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IL-4 Inhibits Mouse Mast Cell FcεRI Expression Through a STAT6-Dependent Mechanism

John J. Ryan,† Shirley DeSimone,‡ Gregory Klisch,‡ Christopher Shelburne,* Lisa J. McReynolds,* Kathy Han,* Reka Kovacs,* Paria Mirmonsef,* and Thomas F. Huff‡

Mast cell activation by IgE-mediated stimuli is a central event in atopic disease. The regulation of the mast cell high affinity receptor, FcεRI, is poorly understood. We show that IL-4 can inhibit FcεRI expression on mouse bone marrow-derived mast cells and fetal liver-derived mast cell progenitors. This effect could be observed at 2.5 ng/ml IL-4 and was dose dependent. IL-4-mediated inhibition of cultured BMMC required 4 days of stimulation and was sustained at maximum levels for at least 21 days. The inhibition of FcεRI expression resulted in decreased sensitivity to IgE-mediated stimulation, as measured by serotonin release, and the induction of mRNA for IL-4, IL-5, IL-6, and IL-13. Additionally, IL-4 could abrogate the IgE-mediated increase in FcεRI expression. Lastly, IL-4-mediated inhibition was dependent upon expression of the STAT6 transcription factor, as STAT6-deficient bone marrow-derived mast cells did not decrease FcεRI levels in response to IL-4. These data argue for a homeostatic role of IL-4 in the regulation of FcεRI expression, a role that could be critical to understanding atopic disease. The Journal of Immunology, 1998, 161: 6915–6923.

Abbreviations used in this paper: BMMC, bone marrow-derived mast cells; SCF, stem cell factor; cRPMI, RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 1 mM sodium pyruvate; WEHI-3-CM, WEHI-3-conditioned medium; RPA, ribonuclease protection assay; EMSA, electrophoretic mobility shift assay.
Materials and Methods

Cells and reagents

Cells were maintained in RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 1 mM sodium pyruvate (all from Biofluids, Rockville, MD; RPMI) or in cRPMI supplemented with 10–20% WEHI-3-conditioned medium (cRPMI/WEHI-3). BMCC were derived from femurs of adult C57BL/6, C57BL/6 x 129 STAT6-deficient or heterozygous littermate mice by culture in cRPMI/WEHI for 3 wk, after which time >99% express FcεRI, c-Kit, and CD13 and have mast cell morphology by histochemical staining. STAT6-deficient and heterozygous littermate mice have been described previously (19) and were provided by Dr. James Ihle (Memphis, TN). The phenotype of BMCC was determined by flow cytometric analysis using Abs specific for c-Kit, CD13, and IgE and by histochemical staining (data not shown).

Cytokines and Abs

Murine SCF, IL-3, and IL-4 were purchased from R&D Systems (Minneapolis, MN). 2.4G2 rat anti-mouse FcγRII/II ascites was a gift from Jane Hu-Li and William Paul (National Institutes of Health, Bethesda, MD) or was purchased from Pharmingen (San Diego, CA). FITC-labeled rat anti-mouse CD13, FITC-labeled rat anti-CD44, FITC-labeled rat anti-CD11c, FITC-labeled rat anti-CD23 (clone B3B4), and murine IgE were purchased from Pharmingen (San Diego, CA). Rat anti-mouse IL-4R Ab was purchased from Genzyme (Cambridge, MA). Goat anti-rat IgG (H+L chains) F(ab')2, and FITC-labeled rat anti-mouse IgE were purchased from Southern Biotechnology Associates (Birmingham, AL). Rabbit-anti mouse STAT6 antisera was a gift from Dr. James Ihle (Memphis, TN).

Tissue culture conditions for inhibition of BMCC FcεRI expression

To examine the regulation of FcεRI expression, BMCC were washed and removed WEHI-3-CM and were incubated for 4–6 h at 37°C. BMCC were then plated at 500,000 cells/ml in a 200-ml volume, using 96-well flat-bottom plates (Costar, Cambridge, MA). Cells were incubated for the indicated times in cRPMI containing 5 mg/ml mouse IL-3 and the indicated concentrations of mouse IL-4 and/or mouse IgE. Cultures were fed every 4 days by replacement of half the culture medium and cytokines. FcεRI levels were determined by flow cytometric analysis.

Culture of fetal liver-derived progenitors

BALB/c mice were bred, and the day of vaginal plug observation was denoted day 0 of gestation. Embryos were harvested on day 14 of gestation, and livers were dissected and dispersed by manual disruption with glass slides. Cells were cultured in cDMEM supplemented with 15% embryonic stem cell FBS (Life Technologies), 100 mg/ml SCF, and 100 mg/ml IL-3 with and without 20 ng/ml IL-4 for 23 days at 37°C in 5% CO2 and 5% O2. Day 23 cultures contained >99% granulated metachromatic cells, as determined by histochemistry and acid toluidine blue.

Flow cytometric analysis

To detect expression of FcεRI on BMCC, cells were incubated with 0.3 ml of 2.4G2 rat anti-mouse FcγRII/II ascites/100 ml of solution for 10 min at 4°C, followed by 10 µg/ml IgE for 30 min at 4°C in PBS/3% FCS/0.1% sodium azide (FACS buffer). Cells were then washed twice and stained with 10 µg/ml FITC-conjugated rat anti-mouse IgE for 30 min at 4°C, washed twice, and analyzed in the absence of FcγRII or using FITC-labeled Becton Dickinson FACScan (Becton Dickinson, San Jose, CA). Control samples were identically stained in the absence of IgE. These BMCC populations do not express detectable levels of the low affinity IgE receptor, CD23, either basally or following IL-4 stimulation, as determined by flow cytometric analysis (data not shown). The percent inhibition of FcεRI expression was determined using mean fluorescence intensities, with cells cultured in IL-3 alone as the baseline for all comparisons. To detect expression of FcεRI on fetal liver-derived mast cells, cells were stained as described above, with the inclusion of 10 ng/ml rat anti-mouse CD23 in combination with 2.4G2 Ab. To detect expression of CD13 or Kit, cells were first incubated with 2.4G2 as described above; then stained with FITC-labeled anti-CD13, anti-Kit, or anti-CD4 (as a control stain); and washed as described above.

Serotonin release assay

Cells were incubated for 11–13 days in cRPMI supplemented with 5 ng/ml IL-3 with or without 20 ng/ml IL-4, then incubated overnight at approxi-

nately 5 x 107 cells/ml with 2 µCi/ml 5-hydroxy-[3H]tryptamine creatinine sulfate (Amersham, Arlington Heights, IL). Cells were washed twice, resuspended at 1 x 107 cells/ml, and incubated for 45 min at 37°C in cRPMI supplemented with 1 ng/ml IL-3 and 2 mg/ml IgE in 96-well V-bottom plates (Costar). Excess IgE was removed by washing twice in cRPMI, after which cells were plated at 1 x 106 cells/well and incubated for 30 min at 37°C with the indicated concentration of rat anti-mouse IgE or with 2 mM ionicomycin. To samples containing rat anti-mouse IgE, goat anti-rat IgG F(ab')2 was added at a 1 mg/ml final concentration for 30 min at 37°C. Control samples contained IgE alone, which resulted in higher background release of serotonin than anti-IgE alone (data not shown). Supernatants were harvested, and lysates were made using 100 µl of lysis buffer (50 mM HEPES (pH 7.5), 0.5% Triton X-100, and 5 mM EDTA). Radioactivity in supernatants and lysates was determined by scintillation counting, and the percent radioactivity in supernatants was calculated. All samples were studied in duplicate.

RNase protection assay (RPA)

For each sample, 5 x 107 BMCC were stimulated with 5 ng/ml IL-3 alone or IL-3 and 10 ng/ml IL-4 as described above for 6–13 days, with feeding of cells every 4 days. Cells were then washed and resuspended at 1 x 107/ml in cRPMI with 1 ng/ml IL-3 (cRPMI/IL-3). IgE (2 mg/ml) and 2.4G2 (5 mg/ml) were added for 45 min at 4°C. Cells were washed twice in cRPMI and resuspended at 1 x 107/ml in cRPMI/IL-3. Cells were then incubated with 1 mg/ml rat anti-mouse IgE for 30 min at 37°C, after which time goat anti-rat IgG F(ab')2 was added to a final concentration of 1 mg/ml for 5 h at 37°C. Control samples were incubated with goat anti-rat IgG F(ab')2 alone or with 2 mM ionicomycin for 5 h. RNA was harvested with RNAzol (Tel-Test, Friendswood, TX). RPA was performed using the mCK-1 probe set from the RiboQuant System (Pharmingen) to the manufacturer’s specifications. Pixel intensity was determined using a Phosphorimaging 445si System (Molecular Dynamics, Sunnyvale, CA).

Proliferation experiments

Cells were washed twice to remove WEHI-3-CM, incubated for 16 h in cRPMI followed by 2 h in RPMI, and plated at 2 x 105 cells/well in a 96-well flat-bottom plate for 48 h in cRPMI with the indicated cytokines. Cultures were pulsed with 1 µCi/ml tritiated thymidine deoxyribonucleotide for 16 h before harvesting. The mean counts per minute of triplicate samples were determined.

Electrophoretic mobility shift assay (EMSA)

BMCC (3 x 107) were resuspended in cRPMI without WEHI-3-CM at 2 x 107/ml, incubated for 4 h at 37°C, washed, and resuspended in RPMI at 2 x 107/ml for 2 h at 37°C. After a final wash, BMCC were resuspended at 6 x 106/ml in RPMI/50 mM sodium orthovanadate (vanadate) and stimulated with 100 ng/ml IL-3 or IL-4 for 10 min at room temperature. Reactions were stopped by addition of cold RPMI/100 mM vanadate. Cell pellets were resuspended in 10 ml of lysis buffer (0.5% Nonidet P-40, 50 mM Tris (pH 8.0), 10% glycerol, 100 µM EDTA (pH 8.0), 50 mM NaF, 150 mM NaCl, 100 µM NaVO3, 1 mM DTT, and 1 X complete protease inhibitor mixture (Boehringer Mannheim, Indianapolis, IN) and incubated on ice for 60 min. Lysates were cleared by centrifugation at 15,000 rpm for 15 min at 4°C, and supernatants were harvested, resuspended to a final concentration of 7.6 µg/ml, and stored at -70°C. For assays, 38 µg of cell lysate was incubated with 100 ng of 32P-labeled oligonucleotide in reaction buffer (40 mM KCl, 1 mM MgCl2, 0.1 mM EDTA, 0.5 mM DTT, 20 mM HEPES (pH 7.9), 6% glycerol, 1 mg/ml BSA, and 0.1 mg/ml poly(dI-dC)) for 15 min room temperature. Reactants were loaded (without loading dye) onto a 6% polyacrylamide/0.22 X TBE gel that had been pre-run at 200 V for 60 min and electrophoresed at 300 V for 2 h. Gels were dried and exposed directly to film. To detect STAT DNA binding activity, a double-stranded oligonucleotide corresponding to a GAS-like element found in the mouse IL-4 promoter element (4G-L3) was used: gatcAAGA CC1627TCACAGGAA193CTTAAACT Tgc. To detect STAT3 and -5 (induced by IL-3), a GAS-like element found in the human CD23 promoter was used: 5'-gatcAAGACCC227TCATCAAG225CTTAACT Tgc. Lysates were confirmed to contain STAT6 by supershift analysis with anti-

STAT6 Abs. Oligonucleotides were synthesized with a 5' ACTG overhang on each end (denoted by lowercase letters) and were labeled using Klenow DNA polymerase and [32P]dCTP by standard techniques.
Results

IL-4 inhibits FcεRI expression on murine BMMC and developing fetal liver progenitor cells

Given the role of IL-4 in IgE synthesis and the recent demonstration that IgE can up-regulate mast cell FcεRI expression (22, 23), we assessed the effect of IL-4 stimulation on BMMC expression of FcεRI. As shown in Fig. 1, BMMC cultured in the presence of IL-3 and IL-4 exhibited diminished FcεRI expression compared with cells cultured in IL-3 alone, as assessed by flow cytometry. This effect varied between different populations of BMMC, with an average inhibition of nearly 60% based on mean fluorescent intensities. IL-4-mediated inhibition of FcεRI expression could be observed at concentrations as low as 2.5 ng/ml IL-4. Maximal activity was observed using 20 ng/ml IL-4, with no further inhibition at higher concentrations (Fig. 1B).

The decrease in FcεRI expression required several days of IL-4 stimulation and was prolonged with continuous exposure to IL-4. As shown in Fig. 1C, maximal inhibition of FcεRI expression was observed after 4 days of stimulation and was sustained for 21 days of culture, the longest time point examined. Thus, the effects of IL-4 appeared to be both sensitive and sustainable. Further, IL-4 stimulation of BMMC did not appear to mediate a generalized decrease in the expression of all surface proteins, as CD13 expression was not diminished by IL-4 stimulation (data not shown).

After observing that IL-4 could inhibit FcεRI expression on differentiated mast cells, we wished to determine whether these effects were similar on developing mast cell progenitors. Bone marrow cells cultured in IL-3 and SCF yielded mast cells, as expected. However, attempts to culture bone marrow-derived progenitors in the presence of IL-3, SCF, and IL-4 led to a complete failure of mast cell development (data not shown). The ability of IL-4 to enhance monocyte development from bone marrow progenitors (26) may explain these results, as our cultures appeared to produce monocytic cells.

Since bone marrow progenitors were unsuitable for these studies, we assessed the effect of IL-4 stimulation on cultures of developing progenitors from day 14 murine fetal liver. As shown in Fig. 2, the addition of IL-4 to cultures of developing mast cells from fetal liver progenitors led to a dose-dependent decrease in FcεRI staining. The inhibitory effect of IL-4 was similar to that observed with BMMC, with an approximately 50% decrease in FcεRI expression levels. Thus, IL-4 is able to inhibit FcεRI expression on both differentiated mast cells and developing mast cell progenitor populations.

FIGURE 1. IL-4 inhibits FcεRI expression by BMMC. A, BMMC were cultured for 4 days in medium containing 5 ng/ml IL-3 with or without 20 ng/ml IL-4. FcεRI expression was determined by flow cytometry as described in Materials and Methods. Results are representative of 23 experiments using 10 separate BMMC populations derived from eight animals. B, Dose response of IL-4-mediated FcεRI inhibition on BMMC. BMMC cells were cultured as described in A with the indicated concentrations of IL-4, and the percent inhibition was determined using mean fluorescence intensities. Data shown are the means and SEs from three separate BMMC populations in one of five representative experiments. C, Time course of IL-4-mediated FcεRI inhibition on BMMC. Cells were cultured as described in A for the indicated time points. Percent inhibition was determined by calculations of mean fluorescence intensities. Data shown are the means and SEs from two (days 2 and 3) or four (days 4 – 21) separate BMMC populations.
IL-4 treatment of BMMC diminishes IgE-mediated mast cell activation

To determine the effect of IL-4 stimulation on FcεRI function, we measured serotonin release in response to FcεRI cross-linkage with anti-IgE Abs in BMMC cultured with IL-3 alone or with 20 ng/ml IL-4. As shown in Fig. 3, IL-4 treatment of BMMC led to only a slight decrease in maximal serotonin release following IgE cross-linkage. However, there was a more potent effect on the concentration of anti-IgE necessary to induce half-maximal serotonin release. Cells cultured in IL-3 and IL-4 required an average 11.5-fold more anti-IgE to reach half-maximal serotonin release than cells cultured in IL-3 alone (0.75 vs 0.065 µg/ml, respectively). Thus, IL-4-mediated inhibition of FcεRI expression coincided with a decrease in sensitivity to IgE-mediated stimulation.

We also investigated the effect of IL-4 treatment on the ability of mast cells to transcribe cytokine mRNA following IgE-mediated activation. Mast cells were cultured in IL-3 alone or with 20 ng/ml IL-4 for 6 or 13 days, then activated by cross-linkage of surface-bound IgE for 5 h. RPA measurements demonstrated an 80% decrease in induction of mRNA encoding IL-4, IL-5, IL-6, and IL-13 when comparing BMMC cultured with IL-3 plus IL-4 vs cells cultured in IL-3 alone (Fig. 4). These data indicate that IL-4 stimulation of BMMC reduces both immediate and delayed effects of IgE-mediated stimulation, and that these effects coincide with the IL-4-mediated inhibition of FcεRI expression.

IL-4 treatment of BMMC inhibits IgE-mediated up-regulation of FcεRI expression

The time required for FcεRI modulation by IL-4 is similar to that reported for IgE-mediated enhancement of receptor expression (24). This led us to examine the coordinated effects of IL-4 and IgE on FcεRI expression. BMMC were cultured in IL-3 alone, in IL-3 with increasing concentrations of IgE, or in IL-3, IgE, and 10 ng/ml IL-4. IL-4 repressed the IgE-mediated increase in FcεRI expression (Fig. 5). This effect was clearly dependent upon the concentration of IgE. BMMC cultured with 1 mg/ml IgE showed a modest reduction in FcεRI levels when costimulated with IL-4, while cells cultured with 0.2 mg/ml IgE expressed FcεRI at control (IL-3 alone) levels when costimulated with IL-4.

Preculturing cells with IL-4 or IgE for 4 days before the addition of IgE or IL-4 altered these results only slightly (Fig. 5). BMMC cultured in IL-3 plus IL-4 for 4 days before the addition of IgE showed lower FcεRI levels than cells cultured in IL-3 alone before the addition of IgE, an effect that closely mirrored the simultaneous addition of IL-4 and IgE. BMMC cultured with IL-3 and IgE for 4 days before the addition of IL-4 showed less reduction in FcεRI levels than cells precultured with IL-3 and IL-4. Thus, we found that IL-4 and IgE have opposing effects on FcεRI expression, with the end effect on FcεRI levels contingent upon the concentration and timing of each stimulus.

STAT6 expression is required for IL-4-mediated inhibition of BMMC FcεRI expression

IL-4R signaling is known to proceed through at least two pathways (reviewed in Ref. 17). Of these, the STAT6 pathway has been shown essential for IL-4-induced gene expression (18–20). While STAT6 is generally thought to be a positive regulator of gene transcription, there is some evidence for its requirement in gene
repression activities (27). Thus, it seemed possible that STAT6 functions in the IL-4-mediated inhibition of FcεRI expression.

To determine the role of STAT6 in IL-4 regulation of FcεRI, we derived BMMC populations from mice bearing a targeted disruption in the STAT6 gene or their heterozygous littermates. STAT6-deficient BMMC derived from WEHI-3CM cultures had mast cell morphology and expressed normal levels of FcεRI, c-Kit receptors, and CD13 (data not shown). However, STAT6-deficient BMMC exhibited little or no inhibition of FcεRI expression in response to IL-4 (Fig. 6A), while wild-type and heterozygous littermate control BMMC responded with a substantial reduction in FcεRI staining.

The lack of IL-4-mediated FcεRI regulation in STAT6-deficient BMMC did not appear to represent a deficiency in IL-4R expression or function. STAT6-deficient BMMC expressed IL-4R at similar levels to wild-type and heterozygote littermate BMMC populations (Fig. 6B), and all populations exhibited similar proliferative responses to IL-4 in combination with suboptimal doses of FcεRI, c-Kit receptors, and CD13 (data not shown). However, STAT6-deficient BMMC exhibited little or no inhibition of FcεRI expression in response to IL-4 (Fig. 6A), while wild-type and heterozygous littermate control BMMC responded with a substantial reduction in FcεRI staining.

The lack of IL-4-mediated FcεRI regulation in STAT6-deficient BMMC did not appear to represent a deficiency in IL-4R expression or function. STAT6-deficient BMMC expressed IL-4R at similar levels to wild-type and heterozygote littermate BMMC populations (Fig. 6B), and all populations exhibited similar proliferative responses to IL-4 in combination with suboptimal doses of IL-3 or SCF (Fig. 6C). The STAT6-deficient phenotype of these BMMC populations was confirmed by EMSA analysis. IL-4, and to a lesser extent IL-3, activated a DNA binding factor in wild-type, but not STAT6-deficient, BMMC that bound an oligonucleotide containing a canonical STAT6 site (Fig. 6D). This DNA:protein complex could be supershifted with anti-STAT6 antisera (data not shown). Using a probe that binds STAT3 and STAT5, wild-type and STAT6-deficient BMMC were shown to activate similar DNA-binding factors in response to IL-3 stimulation (data not shown). Thus, STAT6-deficient BMMC did not appear to have a global loss of transcription factor activation. These data indicate a requirement for STAT6 in IL-4-mediated inhibition of FcεRI staining.

Discussion

IL-4 is known for its central role in atopy, regulating IgE synthesis and Th2 development (reviewed in Ref. 14), both essential components of the allergic response. Thus IL-4 has been thought of as a pro-atopy factor, a hypothesis recently corroborated by the description of a human IL-4R polymorphism that is tightly linked to atopic disease (16). Further, the effects of IL-4 on mast cells are generally positive, enhancing mediator release (28) and cytokine production (29), and acting as a comitogen for mast cell lines (15). Lastly, IL-4 was recently reported to be essential for up-regulation of FcεRI on human mast cells (22, 23).

However, in these experiments using murine mast cells, we found that IL-4 exhibits a dose- and time-dependent inhibition of FcεRI levels. These results were consistent in 30 experiments using 13 individual BMMC populations derived from 10 animals, with an average IL-4-mediated inhibition of nearly 60% compared with that of cells cultured in IL-3. This effect was delayed but sustainable; maximum inhibition required 4 days of stimulation.
and was maintained for 21 days of culture. IL-4-mediated inhibition of Fc[\(\varepsilon\)]RI expression did not appear to be strain dependent, as we have obtained similar results using BMMC populations derived from C57BL/6, C57BL/6\times129, and BALB/c mice.

We also find that IL-4 stimulation of developing mast cell progenitors from day 14 fetal liver yields mast cells with decreased Fc[\(\varepsilon\)]RI levels compared with cells grown in medium lacking IL-4. Conversely, in human fetal liver progenitor cells IL-4 induces, rather than represses, Fc[\(\varepsilon\)]RI expression (22). This discrepancy could be explained by differential cytokine responsiveness across or within species. For example, mouse mast cell progenitors develop in response to IL-3 and SCF, while human progenitors do not respond to IL-3, but develop in response to SCF alone (30). Further, our own findings show a disparity in IL-4 responsiveness in mouse mast cell progenitors derived from different organs. While bone marrow mast cell progenitor development is nearly completely inhibited by IL-4 stimulation, fetal liver progenitors are less dramatically affected in their development, but do show reduced Fc[\(\varepsilon\)]RI expression. The precise explanation for differences in these experimental systems remains to be determined.

The functional consequence of inhibiting Fc[\(\varepsilon\)]RI levels was evident by measurements of serotonin release. We found an 11.5-fold decrease in sensitivity to anti-IgE stimulation when measuring serotonin release of BMMC cultured with IL-3 and IL-4 vs that of cells cultured in IL-3 alone. While IL-4 has been previously reported to enhance serotonin release (28), these studies examined the effects of IL-4 after a 2-day stimulation. Since our data indicate that IL-4 has little or no effect on Fc[\(\varepsilon\)]RI expression at this time point, the difference between our data and those reported earlier is probably due to this timing effect. A diminished response to activation stimuli could play a role in modulating mast cell degranulation, a critical step in allergic disease. What role, if any, this plays in vivo remains to be determined.

Stimulation with IL-4 was also found to result in potent inhibition of IgE-mediated cytokine mRNA induction. RPA studies indicated that BMMC cultured in IL-3 and IL-4 before cross-linkage of surface-bound IgE expressed mRNA for IL-4, IL-5, IL-6, and IL-13 at approximately 20% of the levels elicited by IgE-mediated stimulation of cells cultured in IL-3 alone. Coupled with the decrease in serotonin release, these data argue for a potentially important role of IL-4 in regulating the early and late effects of IgE-mediated mast cell stimulation. Whether these effects are entirely due to a reduction in Fc[\(\varepsilon\)]RI expression or to other effects on cytokine transcription is as yet unknown.

FIGURE 5. IL-4 stimulation of BMMC abrogates the IgE-mediated increase in Fc[\(\varepsilon\)]RI expression. Top, Simultaneous addition of IL-4 and/or IgE. BMMC were cultured for 8 days in the presence of IL-3 alone, IL-3 plus 10 ng/ml IL-4, IL-3 plus the indicated concentrations of IgE, or IL-3, IL-4, and IgE. IL-3 plus IL-4 samples yielded data identical with those shown in Figs. 1 and 6 and are omitted for clarity. Bottom, Delayed addition of IgE or IL-4. BMMC were cultured for 4 days in the presence of IL-3 alone, IL-3 plus IL-4, or IL-3 plus IgE (primary conditions). On day 4 cells were again stimulated under primary conditions and were additionally stimulated with IL-4 or IgE. Cells were harvested on day 8 and assessed for Fc[\(\varepsilon\)]RI expression by flow cytometry. None/IgE, IL-3 alone on days 1–4 and IL-3 plus IgE on days 4–8. IL-4/IgE, IL-3 plus IL-4 on days 1–4 and IL-3, IL-4, plus IgE on days 5–8. IgE/IL-4, IL-3 plus IgE on days 1–4 and IL-3, IgE, plus IL-4 on days 5–8. Data shown are representative of four separate experiments that gave similar results.
In addition to the effects on FceRI signaling, IL-4 was also capable of abrogating the dramatic increase in BMMC FceRI expression driven by long-term incubation with IgE. This effect displayed crucial timing and concentration elements, with maximal inhibitory effects observed under conditions where IL-4 was given before or during IgE stimulation of BMMC. These data may be

**FIGURE 6.** IL-4-mediated inhibition of BMMC FceRI expression is STAT6 dependent. A, BMMC derived from wild-type, STAT6<sup>+/−</sup>, or STAT6<sup>−/−</sup> mice were cultured as described in Fig. 1 and assessed for FceRI expression by flow cytometry. Data shown are representative of eight separate experiments using four individual STAT6<sup>+/−</sup> or STAT6<sup>−/−</sup> BMMC lines. B, IL-4R expression was determined by Ab staining and flow cytometry analysis as described in Materials and Methods. C, IL-4-induced proliferation of BMMC was determined as described in Materials and Methods. Data shown are the means of triplicate samples from one of four representative experiments. D, EMSA analysis of cytokine-induced STAT6 DNA binding activity. The arrow indicates the location of the STAT6/DNA complex.
IgE-mediated responses in a normal host. Thus, IL-4 may serve to both elicit and limit STAT6 DNA binding sequence that the murine FcεRIa chain promoter possesses a canonical STAT6-dependent mechanism, possibly maintaining receptor levels within a basal range. Mast cell development from splenic and bone marrow precursors is inhibited by IL-4, and fetal spleen-derived progenitors display diminished FcεRI expression when stimulated with IL-4. Thus, IL-4 may serve to both elicit and limit IgE-mediated responses in a normal host.

In summary, these data indicate that IL-4 may function to both initiate and dampen atopic responses. Both positive and negative regulatory events appear to require STAT6 activation. While further studies are needed to fully elucidate the mechanism of these responses and understand their in vivo roles in normal and atopic individuals, IL-4 may function as a homeostatic factor in the atopic response.

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