

CUTTING EDGE

Cutting Edge: CD4⁺ T Cell-Mast Cell Interactions Alter IgE Receptor Expression and Signaling¹

Mohit Kashyap,* Angela M. Thornton,[†] Sarah Kennedy Norton,* Brian Barnstein,* Matthew Macey,* Jennifer Brenzovich,* Ethan Shevach,[‡] Warren J. Leonard,[‡] and John J. Ryan^{2*}

Mast cell activation is associated with atopic and inflammatory diseases, but the natural controls of mast cell homeostasis are poorly understood. We hypothesized that CD4⁺CD25⁺ regulatory T cells (Treg) could function in mast cell homeostasis. In this study, we demonstrate that mast cells can recruit both Treg and conventional CD4⁺ T cells (Tconv). Furthermore, Treg, but not Tconv, suppress mast cell FcεRI expression. Despite the known inhibitory functions of IL-10 and TGFβ1, FcεRI suppression was independent of IL-10 and TGFβ1 and required cell contact. Surprisingly, coculture with either Treg or Tconv cells suppressed IgE-mediated leukotriene C₄ production but enhanced cytokine production by mast cells. This was accompanied by a selective increase in FcεRI-mediated Stat5 phosphorylation, which is a critical mediator of IgE-mediated cytokine secretion. These data are the first direct demonstration that mast cells can recruit Treg and illustrate that T cell interactions can alter the mast cell response. The Journal of Immunology, 2008, 180: 2039–2043.

Mast cells play an important role in both innate and adaptive immunity. They are activated by intrinsic pathogen-associated molecules as well as by Ag-specific IgE and IgG. By releasing preformed mediators such as histamine and neutral proteases, along with newly synthesized arachidonic acid metabolites, cytokines, and chemokines, mast cells function in a variety of type I hypersensitivity diseases including rhinitis, asthma, and atopic dermatitis (1). In recent years, the importance of mast cell activation has become appreciated in a broader group of inflammatory conditions, including multiple sclerosis, rheumatoid arthritis, and host resistance to bacterial infections (2–5).

Immune homeostasis appears to be regulated in part by the suppressive actions of some T cell subsets. So-called “natural” regulatory T cells (Treg)³ make up ~10% of the mouse CD4⁺ T cell population and have been shown to express Foxp3 and inhibit autoimmunity in several systems (reviewed in Ref. 6). The mode of Treg-mediated suppression has been difficult to discern. A common in vitro assay, the suppression of conventional CD4⁺CD25[−] T cells (Tconv) proliferation, has been used with considerable success in this regard. In these assays, Treg-mediated suppression requires that the Treg be activated via the TCR and maintain contact with the target population (7). Suppression may be accomplished in part by blocking IL-2 mRNA induction in the target population (8). An interesting feature of Treg is their production of IL-10 and TGFβ1, cytokines with suppressive activities that could explain Treg function. The role of these cytokines, especially TGFβ1, has been controversial, suggesting that their importance is context dependent (7, 9–14). Given that suppression by preactivated Treg is both Ag and MHC nonspecific, these potent inhibitory cytokines may be critical depending upon the disease model used. We have found that IL-10 and TGFβ1 inhibit mast cell development, function, and survival (15–22). We have now investigated the role of Treg in controlling the mast cell response.

Recently, it has been shown that airway inflammation and hyperreactivity can be resolved by CD4⁺CD25⁺ Treg through an IL-10-dependent mechanism (23). In another study, it was shown that Treg alter pulmonary dendritic cell phenotype and function, thereby protecting against experimentally induced asthma (24). Moreover, Treg-mediated mast cell recruitment was demonstrated in a model of tissue graft tolerance, supporting the hypothesis that Treg-mast cell interactions may occur in vivo (25).

We hypothesized that Treg can alter the mast cell response to regulate inflammation. To this end, we investigated mast cell-mediated Treg recruitment and the ability of Treg to control mast cell IgE receptor expression and function. We have further

*Department of Biology, Virginia Commonwealth University, Richmond, VA 23284;

[†]Laboratory of Immunology, National Institute of Allergy and Infectious Diseases and [‡]Laboratory of Molecular Immunology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892

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

³ Abbreviations used in this paper: Treg, regulatory T cell; BMMC, bone marrow-derived mast cell; DNP, 2,4-dinitrophenyl; HA, hemagglutinin; Tconv, conventional CD4⁺CD25[−] T cell.

examined the importance of cell contact and Treg-derived cytokines and have compared Treg activity to that of Tconv. These data comprise the first direct study of Treg-mediated mast cell activation.

Materials and Methods

Cell culture

Bone marrow mast cells (BMMC) as well as wild-type, IL-10-deficient, or hemagglutinin (HA)-specific TCR transgenic mouse lymph node CD4⁺CD25⁺ and CD4⁺CD25⁻ T cells were cultured as described previously (8, 16). CD4⁺CD25⁺ Treg cells were purified using magnetic beads and yielded ~90% Foxp3⁺ cells (data not shown).

Reagents

Murine stem cell factor and IL-3 were purchased from Peprotech. IgE (clone C38-2), anti-CD3 (clone 2C11), FITC anti-CD4, and anti-CD16/CD32 (clone 2.4G2) were purchased from BD Biosciences. PE-labeled Rat IgG isotype control was purchased from eBioscience. FITC-labeled rat IgG isotype control and FITC-labeled rat anti-mouse IgE were purchased from Southern Biotechnology Associates. 2,4-Dinitrophenyl (DNP)-human serum albumin was purchased from Sigma-Aldrich. Control IgG and anti-TGFβ1 (catalog no. 240-B) Abs were purchased from R&D Systems. Anti-IL-10 receptor Ab was purchased from BD Pharmingen.

T cell migration assay

Culture supernatants were harvested from BMMC (2.5×10^6 cells/ml) activated for 24 h with DNP-albumin (50 ng/ml) in RPMI 1640/BSA. Transwell plates (5-μm pore size; Greiner Bio-One) were preincubated with RPMI 1640/BSA for 30 min at 37°C, after which CD4⁺ T cells (5×10^5 cells in 100 μl) were placed into the top chamber. Either mast cell culture supernatant, RPMI 1640/BSA plus IL-16 (10 ng/ml), or RPMI 1640/BSA alone was added to the bottom chamber. T cells resuspended in activated mast cell culture supernatant were a migration control. All chambers received anti-CD3 and IL-2. Migration was assessed 16 h later by harvesting triplicate samples from each well and counting cells by timed flow cytometry with propidium iodide exclusion. Net migration for the IL-16-induced migration was determined by subtracting the number of cells migrating in RPMI 1640/BSA from those that migrated in response to IL-16. In some experiments, IL-16-mediated migration was also calculated using IL-16 in the top and bottom wells as a control, which yielded similar results. Net migration in response to mast cell culture supernatant was calculated by subtracting the number of cells migrating when mast cell supernatant was present in both the top and bottom wells from the number of T cells migrating with mast cell supernatant in the bottom well only.

Coculture and Transwell assay

BMMC and Treg were cocultured at a 1:1 ratio (5×10^5 cells/ml) for 3–5 days in complete RPMI 1640 medium containing IL-2 and IL-3. Anti-CD3 (1 μg/ml) was added where indicated. FcεRI surface expression was determined by flow cytometry as described (15). In experiments designed to prevent cell contact, Treg and BMMC were separated by 0.4-μm membranes in Transwell plates (Greiner Bio-One). In experiments using TCR transgenic cells, T-depleted spleen cells (5×10^4) and 8 μM HA_{110–119} peptide were included. HA peptide was synthesized and purified by the Laboratory of Molecular Structure, Peptide Synthesis Laboratory (National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD). In experiments where cytokine-blocking Abs were added, these were used at a final concentration of 10 μg/ml at the start of the assay.

ELISAs

Mast cells and T cells were cultured alone or together for 4 days. Cells were re-plated at 1×10^6 live BMMC per milliliter and activated with IgE plus DNP-albumin. Culture supernatant collected after 18 h was used to determine the concentration of TNF, IL-13, and MIP-1α using OptEIA ELISA kits (BD Biosciences). Supernatants collected after 60 min were used to detect histamine (Neogen) and leukotriene C₄ (LTC₄; Cayman Chemical). p65 NFκB activation was also measured after 5–15 min of IgE stimulation using an ELISA (TransAm kit; Active Motif).

Alterations in FcεRI signaling

BMMC were cocultured with Treg or Tconv cells for 4 days. Cells were replated at 3×10^6 BMMC/ml and activated with IgE plus DNP-albumin for 5 or 15 min. Total cell lysates were prepared and used to detect protein expression by Western blotting. Abs used included anti-phosphotyrosine (pY694) Stat5, anti-

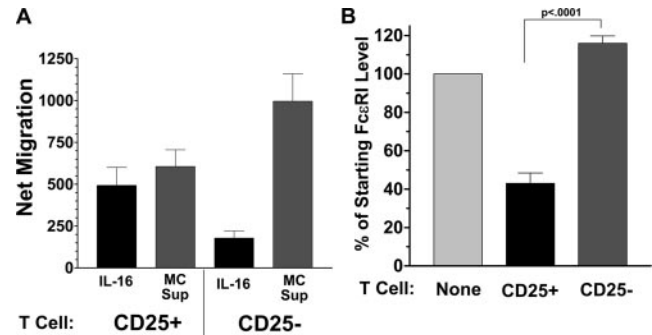


FIGURE 1. Mast cells induce T cell migration, but FcεRI suppression is restricted to Treg. *A*, Culture supernatants (Sup) from IgE plus Ag-activated BMMC (MC) or recombinant IL-16 were used to induce migration of CD4⁺CD25⁺ or CD4⁺CD25⁻ T cells as described in *Materials and Methods*. Data shown are means and SE values obtained using three separate BMMC populations, each harvested in triplicate. *B*, FcεRI suppression is restricted to CD25⁺ T cells. BMMC were cultured alone or with anti-CD3-activated CD25⁺ or CD25⁻ T cells for 5–6 days, after which the percentage of starting FcεRI expression was determined by flow cytometry. Data shown are mean ± SEM of at least eight samples from three experiments.

phosphothreonine/tyrosine (pT202/pY204) ERK-1/2, anti-phosphoserine (pS473) Akt, and anti-phosphothreonine/tyrosine (pT180/pY182) p38 MAPK. These Abs were purchased from Cell Signaling Technology and used at a final dilution of 1/1000. Anti-actin was purchased from Sigma-Aldrich and used at 1/5000 dilution.

Statistics

Results are the mean and SD for experiments with 5–6 samples. SE measurements are shown for data sets where greater than five measurements were made. $p < 0.05$, as determined by Student's *t* test, was considered significant.

Results and Discussion

Mast cells induce migration of CD25⁺ and Tconv cells, but FcεRI suppression is CD25 restricted

Recently, Treg-mediated mast cell recruitment was noted in a tissue graft model (25). Because we have found that the cytokines IL-10 and TGFβ1, which are produced by Treg, can suppress mast cell function (15–22), we investigated how Treg-mast cell interactions affect the mast cell response. Mast cell activation by IgE logically precedes inflammation in atopic disease, suggesting that mast cells could elicit Treg migration. Furthermore, T cell recruitment in mast cell-related inflammatory syndromes such as asthma is established (26, 27). Hence, we tested mast cell-mediated recruitment of CD4⁺CD25⁺ Treg and Tconv cells in vitro. CD4⁺CD25⁺ and Tconv cells both exhibited migration in response to supernatant from IgE-activated BMMC to an equal or greater extent than that noted with the known chemotactic factor IL-16 (Fig. 1*A*). Although the migration of Tconv cells was expected, these data are the first to show mast cell-mediated Treg recruitment. These results illustrate that Treg may migrate to an area of ongoing mast cell activation. Mast cells produce numerous cytokines, chemokines, arachidonic acid metabolites, and proteases. The identity of the Treg-recruiting factor is under investigation.

Our previous studies illustrated that FcεRI expression can be inhibited by IL-10 or TGFβ1 to regulate mast cell activation (16, 19). To determine whether T cells could cause a similar suppression, we cocultured mast cells with CD4⁺CD25⁺ Treg or Tconv in the presence of anti-CD3. As shown in Fig. 1*B*, Treg but not Tconv lymphocytes significantly suppressed

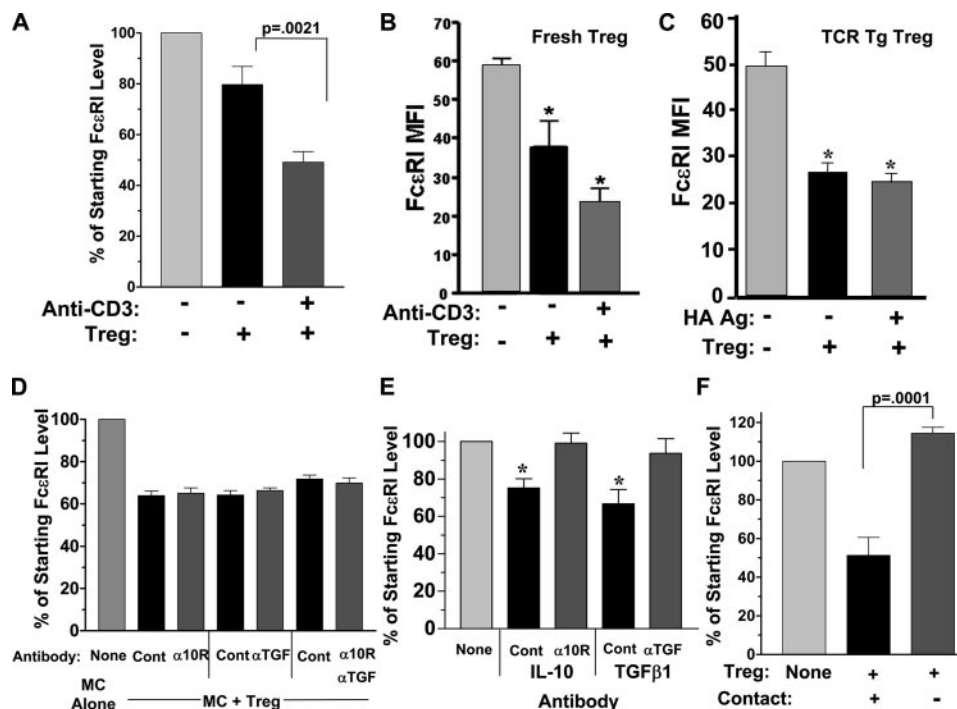


FIGURE 2. Activated Treg suppress mast cell FcεRI expression. *A*, BMMC were cultured alone or with Treg with or without anti-CD3 (1 μg/ml) for 5–9 days. The percentage of starting FcεRI expression was determined by comparing the mean fluorescence intensity of mast cells cultured without Treg. Data shown are mean ± SEM of at least five samples from three experiments. *B*, Freshly isolated Treg were cocultured with BMMC as in *A* for 4 days. Anti-CD3 was added where indicated. FcεRI expression was measured by flow cytometry. Data shown are mean fluorescence intensity ± SE values from four samples harvested in two experiments. *C*, BMMC were cocultured as in *B* with Treg purified from TCR transgenic mice specific for HA peptide, which was added in place of anti-CD3. All wells also included irradiated APC. Data shown are mean ± SEM for three BMMC populations. *D*, BMMC (MC) were cultured for 5 days alone or with anti-CD3-stimulated Treg in the presence of control IgG, anti-IL-10 receptor, and/or anti-TGFβ1/2/3 Abs (10 μg/ml), and reduction in FcεRI expression was determined as in *A*. Data shown are mean ± SEM for four BMMC populations. *E*, BMMC were cultured for 4 days with IL-3 alone or with IL-10 (10 ng/ml) or TGFβ-1 (5 ng/ml). Where indicated, anti-IL-10 receptor or anti-TGF Abs were added as in *D*. Inhibition of FcεRI expression was determined by flow cytometry staining. Data shown are mean ± SEM for three BMMC populations. *F*, BMMC were cocultured with anti-CD3-activated Treg for 4 days in standard 24-well plates or separated by 0.4-μm filters to prevent cell contact. Mast cell FcεRI expression was determined by comparing the mean fluorescence intensity of mast cells cultured without Treg. Data shown are mean ± SEM of 8–10 samples. *, $p < 0.05$ when compared with control samples.

FcεRI expression on mast cells in these cocultures, suggesting that Treg-mast cell interactions could alter IgE responses.

Treg inhibit mast cell FcεRI expression through a contact-mediated process

The means by which Treg suppress FcεRI expression was examined via coculture studies. Treg purified from peripheral lymph nodes were activated with IL-2/anti-CD3 for 3 days, followed by further expansion in IL-2 for one week. Although anti-CD3 stimulation during the coculture period was not required for inhibition, it greatly enhanced this effect (Fig. 2*A*). Coculturing freshly isolated Treg with mast cells similarly suppressed IgE receptor (Fig. 2*B*). Also, HA-activated Tregs from animals expressing an HA-specific TCR were as capable of suppressing FcεRI expression as polyclonal Tregs activated with anti-CD3 (Fig. 2*C*). These data indicate that Treg-mediated FcεRI suppression is not restricted to precultured or Ag-specific T cells.

Because Treg can produce IL-10 and TGFβ1, we examined the effect of cytokine-blocking Abs on FcεRI inhibition. As shown in Fig. 2*D*, the addition of anti-IL-10 receptor and/or anti-TGFβ Abs had no effect on Treg-mediated FcεRI suppression. We confirmed that these Abs were effective in blocking their target cytokines by assessing IL-10- or TGFβ1-mediated FcεRI suppression (Fig. 2*E*).

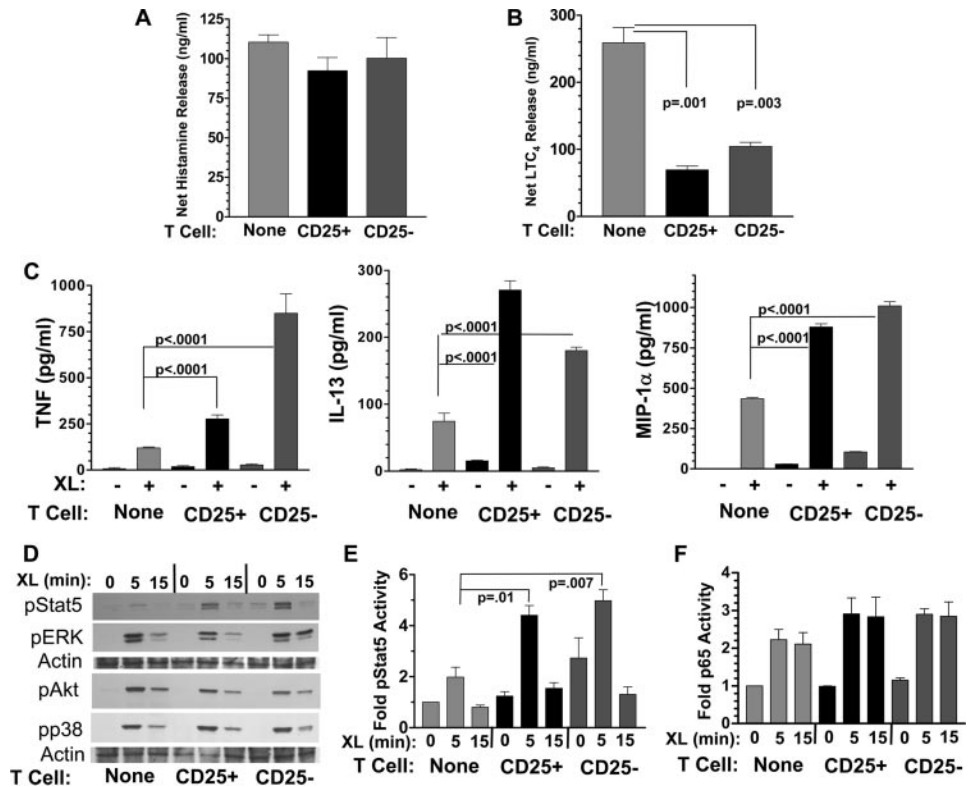
Treg-mediated effects have been shown to require cell contact in some systems (reviewed in Ref. 6). We therefore tested

the importance of Treg-mast cell contact. Mast cells were cocultured with anti-CD3-activated Treg in standard wells or separated by a 0.4-μm membrane. After 4 days of culture, FcεRI expression was measured by flow cytometry. As shown in Fig. 2*F*, cell contact was absolutely required for FcεRI suppression. Because the 0.4-μm membrane pore size would prevent cell contact but allow cytokine dispersion in the medium, these data corroborated our Ab neutralization studies, suggesting that Treg-secreted cytokines, including IL-10 or TGFβ, are not the likely means by which Treg inhibit IgE receptor expression. Rather, some form of contact-mediated regulation is occurring between these two cell types. FcεRI signaling is perhaps the most common and certainly the best-studied means by which mast cells are triggered to induce inflammation. These data are the first demonstration that Treg can alter FcεRI levels and suggest a novel mechanism not requiring a secreted factor.

T cells alter the strength of IgE-mediated mast cell activation

FcεRI transduces signals that directly result in allergic inflammation by eliciting histamine release, arachidonic acid metabolism, and cytokine secretion. Because mast cells recruited both CD25⁺ and Tconv cells, we investigated the effect of coculturing mast cells with these populations before IgE-mediated activation. As shown in Fig. 3, *A* and *B*, the presence of T cells had no effect on IgE-induced histamine release but significantly suppressed leukotriene C₄ production. It was interesting to

FIGURE 3. T cell interactions alter IgE-mediated mast cell activation. *A–C*, BMHC were cultured for 4–5 days with anti-CD3-stimulated CD25⁺ or CD25⁻ T cells and then activated with IgE plus Ag as described in *Materials and Methods* for 60 min (*A* and *B*) or 18 h (*C*) before harvesting culture supernatants. Data shown are means $\pm$ SE values of 3–6 samples obtained in three independent experiments. *D–F*, BMHC were cultured alone or with CD25⁺ or CD25⁻ Th cells as in *A* and then activated with IgE plus Ag as described in *Materials and Methods*. Cell lysates were examined for the expression of the indicated proteins by Western blotting in *D* or by ELISA in *E*. The degree of Stat5 tyrosine phosphorylation was determined by densitometry analysis of Western blots from three separate groups of samples as shown in *E*. Fold activation was determined by normalizing all bands to the phosphorylated Stat5 (pStat5) bands in unstimulated BMHC alone (*lane 1*). XL, IgE crosslinkage.



note that this inhibitory effect was observed with both populations of T cells and, hence, did not shown concordance with Fc ϵ RI suppression.

We next examined IgE-elicited cytokine secretion, a hallmark of the late phase atopic response. These data were striking in that the presence of either Treg or Tconv cells significantly enhanced the production of TNF, IL-13, and MIP-1 α (Fig. 3C) compared with mast cells cultured alone. As expected, T cells cultured alone had no response to IgE cross-linkage and produced cytokines at levels below the limits of detection (not shown). This increase in IgE-mediated cytokine production was unexpected given the reduced Fc ϵ RI levels noted when coculturing mast cells and Treg. However, the ability of T cells to augment mast cell activation agrees with the previous observation that mast cells recruited by Treg assist in tissue graft tolerance (25). Furthermore, T cell-mediated augmentation of the mast cell response fits well with mast cell-related immunopathology, which can involve both a mast cell and a T cell component (27).

Our data suggest that T cells may dampen some aspects of the immediate response (leukotriene secretion) while augmenting the late phase response. The lack of concordance between changes in Fc ϵ RI expression and IgE-mediated function may be explained by the great sensitivity with which the IgE receptor functions. Although mast cells express at least 100,000 Fc ϵ RI molecules per cell, perhaps only a few hundred of these need to be aggregated to yield a functional signal (28). Hence, the 50% reduction in Fc ϵ RI levels noted with Treg coculture is apparently not sufficient to block activation. The role of this suppression is unclear, but it could be postulated that the suppression reduces Ag sensitivity or possibly serves to mitigate an even greater signaling augmentation that would otherwise be conveyed by T cells. The enhancement of IgE-mediated cytokine production by T cells could have direct and important effects. Cytokines control leuko-

cyte infiltration and function; hence, regulating the late phase response could profoundly alter mast cell-mediated diseases.

We investigated the mechanism by which IgE-mediated cytokine production could be altered by T cells by examining Fc ϵ RI signaling pathways. Mast cells were cocultured with Treg or Tconv cells for 4 days and then activated with IgE plus Ag. Total cell lysates were examined for the activation status of Stat5, ERK-1/ERK-2, Akt, and p38 (Fig. 3D). We found that Stat5 tyrosine phosphorylation was consistently enhanced by previous coculture with T cells while the other proteins examined showed little or no difference in activation. We quantified Stat5 phosphorylation via densitometry (Fig. 3E) and found that T cell coculture typically enhanced IgE-mediated Stat5 activation >2-fold above the control culture, which correlated with the increase in cytokine production. Stat5 selectivity in this process was further confirmed by measuring IgE-mediated p65 NF- κ B activation, which was unchanged by the presence of T cells (Fig. 3F). Our group has previously found that Stat5 is a focal point for mast cell function (28–30); thus, enhanced Stat5 activation is a logical means by which cytokine production could be augmented. It is striking that Stat5 is hyperactivated after IgE cross-linkage even in cocultures that contained Treg, which suppressed Fc ϵ RI levels. How T cells regulate Stat5 activation is a focus of our current studies.

IgE signaling is central to atopic diseases and represents a well-studied model of mast cell-mediated inflammation that is being extrapolated into autoimmune disorders. The importance of T cell-mast cell interactions is becoming more fully appreciated, as new work has uncovered a role for mast cells in T cell activation and dendritic cell function (28, 31, 32). Investigating these interactions may improve our understanding and treatment of atopic and autoimmune disorders.

Disclosures

The authors have no financial conflict of interest.

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