



FcεRI-dependent gene expression in human mast cells is differentially controlled by T helper type 2 cytokines

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Background: Mast cells (MCs) proliferate in response to T_H2 cytokines and express genes de novo after activation. Limited information is available concerning the interplay between these events.

Objective: We explored the potential for T_H2 cytokines to alter activation-dependent gene expression by MCs.

Methods: Cord blood-derived human (h)MCs maintained in stem cell factor (SCF) alone were compared with replicates treated with IL-4, IL-5, or IL-9, respectively, for their patterns of FcεRI-dependent gene induction using microarray technology.

Results: Activation of SCF-treated hMCs upregulated their expression of roughly 140 transcripts at 2 hours, including genes involved in cell cycle progression and arrest. Each cytokine substantially modified this profile; ~800 inducible genes apiece were controlled by IL-5 or IL-9, whereas 169 inducible genes were controlled by IL-4. IL-4 favored the induction of cytokines and of genes associated with cell growth arrest (GADD34, GAS-1, CIDE-A, INK4D, and BAX) and completely abolished the enhanced proliferation observed in the other 3 groups after activation. Conversely, IL-5 priming induced preferential upregulation of genes involved in cell proliferation and did not abolish thymidine incorporation.

Conclusions: T_H2 cytokines differentially modulate gene induction in hMCs after FcεRI cross-linkage. IL-4 uniquely controls cytokine gene expression by hMCs and might also limit their activation-driven proliferation. (*J Allergy Clin Immunol* 2003;112:1119-26.)

Key words: Cytokines, activation, mast cells, asthma

Abbreviations used

FcεRI: High-affinity Fc receptor for immunoglobulin E
hMC: Cultured human mast cell
MC: Mast cell
MAPK: Mitogen-activated kinase
SCF: Stem cell factor

Mast cells (MCs) are central to allergic reactions and asthma.^{1,2} When activated through their high-affinity Fc receptor for immunoglobulin E (FcεRI), MCs rapidly release preformed granule-associated mediators, generate newly formed eicosanoids, and also generate a diverse array of proinflammatory and immunomodulatory cytokines, growth factors, and chemokines over a period of hours,³⁻⁵ reflecting de novo transcriptional activity. In vitro studies of both human⁶ and mouse⁷ MCs indicate that they survive and regranulate after activation. Furthermore, localized anaphylaxis in mice is followed 24 hours later by a 5-fold increase in the numbers of local MCs, suggesting potential in situ proliferation after activation.⁷ Transcriptional and proliferative events in activated MCs could relate to their putative, yet poorly understood, functions in persistent inflammation and suggest that their functional phenotype might change profoundly during an inflammatory response.

All mammalian MC development requires an interaction between the Kit membrane tyrosine kinase and its stromal cell-derived ligand, stem cell factor (SCF).^{8,9} Phenotypically heterogeneous MC subpopulations exist within this SCF/Kit-dependent lineage. Although mouse MC subpopulations differ in fixation properties and differential expression of at least 10 different proteases,¹⁰ human MCs (hMCs) express a more limited repertoire of proteases than their rodent counterparts; a family of homologous tryptases,¹¹ a single MC-specific chymase,¹² cathepsin G,¹³ and a carboxypeptidase A.¹⁴ hMC subpopulations either express tryptases only (commonly referred to as "MCT") or tryptases plus chymase, carboxypeptidase A, and cathepsin G ("MCTC"). One critical feature of MC heterogeneity is strikingly conserved between species; a requirement for T-cell-derived cytokines in the development of MCs in the intestinal (and likely respiratory) mucosal surfaces but not for the

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adjacent submucosa. T-cell-deficient mice¹⁵ and humans¹⁶ lack MCs in these mucosal surfaces, despite normal MC numbers in the adjacent submucosa. Moreover, intestinal helminths elicit a T-cell-dependent reactive intraepithelial mastocytosis in mice.^{15,17} This feature is similar to intraepithelial mastocytosis observed in several human allergic diseases, including asthma, rhinitis, allergic conjunctivitis, and eosinophilic gastroenteritis.¹⁸⁻²² A number of recombinant T-cell-derived cytokines (IL-3, IL-4, IL-5, and IL-9) either enhance SCF-dependent proliferation²²⁻²⁵ or sustain survival²⁶ of hMCs and their progenitors from a number of sources in vitro. IL-4 also induces expression of key effector genes in hMCs²⁷ and primes for augmented activation responses.^{24,28} Thus, allergy-associated T-cell-derived cytokines could contribute to the phenotypic diversity of MCs in humans by altering basal and activation-dependent gene expression profiles. This pathogenetically relevant issue has not been experimentally explored in hMCs.

In this study, we examined cytokine-dependent modulation of a transcriptional repertoire of mature cytokine-responsive hMCs derived from umbilical cord blood progenitors²³ after FcεRI-mediated activation. We found that the pattern of activation-dependent gene expression by hMCs was selectively altered by priming cytokines; in particular, the profile of genes induced by activation after IL-4 priming contrasted markedly to the pattern of inducible gene expression emerging in the groups primed with either IL-5 or IL-9, with IL-4 preferentially inducing growth arrest genes after hMC activation. Moreover, these cytokine-dependent differences were associated with contrasting patterns of proliferation after activation. The data might provide information relevant to the mechanisms by which IgE-dependent inflammation is controlled and reactive MC hyperplasia is induced and resolved.

METHODS

Cytokines

Recombinant human SCF and IL-6 were purchased from R & D Systems (Minneapolis, Minn). Recombinant human IL-4, IL-5, IL-9, and IL-10 were purchased from Endogen (Woburn, Mass).

Cell culture

hMCs were derived in vitro from umbilical cord blood mononuclear cells as described.²³ Cells were used for this study when they reached > 95% toluidine blue positivity (generally 7-9 weeks of culture).

Priming, sensitization, and anti-IgE activation

hMCs were washed into new medium containing SCF (100 ng/mL) with or without IL-4 (10 ng/mL, Endogen), IL-5 (10 ng/mL, Endogen), and IL-9 (50 ng/mL, R & D) and cultured for 5 days. Cytokine doses were chosen on the basis of the optimal concentrations required for synergistic proliferation with SCF,²³ and, in the case of IL-4, for induction of leukotriene C₄ synthase.²⁷ On the fourth day, semipurified human myeloma IgE (2 μg/mL) (Chemicon, Temecula, Calif) was added to the cells to saturate FcεRI. The hMCs were washed extensively and stimulated with a rabbit anti-human IgE antibody (ICN, Costa Mesa, Calif, 1 μg/mL). Preliminary experiments showed no differences in histamine release between samples treated with irrelevant IgG or medium alone.

Thus, for all groups, medium alone was used as a negative control. All activated samples and controls were incubated for 2 hours (or, when indicated, for 6 hours) at 37°C before harvesting for RNA. Cytokines were measured in the supernatants collected 6 hours after activation by commercial ELISA as detailed elsewhere.²⁸

Microarray expression analysis

RNA isolation, reverse transcription, and small-scale DNA synthesis for the microarray were prepared as described elsewhere.^{29,30} The custom-made in-house arrays contain ~10,000 nonredundant genes, representing ~30% of the genome,³¹ composed of PCR products (0.2-2 kB in length) generated from cDNA libraries representing a wide variety of human tissues and cell types. Double-stranded ³²P-labeled cDNA probes were derived from hMC RNA and hybridized to each nylon array in duplicate.³⁰ Membranes were then scanned by a phosphorimager, and the spots were quantified. Intensities of each array were normalized to the median of the array, and the average was taken of the replicates. Self-organizing map analysis and hierarchical clustering was performed on data filtered so that the only genes with expression intensities ≥1.0 and at least a 1.5-fold difference between the probe with the highest intensity and the lowest intensity were analyzed. Self-organizing maps³² arrange genes into groups with statistically similar expression patterns and were generated as described using a clustering algorithm to analyze all the intensity values for each gene in each RNA sample.³¹ Hierarchical clustering was done using GeneMaths (Applied Maths, Austin, Tex). In this analysis, the intensity of expression for each gene in each RNA sample was assigned an intensity-based color code indicating fold upregulation (red) or downregulation (green) compared with the mean for each particular gene across all samples. A dendrogram was built to show expression-based relationships across the entire set of regulated genes and samples used in the experiment. Scatter plots were calculated using internally designed software.

Proliferation and apoptosis

Proliferation of activated and nonactivated hMCs was measured in triplicate samples of 2×10^4 passively sensitized hMCs maintained in SCF, stimulated with 1 μg/mL anti-IgE or medium only, in the wells of 96-well microtiter assay plates (Costar, Cambridge, Mass). Cells were immediately pulsed with 0.1 μCi of [³H]thymidine (6.7 Ci/mmol, Dupont NEN, Newton, Mass), incorporation was determined as described.²³ Proliferation was assessed at 6 and 24 hours after stimulation and expressed as absolute counts per minute and as percent change in counts per minute. Apoptosis was determined by use of flow cytometry with Annexin V-FITC conjugate and propidium iodide according to the manufacturer's protocol (Pharmingen, Carlsbad, Calif). Flow cytometry analysis for Kit expression was performed as previously described²³ with the mAb K69 (Pharmingen) and an isotype-specific control. Results are displayed as overlaid histograms.

Statistical analysis

Data are reported as the mean ± SEM from at least 3 independent experiments with the cells from different donors. Statistical differences were determined with a 2-tailed Student *t* test.

RESULTS

Transcriptional regulation mediated by activation through FcεRI

Microarray analysis showed highly reproducible results (variability in the duplicate hybridizations of $5.5\% \pm 1.7\%$, mean ± SD) and was restricted to genes with very low expression levels (Fig E1, supplemental

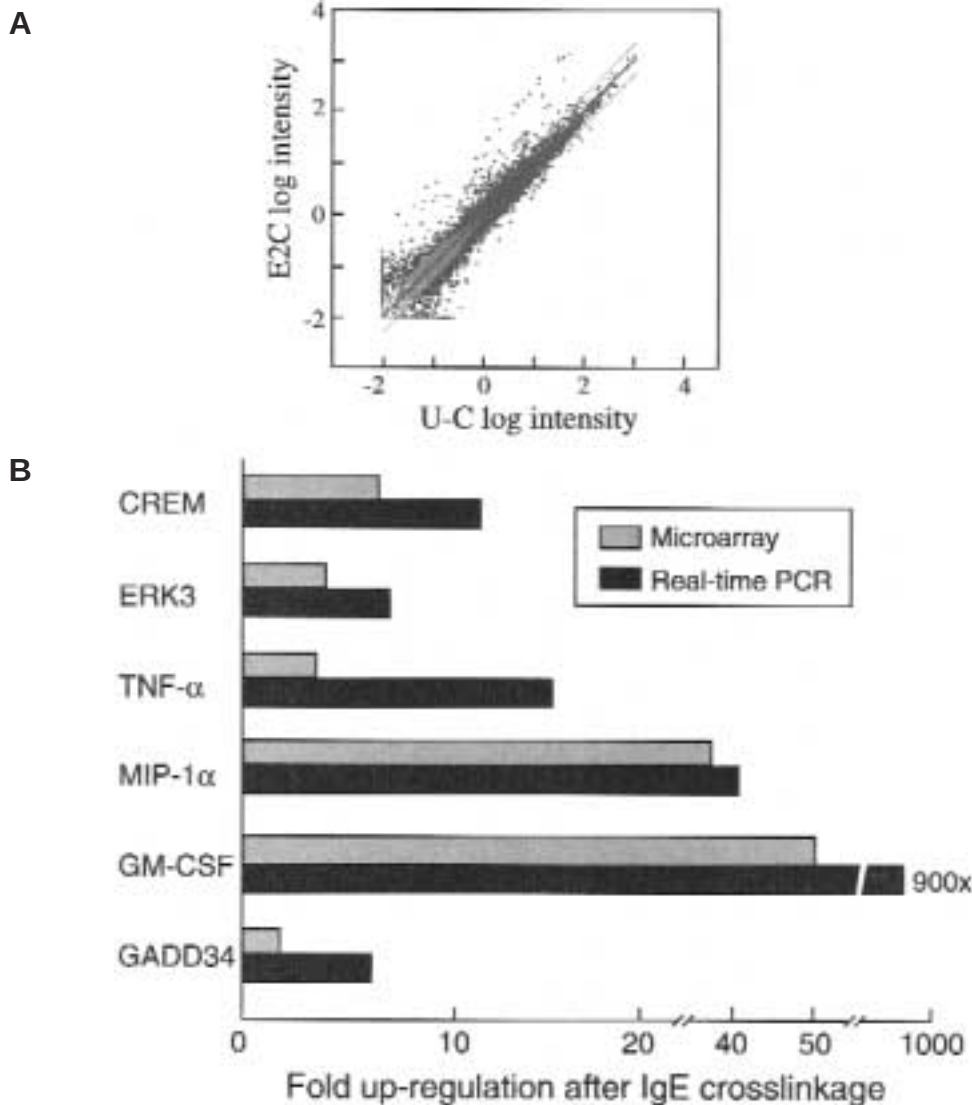


FIG 1. A, Scatter plot analysis of gene induction on Fc ϵ RI cross-linkage of cultured human mast cells (hMCs). The horizontal axis indicates absolute expression levels for the resting hMCs, and the vertical axis shows expression levels for the activated hMCs. Each dot represents 1 gene on the array. The diagonal line represents identical expression levels for any given gene in both samples, and the parallel blue lines indicate the ± 2 -fold difference cutoff chosen for the analysis. Dots falling within the space defined between the blue lines are considered constitutive genes, and dots outside that space are considered upregulated or downregulated genes (≥ 2 -fold change in expression). **B**, Comparison of magnitude in upregulation of a subset of genes as determined by array results (gray bars) and real-time PCR (TaqMan) (black bars). Results were similar on a second analysis. The same RNA was used for microarray and real-time PCR.

information). Scatter plot analysis of unprimed hMCs (Fig 1, A) showed ~140 transcripts (~1.5% of the genome), with a steady-state accumulation of at least 2-fold on activation by means of the Fc ϵ RI, including several genes that were upregulated at least 4-fold (Table E1, supplemental information). Real-time PCR of the same RNA samples reproducibly showed the same pattern of representative gene expression as the microarray (Fig 1, B), generally at higher levels than when detected by the less quantitative microarray. Although ~180 genes were downregulated by Fc ϵ RI-dependent activation, only 4 of these were downregulated 4-fold or more (Fig 2, A).

Notably, *c-kit* was 1 of these 4 genes. The downregulation of *c-kit*, confirmed by real-time PCR, was time-dependent and enhanced by both IL-4 and IL-5 priming (Fig 2, B). The corresponding Kit protein was also transiently decreased under these conditions (Fig 2, C).

Among the 140 activation-dependent transcripts, the largest group (~one quarter of the total) coded for cell surface molecules (listed in Table E1, supplemental material). Enzymes, with roughly half being proteases, accounted for ~20%, including tryptase- β 1 (3-fold induction), granzyme B (8-fold induction), airway trypsin-like protease (3-fold induction), and the disinte-

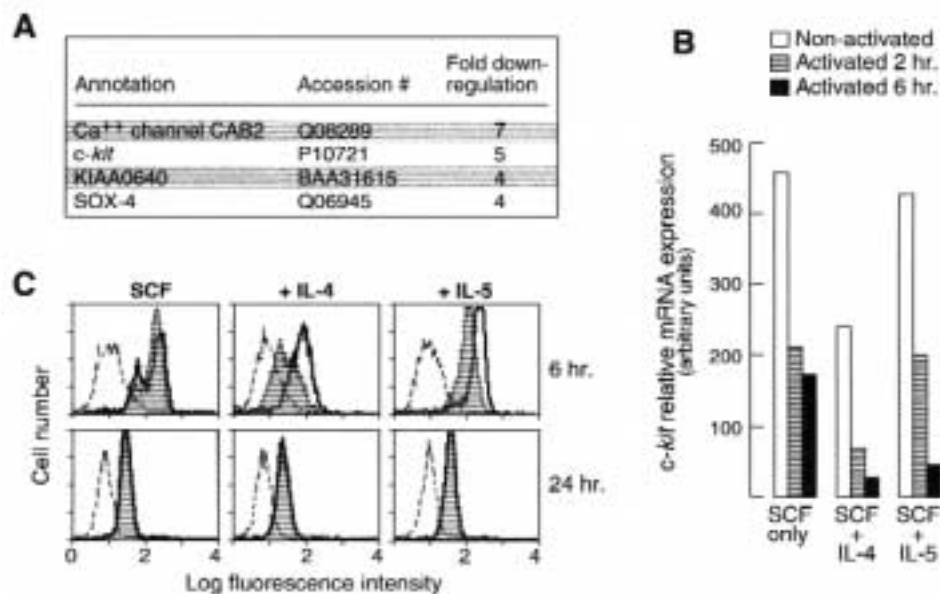


FIG 2. Activation-dependent changes in *c-kit* mRNA and surface Kit expression. **A**, Identity of the 4 highly expressed transcripts that were downregulated by at least 4-fold in response to FcεRI cross-linkage as determined by microarray. The data were confirmed by real-time PCR. **B**, Differential effects of IL-4 and IL-5 priming on activation-dependent decreases in *c-kit* mRNA as determined by real-time PCR. **C**, Cytofluorographic analysis for surface Kit expression at 6 hours (*top*) and 24 hours (*bottom*) after FcεRI cross-linkage. Histograms for Kit expression in activated cultured human mast cells (*bold tracings*) are overlaid with their nonactivated replicates (*gray tracings*) and with the negative controls (*dashed tracings*). Note that the gray and bold tracings are superimposed by 24 hours. Data are from 1 experiment representative of 2 performed.

grin ADAM-TS-9 (3-fold induction). Signaling molecules (~20% of the induced genes) included 2 mitogen-activated protein kinases (MAPKs), namely MAPK-extracellular-signal-regulated kinase-1 and extracellular-signal-regulated kinase-3; the former was among the genes with the highest levels of FcεRI-mediated induction (35.6-fold). Finally, 13% of upregulated genes in the unprimed hMCs were cytokines, including some among the genes exhibiting the highest absolute levels of expression (eg, macrophage inflammatory protein-1β), and some among the most strongly induced in activated compared with resting cells (eg, 45-fold, 38-fold, and 33-fold induction of mRNA encoding GM-CSF, macrophage inflammatory protein-1α, and monocyte chemoattractant protein-1, respectively; [Table I](#)).

Distinct effects of IL-4 and IL-5 in modulating activation-dependent gene expression

We next studied how cytokine priming affected gene expression after FcεRI-mediated activation by use of hierarchical clustering analysis. Again, results from duplicate experiments were highly reproducible. Each cytokine elicited a distinct “signature” of gene expression in the nonactivated samples and modulated the expression pattern induced by FcεRI activation ([Fig 3, A](#)). Although IL-5 and IL-9 elicited different profiles of gene expression without activation, they favored strikingly similar profiles and numbers (~800 apiece) of activation-dependent gene expression. In contrast, IL-4 primed for the upregulation of ~5-fold fewer genes, most of which did not overlap with

the other groups ([Fig 3, A](#)). For simplicity, we focused subsequent analysis on contrasts between the IL-4–primed and IL-5–primed groups, with nonprimed, nonactivated hMCs used as an additional control for the comparison. Self-organizing maps revealed 3 clusters dissecting out the differential contributions of IL-4 and IL-5 priming ([Fig 3, B](#)): (1) genes controlled by IL-4 (169 genes); (2) genes controlled by IL-5 (763 genes); and (3) genes controlled by both IL-4 and IL-5 (162 genes). Thus, 1093 genes, or, when corrected for the entire genome, ~10% of the genome was altered by priming and activation, and IL-5 primed for 4.5-fold more genes than did IL-4.

Although a complete analysis of the >1000 genes induced by priming in these data sets is beyond the scope of this study, there were striking contrasts in the gene signatures favored, respectively, by IL-4 and IL-5. First, whereas cytokines were induced in both IL-4 and IL-5 samples, these transcript levels were higher in the IL-4–primed groups. IL-4 primed for greater levels of IL-3 mRNA (10-fold greater activation-dependent induction in IL-4–primed than IL-5–primed hMCs), followed by eotaxin-3, IL-4, GM-CSF, TNF-α, single cysteine motif-1β, IL-4, IL-5, and IL-13 (5-fold, 4-fold, 3-fold, 2.5-fold, 2.5-fold, 1.7-fold, and 1.7-fold stronger induction in the IL-4–primed group than in the IL-5–primed group, respectively). Supernatants collected from IL-4–primed hMCs 6 hours after activation contained IL-3 (120 ± 38 pg/10⁶ hMCs), IL-5 (100 ± 12 pg), and IL-13 (142 ± 22 pg) proteins at levels that were 2-fold, 5-fold, and 5-fold higher than in supernatants

from activated, IL-5–primed hMCs ($n = 1$, data not shown). These data are consistent with our prior report.²⁸ Second, IL-4–primed hMCs showed higher levels of transcripts involved in growth arrest and/or apoptosis, such as GADD45, GADD34 (GB:U83981), GAS-1 (SP:P54826), CIDE-A (GB: AF041378), INK4D (SP: P55273), and BAX (SP: Q07814)^{33–37} (Table I). In contrast, the IL-5–primed group expressed higher levels of inducible genes involved in cell cycle progression, DNA replication, gene transcription, and RNA translation; a total of 34 such genes were expressed at higher levels in the activated IL-5–primed group compared with their IL-4–primed counterparts (Table I). Importantly, all groups showed induction of the anti-apoptotic bcl-2 family member A1,⁷ maximally so for the IL-4–primed group (4-fold induction, confirmed by real-time PCR, data not shown).

Differential effects of activation on survival and proliferation of primed hMCs

The differential modulation of activation-dependent genes potentially germane to MC proliferation prompted measurements of proliferation and entry into apoptosis with and without FcεRI cross-linkage. All hMCs were maintained in the continued presence of SCF to support basal proliferation and survival. Without activation, both the IL-4–primed and IL-5–primed groups tended to proliferate more than their unprimed or IL-9–primed replicates at 6 hours (1111 ± 621 , 2604 ± 1216 , 3088 ± 3057 , and 1377 ± 994 counts per minute for the nonactivated unprimed, IL-4–primed, IL-5–primed, and IL-9–primed hMCs, respectively, means $\pm$ SD, $n = 3$, data not shown) and at 24 hours (Fig 4, A) after the thymidine pulse, and consistently contained 10% to 20% more cells than their cohorts at 24 hours. Although activation did not alter proliferation in any of the groups at 6 hours (not shown), it incrementally increased proliferation at 24 hours in the unprimed, IL-9–primed, and IL-5–primed cohorts relative to nonactivated replicates ($n = 6$, Fig 4). Although the nonactivated IL-4–primed and IL-5–primed hMCs showed similarly high proliferative rates at 24 hours (Fig 4, A), only the IL-5–primed group showed the activation-dependent increment in proliferation seen in the other groups, whereas this increment was abolished in the IL-4–primed group (Fig 4, B). Nonadherent cell counts were ~20% lower in the IL-4–primed groups than in the other groups at 24 hours after activation, although extensive clumping and adhesion of the cells limited the accuracy. Although slightly higher numbers of apoptotic cells were observed at in the nonactivated unprimed ($8\% \pm 4\%$ at 6 hours, $n = 3$) and IL-4–treated ($9\% \pm 5\%$) groups than in either the IL-5–treated or IL-9–treated groups ($4\% \pm 3\%$ and $3\% \pm 1\%$, respectively), these percentages were not increased by activation out to 48 hours and did not change during the experiment.

DISCUSSION

This study reveals that activation-dependent gene expression by hMCs is altered by T_H2 cytokines that are

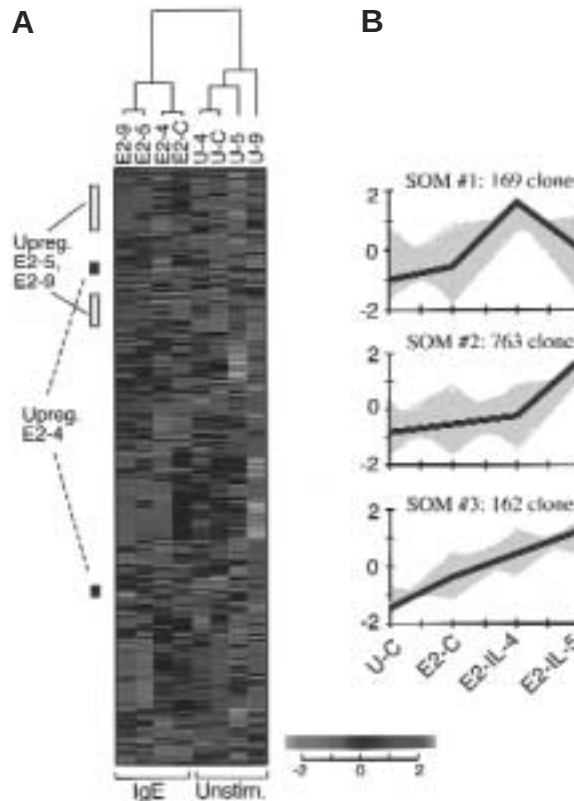


FIG 3. A, Hierarchical clustering of regulated genes from the microarray experiment. The branching pattern (top) shows the relationships between expression profiles of the different samples. U-C, Unstimulated, unprimed cultured human mast cells (hMCs); U-4, unstimulated, IL-4–primed hMCs; U-5, unstimulated, IL-5–primed hMCs; U-9, unstimulated, IL-9–primed hMCs; E2-C, activated, unprimed hMCs; E2-4, activated, IL-4–primed hMCs; E2-5, activated, IL-5–primed hMCs; E2-9, activated, IL-9–primed hMCs. The regions labeled *Upreg. E2-5*, *E2-9* indicate genes with similar upregulation profiles in the activated IL-5–primed and IL-9–primed groups, whereas *Upreg. E2-4* indicates genes whose upregulation was maximal in the IL-4–primed hMCs. The color-coded scale indicates the degree of expression for each gene on the array compared with its average expression across the panel of samples. B, Three Self Organizing Maps (SOMs) (see “Methods”) showing genes with peak expression in activated IL-4–primed hMCs (SOM #1, 169 genes), in activated IL-5–primed hMCs (SOM #2, 769 genes), and in either IL-4–primed or IL-5–primed activated hMCs (SOM #3, 162). The black lines indicate the expression pattern representative of the SOM, whereas the blue lines show the range of expression intensities for the genes in each SOM compared with their averaged expression across all samples.

prominent in allergic inflammation. Studies in mice using knockouts, antibody neutralization, or targeted overexpression strategies *in vivo* have confirmed that T_H2 cytokines regulate MC hyperplasia associated with helminth-induced mucosal inflammation,^{38–41} during which MCs undergo striking changes in granule ultrastructure, protease expression profile, and survival kinetics.⁴² T_H2 cytokines also figure prominently in human allergic diseases, and human MCs express functional receptors for IL-3,^{23,43} IL-4,⁴⁴ IL-5,²³ and IL-9.²⁵ The corresponding cytokines are either cytoprotective or comitogenic for hMCs *in vitro*. IL-4 also upregulates expression of FcεRI⁴⁴ and leukotriene C₄ synthase²⁸ in cultured

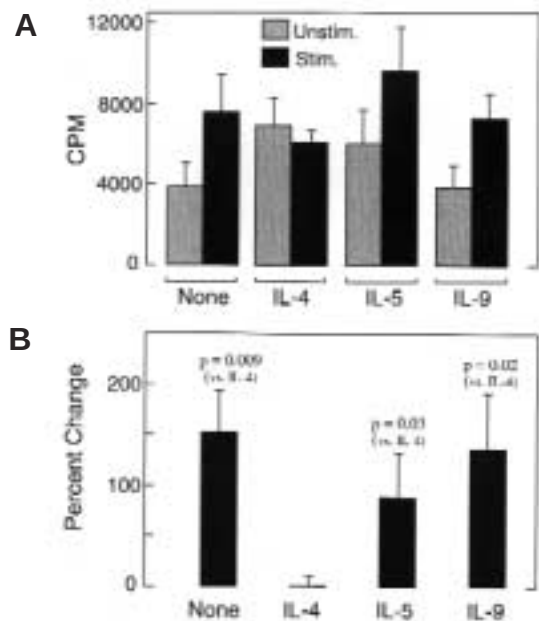


FIG 4. Effect of cytokine priming on basal and activation-dependent proliferation. **A**, Absolute counts per minute of [^{3} H]thymidine incorporated by cultured human mast cells (hMCs) 24 hours after Fc ϵ RI cross-linkage (black columns) or treatment with buffer control (gray columns), both in the constant presence of stem cell factor (100 ng/mL) to maintain basal proliferation and survival. The values obtained at 6 hours are presented in the text. **B**, Percent changes in [^{3} H]thymidine incorporation by hMCs activated by Fc ϵ RI cross-link compared with nonactivated controls at 24 hours. The percent change in thymidine incorporation was determined for each group of hMCs in each experiment. Both **A** and **B** are the mean $\pm$ SD for $n = 6$ experiments.

hMCs (which resemble “MCTC” based on protease content)²³ and primes them for augmented cytokine secretion,²⁸ indicating a flexible program of gene expression that is potentially modified by cytokines relevant to the pathophysiology of allergy. Recent gene profiling studies demonstrated that both human and mouse MCs display an overlapping profile of activation-induced cytokine and chemokine gene expression.^{45,46} To date, however, no study has explored the potential modification of this gene expression profile by cytokines relevant to mucosal inflammation. We therefore undertook this analysis.

We first sought to establish the profile of genes that were upregulated by Fc ϵ RI cross-linkage in the absence of cytokine priming, selecting only genes with sufficiently robust and reproducible expression (Fig E1, supplemental information). Approximately 140 upregulated genes and an approximately equal number of suppressed genes were identified on the microarray (Fig 1, A), with real-time PCR confirming the pattern (Fig 1, B). The induced expression of multiple cytokines and chemokines, as well as surface receptors, enzymes, and genes involved growth and growth arrest, is consistent with previous gene profiling experiments in both human and mouse MCs,⁴⁵ although growth genes were not the focus of these studies. Thus, activation likely causes functional changes in hMCs that are not directly related to their secretory activity. Of the suppressed genes, only a small number were downregulated by 4-fold

or more, including *c-kit* (Fig 2, A and B), and activation decreased Kit surface protein in the IL-4–primed and IL-5–primed groups (Fig 2, C). The transient decrease in Kit could facilitate the mobility of MCs after activation, because Kit signaling facilitates adhesion.⁴⁷

We next determined the respective effects of IL-4, IL-5, and IL-9 on the profile of inducible genes. Microarray revealed a characteristic “signature” for each priming cytokine, both with and without activation (Fig 3, A). The more limited impact of IL-4 on activation-dependent gene induction (4-fold fewer activation-induced genes than in the IL-5–primed or IL-9–primed groups) suggest that these differences are not entirely due to the enhanced “strength of signal” anticipated as a result of IL-4–induced upregulation of Fc ϵ RI.⁴⁴ Nevertheless, IL-4 both increased the strength of induction for cytokine genes that were expressed by the unprimed hMCs and expanded the profile to include some transcripts (eg, eotaxin 3) not previously reported as MC products. Other studies have noted that IL-4 not only enhances Fc ϵ RI-dependent cytokine generation in hMCs from several sources^{24,28,44,48} but also primes them for cytokine generation in response to challenge with bacteria⁴⁹ or with cysteinyl leukotrienes.⁵⁰ The IL-4 effect likely alters signal transduction or transcriptional machinery germane to cytokine production through all of these receptors. It is noteworthy that MAPK-extracellular-signal-regulated kinase-1, an upstream effector of p38 MAPK involved in cytokine gene induction,⁵¹ was upregulated ~5-fold by IL-4 priming even without activation (not shown). Through these actions, IL-4 could amplify leukocyte recruitment and inflammation downstream of MC activation, particularly in the context of allergic inflammation.

Whereas IL-5 is cytoprotective for cord blood–derived hMCs ex vivo,²³ IL-4 induces their apoptosis under conditions where IL-6 is not used.⁵² In our study, IL-4 itself did not induce apoptosis of hMCs, possibly because IL-6 was used in their derivation. The higher basal proliferative rates of the IL-4–primed and IL-5–primed hMCs (Fig 4) is consistent with the recognized capacity for both of these cytokines to amplify SCF-dependent proliferation of hMCs in vitro.^{23,26} The contrasting patterns of gene expression involved in cell cycle arrest and progression (Table I) could relate to the different responses of these 2 proliferating groups to activation (Fig 4, B). In contrast, differences in apoptosis were not detected under these conditions. This could reflect overriding function of the inducible Bcl homologue A1, which renders mouse MCs resistant to apoptosis after Fc ϵ RI-dependent activation.⁷ It is also noteworthy that survival rates were high despite transiently decreased Kit expression (Fig 2, C). Prior studies suggested that mouse MCs can proliferate in response to IgE-dependent activation itself.^{53,54} Indeed, activation modestly enhanced thymidine incorporation in each hMC cohort, with the noteworthy exception of the IL-4–primed group (Fig 4, B). Thus, IL-4 exerts complex effects on MC growth, favoring SCF-driven proliferation of mouse⁵⁵ and human²⁴ MCs ex vivo, yet inducing apoptosis under certain conditions⁵² and blunting additional growth after an activation signal (Fig 4, B).

TABLE I. Modulation by IL-5 and IL-4, respectively, of cell proliferation– (top) and growth arrest– (bottom) gene expression by activated cultured human mast cells

Gene	Fold expression E2-5/E2-4	Functional group
Cyclin A2	2	Cell cycle
Cyclin E	2	Cell cycle
Cyclin G2	2	Cell cycle
CDC21	2	Cell cycle
RGS1	3.5	Cell cycle
G0/G1 switch regulator RGS2	2	Cell cycle
G1 to S phase transition protein1 homolog	2.5	Cell cycle
RAB-3A	2	Cell cycle
RAB-9	2.5	Cell cycle
ETS-1 transcription factor	2.5	Cell cycle
DNA polymerase beta sub	2	DNA replication
DNA polymerase delta small sub	3	DNA replication
Replication protein 70-kd DNA-binding sub	2	DNA replication
DNA replication licensing factor MCM2	2	DNA replication
DNA replication licensing factor MCM6	2	DNA replication
DNA replication licensing factor MCM7	2	DNA replication
ATP-dependent DNA helicase II	2	DNA replication
DNA ligase III	2	DNA replication
DNA-directed RNA polymerase II large sub	3	Transcription
DNA-directed RNA polymerase II 7.6-kd pept	3	Transcription
DNA-directed RNA polymerase II 23-kd pept	2	Transcription
Transcription initiation factor IIB (TFIIB)	2	Transcription
Ribosomal protein S5	2	Translation
Ribosomal protein SA	2.5	Translation
Ribosomal protein L4	2	Translation
Ribosomal protein L5	2.5	Translation
Ribosomal protein L10	2	Translation
Ribosomal protein L14	2	Translation
Elongation factor 1-γ	2	Translation
Initiation factor EIF-2β	2	Translation
Initiation factor EIF-3β	2	Translation
Initiation factor EIF-4A-I	2	Translation
Initiation factor EIF-4GII	3	Translation
Initiation factor EIF-6	2	Translation

Gene	Fold expression E2-4/E2-5	Functional group
Apoptosis associated protein GADD34	1.5	Growth arrest/apoptosis
Growth-arrest–specific protein GAS-1	1.5	Growth arrest/apoptosis
Cell death activator CIDE-A	2	Growth arrest/apoptosis
Apoptosis regulator BAX	1.5	Growth arrest/apoptosis
Cyclin-dependent kinase 4 inhibitor D INK4D	1.5	Growth arrest/apoptosis

Fold expression refers to the gene expression level in activated IL-5–primed cultured human mast cells (E2-5) over that seen in the activated IL-4–primed cultured human mast cells (E2-4), and vice versa. All listed genes had absolute expression levels of 1 or greater.

Our study confirms that T_H2 cytokines differentially alter the expression of functionally relevant genes that could relate to disease-associated alterations in MC numbers, fate, and function. Although the homeostatic functions of SCF and Kit ensure the presence of MCs in all healthy tissues, the increased numbers of MCs observed in asthma and other allergic human diseases^{2,18-22} could reflect the ability of the T_H2 cytokines to amplify their growth and alter profiles of their gene expression. We speculate that IL-4, while amplifying the secretory properties of MCs (cys-LT generation, cytokine production), might also limit their expansion in the context of activation by favoring growth arrest.

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