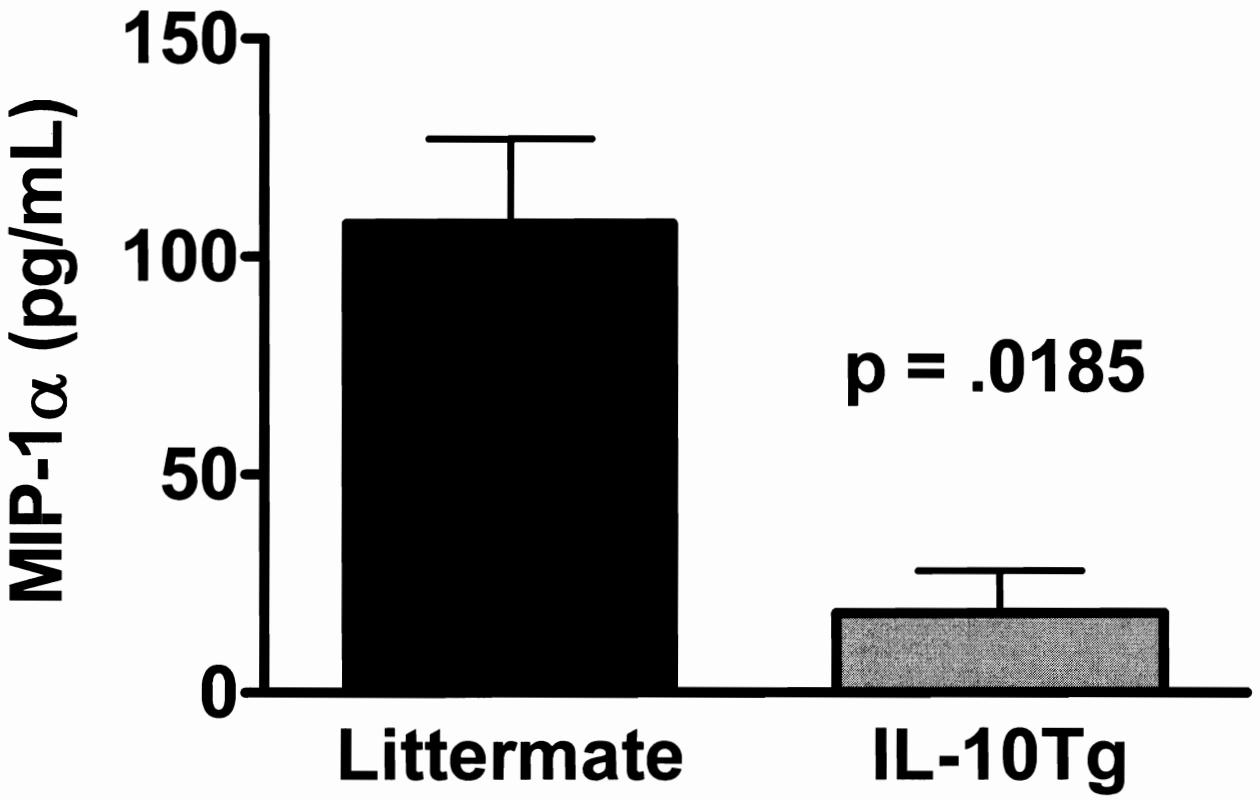


Figure 5E

## Vita

Sarah Kennedy was born in Arlington, Virginia on April 25, 1981. She graduated from McLean High School in McLean, Virginia in 1999. She then attended the University of Virginia and graduated with a B.S. degree in biology in 2003. For the next year, she worked in Dr. Theresa Pizarro's laboratory researching Crohn's disease and colitis. She then spent a year working for American Type Culture Collection (ATCC) in Manassas, Virginia. In the fall of 2005, she began work on a Master of Science in biology. She worked in the Laboratory of Molecular Immunology and completed her degree in May of 2007.