

Human cord blood-derived mast cells synthesize and release I-309 in response to IgE

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Abstract

Mast cells are the central mediating cells of allergic reactions. Binding of allergen specific IgE to high affinity IgE receptor (FcεRI) and subsequent binding of allergen by the IgE causes receptor cross-linking and activation. In a study examining the differential gene expression in human cord blood-derived mast cells (CBMCs) mediated by activation of FcεRI both with IgE and IgE followed by cross-linking with α-IgE, the chemokine I-309 was found to be upregulated.

I-309 is the ligand for the CCR8 receptor and is responsible for chemoattraction of TH2 type T-cells. Interestingly, I-309 RNA and protein levels were elevated not only in response to IgE/α-IgE activation but also by IgE alone. In addition, the I-309 levels were augmented by growth of the CBMCs in the presence of the proinflammatory cytokine IL-4. GM-CSF and MIP-1α secretion was also induced by IgE. These results suggest that IgE, through the production and release of cytokines such as I-309, GM-CSF and MIP-1α could promote an inflammatory reaction in the absence of antigen stimulation of mast cells.

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Introduction

Mast cells are the central mediating cells of allergic reactions. Binding of allergen specific IgE to high affinity IgE receptor (FcεRI) and subsequent binding of allergen by the IgE causes receptor cross-linking and activation (Yong, 1997). This activation leads to the release of preformed mediators stored in secretory granules. Among these mediators are histamine and mast cell proteases such as tryptase and chymase

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(Yong, 1997). In addition, activation leads to the de-novo synthesis of other mediators such as leukotrienes and prostaglandins and the production and release of various cytokines and chemokines including tumor necrosis factor- α (TNF- α), granulocyte/macrophage colony-stimulating factor (GM-CSF), monocyte chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8) and interleukin-10 (IL-10) (Yong, 1997). The profile of mediators released from activated mast cells in vivo influences the responses of local structural cells but also profoundly contributes to the nature of inflammatory cell infiltrates (Yong, 1997).

Allergic diseases are generally characterized by two phases (Umetsu and DeKruyff, 1997). The early phase allergic response, such as the anaphylactic response, is likely directly manifested through release of preformed mediators from mast cells (Bingham and Austen, 2000). The second, or late phase, response is characterized by inflammatory cell infiltrate and the development of hyperreactivity of various tissues. The cellular influx promoted during the late phase includes eosinophils, T and B lymphocytes, mast cells, basophils and monocytes (Umetsu and DeKruyff, 1997). A prominent modulator of this overall inflammatory response is the CD4⁺ T-cell (Umetsu and DeKruyff, 1997). In particular, the T-cell component of allergic diseases, such as asthma and allergic rhinitis, is predominantly T-helper type 2 (TH2) cells (Kaplan, 2001; Umetsu and DeKruyff, 1997). These TH2 cells elaborate a spectrum of cytokines and chemokines (namely IL-4, IL-13, IL-5 and eotaxin) that promote the infiltration of other cell types (Kaplan, 2001; Umetsu and DeKruyff, 1997).

I-309 is a C-C chemokine that is the ligand for the chemokine receptor CCR8. I-309 is chemotactic for T-cells, particularly TH2 cells through restricted expression of the CCR8 receptor (Kaplan, 2001). It is postulated that I-309 plays a key role in the progression of various diseases such as asthma, which have a TH2 component. Indeed, a subpopulation of the T-cells infiltrating lung mucosa in allergen-challenged asthmatics express CCR8 (Panina-Bordignon et al., 2001).

In this report we describe the observation that human umbilical cord blood-derived mast cells (CBMCs) produce the T-cell chemotactic chemokine I-309 in response to Fc ϵ RI cross-linking. In addition we explore the observation that IgE can induce significant production of I-309, and other cytokines, in the absence of mast cell degranulation.

Methods

Reagents

All chemicals were purchased from Sigma (St. Louis, MO) unless otherwise noted. Tissue culture medium and fetal bovine serum (FBS) were purchased from Invitrogen/Life-Technologies (Carlsbad, CA). Stem cell factor (SCF), interleukin-6 (IL-6) and interleukin-4 (IL-4) were purchased from R&D Systems (Minneapolis, MN). Human IgE and sheep anti-human IgE (α -IgE) were purchased from Biodesign (Saco, MA).

Cord blood derived mast cells (CBMCs)

Human umbilical cord blood mononuclear progenitors (Poeitic Technologies, Gaithersburg, MD) were placed into culture medium (RPMI/10% FBS) in the presence of stem cell factor and IL-6 both at 100 ng/ml, at a density of 1×10^6 cells/ml. One half of the culture medium was changed each week. After 5 weeks IL-4 was introduced into the medium at 100 ng/ml. Cultures were continued for a total of 8 weeks and

evaluated by staining with Toluidine blue dye. Cells used for functional assays were a minimum of 90% Toluidine blue positive. For functional studies cells were primed by the addition of human IgE (various concentrations) for various times. Prior to addition IgE was centrifuged to remove aggregates. Primed cells were triggered by the addition of α -IgE (10 μ g/ml) for various amounts of time.

Microarray analysis of gene expression

RNA was isolated using the methods specified in the Clontech Atlas Pure Total RNA Labeling Kit (BD Biosciences Clontech, Palo Alto, Ca), specifically, denaturation followed by 3 rounds of phenol:chloroform extraction followed by DNAase treatment. Probes labeled with dCTP³³ for microarray analysis were synthesized using the protocol specified in the Atlas Smart Probe cDNA Synthesis Kit (BD Biosciences Clontech, Palo Alto, Ca). Blots were hybridized overnight with labeled probe in ExpressHyb at 68 °C with continuous agitation. Following Hybridization, blots were washed 4 times according to the Clontech Atlas Array protocol and exposed to Phosphor Imaging Screens (BioRad, Hercules, CA) for the Molecular Imager FX for 4–7 days and images read using the BioRad Molecular Imager. Phosphor Images were analyzed using AtlasImage software (BD Biosciences Clontech, Palo Alto, Ca).

Real-time reverse transcriptase polymerase-chain reaction analysis

Total RNA was isolated using the RNeasy Kit (Qiagen, Valencia, CA) and DNAase treated according to the RNeasy protocol. First strand cDNA was reverse transcribed from 1 μ g total RNA using the First Strand Synthesis kit (BD Biosciences Clontech, Palo Alto, CA). SYBR Green PCR Master Mix (Applied Biosystems) was used to amplify I-309 and G3PDH. Quantitation was done in a total volume of 30 μ l, using 5 μ l of a 1:5 dilution of RT reaction in an ABI PRISM Sequence Detector (Perkin Elmer) using the following protocol: *Stage 1*: 50°/2 min (to activate the AmpEraseTM (UNG) enzyme and inactivate contaminating DNA); *Stage 2*: 95°/10 min Denaturation; *Stage 3 (40 cycles)*: 95°/15 sec denaturation followed by 60°/1 min annealing/extension.

Oligonucleotide primers used to detect G3PDH and I-309 (Invitrogen Carlsbad, CA) were as follows:

G3PDH forward 5'CCTCTGACTTCAACAGCGAC3',
G3PDH reverse 5'CATGACAAGGTGCGGCTCCC3',
I-309 forward 5'GGAAGATGTGGACAGCAAGAGC3',
I-309 reverse 5'TGTAGGGCTGGTAGTTTCGG3'.

Real-time PCR data was analyzed by using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001; Winer et al., 1999). The mean sample cycle thresholds (CT) were normalized to those of G3PDH for the corresponding samples. Normalized samples were then compared to one another and the data expressed as relative expression levels.

Determination of cytokine and histamine levels

I-309 levels were determined by sandwich ELISA using the following procedure. 96-well plates (Pierce, Rockford, IL) were coated with 1 μ g/ml rabbit anti-human I-309 polyclonal antibody (PeproTech Inc. Rocky Hill, NJ) in PBS (diluent) overnight. All incubations were performed at room temperature. The

plate was washed with 0.5% Tween-20 in PBS (wash buffer) and blocked with 1% BSA for at least 1 h. Plates were again washed and used to assay CBMC culture supernatants or human I-309 standard (PeproTech Inc.).

100 μ l of cell supernatant or I-309 standards was incubated for 2 h followed by washing to remove unbound antigen. 100 μ l biotinylated rabbit anti-human I-309 polyclonal antibody (PeproTech Inc.) was added for 2 h at a concentration of 0.5 mg/ml in PBS. 100 μ l of a 1:2000 dilution of avidin peroxidase (Sigma) was added and the plate incubated for 30 min. Avidin peroxidase was then removed and the plate washed 10 times. ABTS liquid substrate (Sigma) was added to the wells and the plate was read after 20 minutes in a SpectraMax Plus microplate reader (Molecular Devices) at 405 nm. I-309 levels in samples were determined from the human I-309 standard curve. This ELISA protocol was tested and did not detect purified human GM-CSF, IL-8, MIP-1 α , TARC, TNF- α or MCP-1. Other cytokine levels were determined using Quantikine ELISA kits from R&D Systems (Minneapolis, MN). Histamine levels were determined using an ELISA kit from Immuno Biological Laboratories (Hamburg, Germany).

Statistical analysis

Statistical comparisons were carried out using Graphpad InStat software. Mean values were compared using analysis of variance (ANOVA).

Results

I-309 gene expression in cord blood-derived mast cells

Microarray analysis revealed that I-309 RNA expression was increased approximately 9-fold in cord blood-derived mast cells (CBMCs) cultured with IL-4 and activated with IgE/ α -IgE compared to resting cells (data not shown). Further characterization of I-309 gene expression was carried out in both primed (IgE alone) and activated (IgE followed by α -IgE) CBMCs.

CBMCs grown in the presence or absence of IL-4 were primed with IgE (1 μ g/ml, 24 hr) and activated with α -IgE (10 μ g/ml). Total RNA was isolated at various times after activation and RT-PCR was performed with primers specific for human I-309. Activation of CBMCs grown in the absence of IL-4 resulted in a transient upregulation of I-309 gene expression with peak expression at 6 h and a decline to baseline by 24 h (Fig. 1A). In contrast, I-309 gene expression was highly induced in cells grown in the presence of IL-4 (100 ng/ml) (Fig. 1B). The peak of expression occurred between 3 and 6 h and remained elevated compared to the non-IL-4 grown cells.

In a follow-up experiment, total RNA was isolated from CBMCs grown in the presence or absence of IL-4 at 0, 3, 6 and 24 h after the addition of IgE alone (1 μ g/ml). Real-time PCR analysis demonstrated that I-309 gene expression was increased in a time-dependent manner in cells grown in the presence of IL-4 but not in the absence of IL-4 (Fig. 2). The peak of expression occurred at 3 h with resolution by 24 h.

Control of I-309 protein expression

The amount of I-309 protein secreted into the growth medium of various CBMC cultures was determined. Fig. 3 shows the time-dependent secretion of I-309 from CBMCs grown without IL-4, with

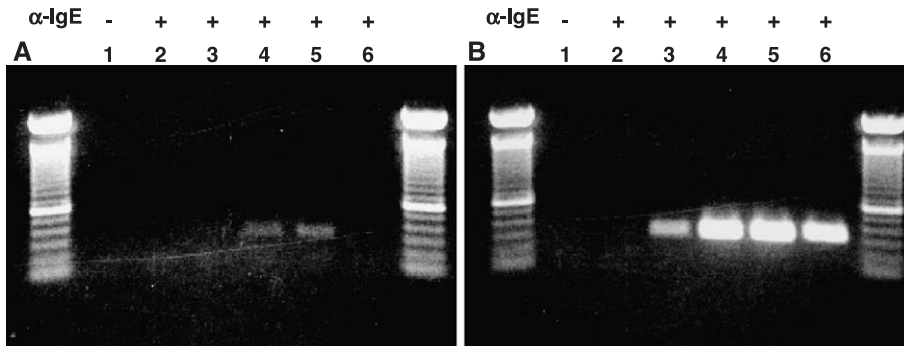


Fig. 1. Total RNA isolated from CBMCs grown either in the absence (A) or presence (B) of IL-4 (100 ng/ml) was used as template for RT-PCR reactions with primers specific for I-309. Cells were harvested and RNA isolated 24 h after addition of IgE (1 μ g/ml) (lane 1), and at 1, 2, 3, 6 and 24 h after addition of α -IgE (10 μ g/ml) (lanes 2–6 respectively). The G3PDH bands were of comparable intensity for all samples (not shown).

IL-4 (100 ng/ml) or with IL-13 (100 ng/ml). Activation with IgE/ α -IgE did not lead to increased I-309 secretion in cells grown in the absence of IL-4. Activation of IL-4 grown cells, however, resulted in a time dependent accumulation of I-309 in the growth medium peaking at 24 h at 418 pg/ml. This represents an approximately 4-fold increase over the 0 h level of 98 pg/ml. The ability of IL-13 to enhance I-309 secretion was also examined since IL-4 and IL-13 share a common signaling pathway. IL-13 failed to promote as great an increase in I-309 secretion from activated cells as did IL-4. However, there was

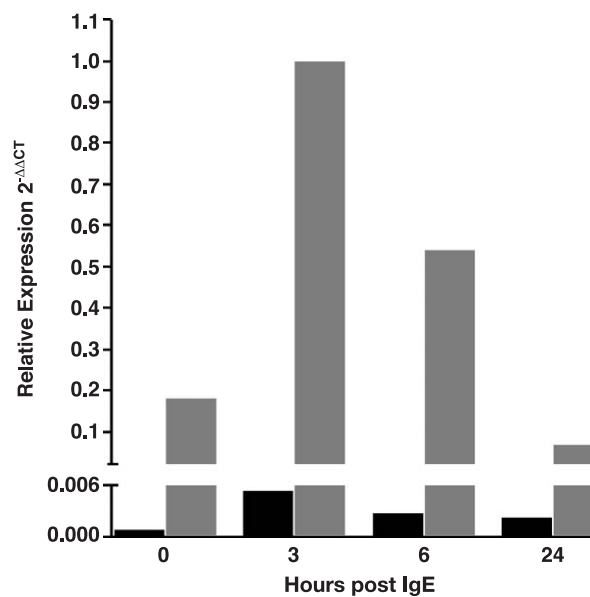


Fig. 2. CBMCs were grown either in the absence (black bars) or presence (gray bars) of IL-4 (100 ng/ml). Total RNA, isolated at various times after the addition of IgE (1 μ g/ml), was used as template for real-time quantitative PCR analysis with primers specific for I-309. Expression is indicated relative to the highest expressing sample.

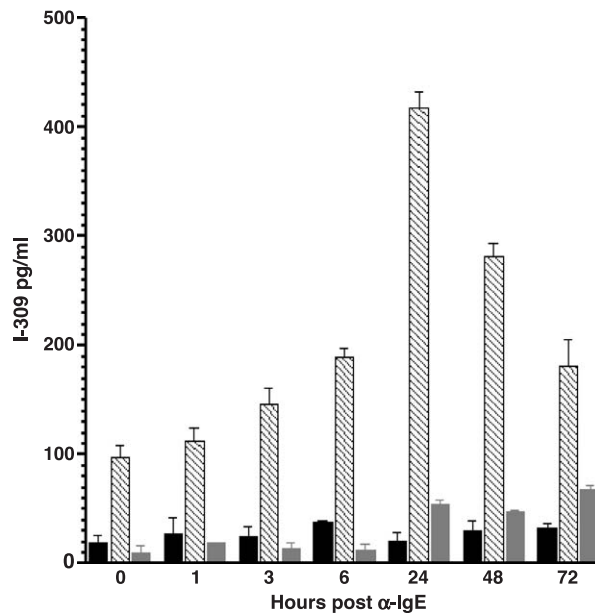


Fig. 3. The amount of human I-309 in cell supernatant from CBMCs grown in the absence of IL-4 (black bars), the presence of IL-4 (100 ng/ml) (hatched bars) or IL-13 (100 ng/ml) (gray bars) was determined as described in Material and Methods. Cells were primed for 24 h with IgE (1 μ g/ml) followed by activation with α -IgE (10 μ g/ml). Samples were assayed at 0, 1, 3, 6, 24, 48 and 72 h after addition of α -IgE.

significantly more I-309 in the growth medium of IL-13 grown cells at the 24, 48 and 72 h time points compared to the non-IL-4 grown cells (55–68 pg/ml from IL-13 cells, 21–33 pg/ml from non-IL-4 cells, $p < 0.05$).

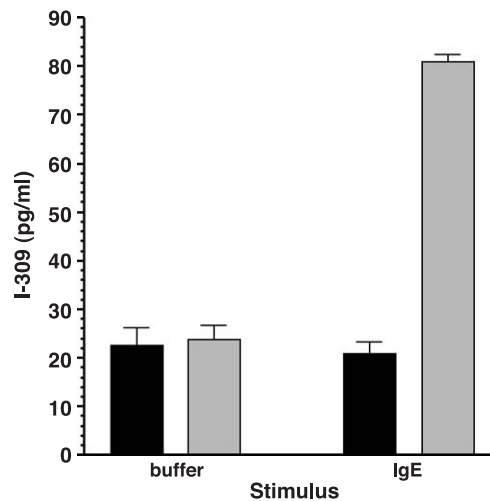


Fig. 4. CBMCs were grown in the absence (black bars) or presence (gray bars) of IL-4 (100 ng/ml). Both groups of cells were treated with either buffer alone or buffer containing 1 μ g/ml IgE. After 6 h samples were removed and supernatants were analyzed for release of I-309 as described in Materials and Methods.

A more detailed analysis of the data in Fig. 3 reveals that the level of I-309 was increased in the growth medium of IL-4 cells prior to the addition of α -IgE (0 h time point). These data suggest either that IL-4 alone or IgE can induce I-309 secretion from CBMCs. As noted above, IgE alone induced the expression of I-309 RNA in IL-4 grown cells; therefore, the induction of I-309 protein secretion by IgE was examined.

CBMCs grown either in the presence or absence of IL-4 were treated with buffer or buffer plus IgE (1 μ g/ml). Samples of growth medium were taken 24 h after stimulation and the levels of I-309 protein were determined. I-309 levels were increased only from cells that were grown in IL-4 and treated with IgE. None of the other conditions led to an increase in I-309 secretion (Fig. 4).

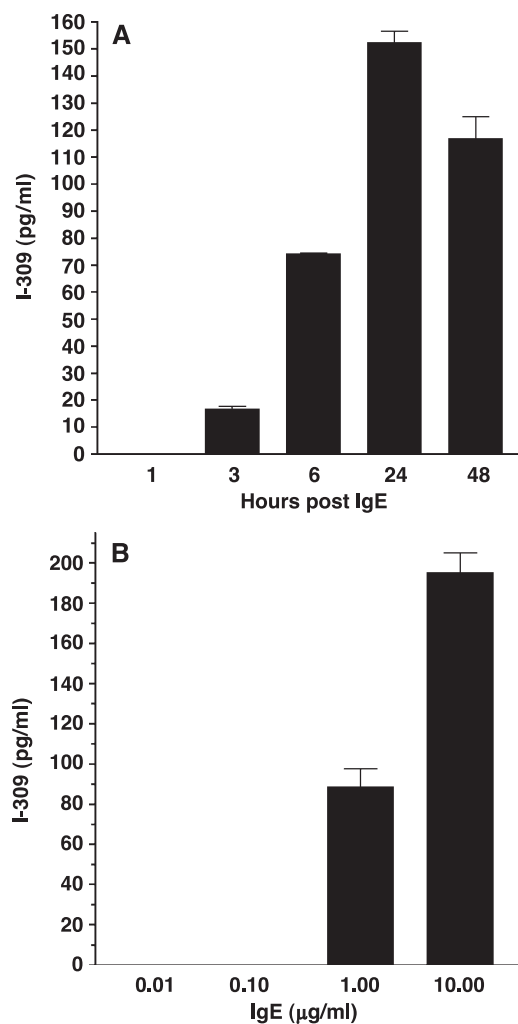


Fig. 5. A. IgE (1 μ g/ml) was added to IL-4 (100 ng/ml) grown CBMCs. Cell supernatants were sampled at intervals from 0–48 h and assayed for I-309. B. IgE (0.01–10 μ g/ml) was added to IL-4 grown cells. Cell supernatants were sampled after 24 h and assayed for I-309.

Table 1

Peak levels of cytokine, chemokine and histamine released from IL-4 treated CBMCs

	GM-CSF (pg/ml)	I-309 (pg/ml)	IL-8 (ng/ml)	MCP-1 (pg/ml)	MIP-1 α (pg/ml)	TNF- α (pg/ml)	Histamine (ng/ml)
Resting	154 $\pm$ 67*	9 $\pm$ 3	12 $\pm$ 3	9 $\pm$ 1	100 $\pm$ 4	ND [#]	9 $\pm$ 1
IgE	499 $\pm$ 136	140 $\pm$ 29	14 $\pm$ 4	11 $\pm$ 3	359 $\pm$ 98	ND	10 $\pm$ 2
α -IgE	3662 $\pm$ 654	490 $\pm$ 59	65 $\pm$ 12	11 $\pm$ 6	1346 $\pm$ 321	33 $\pm$ 5	379 $\pm$ 30

*Units are indicated at the top of each column, data are shown $\pm$ standard error.[#] Not detected.

The time course and dose response of IgE-induced I-309 secretion were determined. As shown in Fig. 5A, I-309 levels peaked at 24 h and began to decline by 48 h after addition of IgE. Fig. 5B shows a dose response of I-309 release to various concentrations of IgE in IL-4 grown CBMCs.

Regulation of other cytokines/chemokines by IgE

In addition to I-309 the production of a number of other cytokines and chemokines was analyzed. IgE induced the production and release of GM-CSF, and MIP-1 α above the level released by resting cells (Table 1, 3.2-fold and 3.6-fold increases respectively). However, IL-8, MCP-1 and TNF- α levels were not increased by IgE (Table 1). Furthermore, while GM-CSF, IL-8, MIP-1 α and TNF- α release were induced by α -IgE activation, MCP-1 levels remained constant (Table 1). The peak level of all of these proteins was decreased when CBMCs were grown in the absence of IL-4 (data not shown).

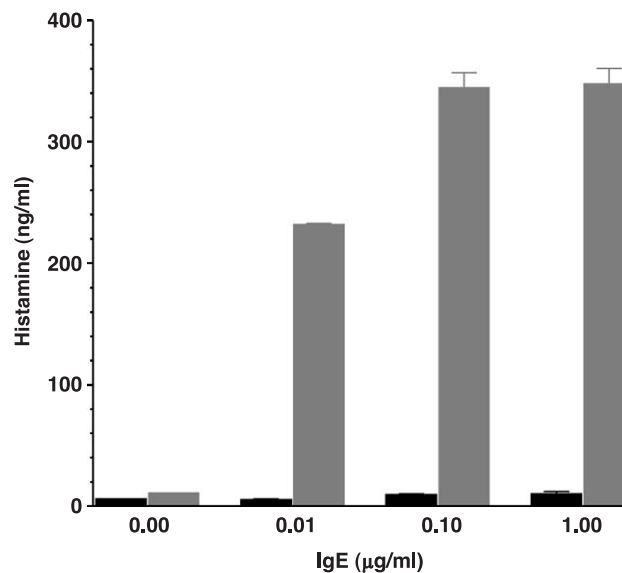


Fig. 6. IgE (0.01–10 μ g/ml) was added to IL-4 (100 ng/ml) grown CBMCs. After 24 h, cells were pelleted and resuspended in HBSS with 2% BSA and activated with α -IgE (10 μ g/ml) (control samples, indicated by the black bars, received IgE alone) followed by incubation at 37 °C for 30 min. Released histamine was quantitated as described in Materials and Methods.

IgE alone does not induce histamine release

One possible explanation for the effect of IgE on I-309 release is that the preparation of IgE used in this study contained extensive aggregates or other substances that could promote activation of the CBMCs. (In all cases the IgE preparation was centrifuged to avoid large aggregates.) Potential activation by the IgE preparation was tested by examining the release of histamine from CBMCs grown in the presence of IL-4. Cells that were primed for 24 h with various concentrations of IgE were activated with α -IgE (10 μ g/ml) and released histamine levels were determined after 30 min (Fig. 6, Table 1). For comparison, resting cells were treated with various concentrations of IgE for 30 min and released histamine levels were determined (Fig. 6, Table 1). Significant release of histamine occurred only in those cells that had been primed with IgE and subsequently activated with α -IgE. Histamine released from activated CBMCs was 10–20% of the total intracellular histamine (data not shown). IgE did not cause the release of histamine from resting cells.

Discussion

This report demonstrates that human umbilical cord blood-derived mast cells are capable of secreting the T-cell chemotactic chemokine I-309. Not only did CBMCs secrete I-309 in response to activation of Fc ϵ RI by IgE/ α -IgE but also by priming with IgE alone. The response to IgE was both dose and time dependent and was not accompanied by release of histamine from these cells. The upregulation of I-309 occurred at the level of transcript accumulation (as indicated by real-time PCR analysis) and was greatly augmented when cells were grown in the presence of IL-4. In addition, IgE also induced the secretion of GM-CSF and MIP-1 α but not IL-8, MCP-1 or TNF- α .

A previous study suggested that human mast cells may indeed secrete I-309. The human mast cell leukemia line, HMC-1, was shown to secrete both I-309 and MCP-1 in response to stimulation with phorbol ester (PMA) (Selvan et al., 1994). In addition, gene expression was also increased by PMA for MIP-1 α , MIP-1 β , Rantes and IL-8. The HMC-1 cell line, however, lacks an intact high-affinity IgE receptor and so can not be stimulated by Fc ϵ RI cross-linking (Butterfield et al., 1988). The data presented in the current study corroborate the findings in HMC-1 cells regarding I-309 and are the first demonstration of I-309 release from human mast cells in response to activation through Fc ϵ RI. Interestingly, unlike the HMC-1 cells, CBMCs did not release MCP-1 (Table 1).

Of particular interest is the finding that IgE triggered the gene expression and subsequent release of I-309, and other proteins, from CBMCs grown in the presence of IL-4, without detectable degranulation. The notion that IgE can stimulate cells through Fc ϵ RI is relatively recent but has its roots in the observation that the expression level of receptor on human basophils is well correlated with the circulating concentration of IgE in humans (Malveaux et al., 1978). More recent studies have shown that IgE modulates the expression of Fc ϵ RI both on murine and human mast cells (Hsu and MacGlashan, 1996; Xia et al., 1997; Yamaguchi et al., 1997; Yamaguchi et al., 1999). In addition, IgE has been shown to regulate the survival of mast cell cultures at least partially through the inhibition of apoptosis (Asai et al., 2001; Kalesnikoff et al., 2001). IgE has also been shown to upregulate the expression of IL-6, TNF- α , IL-4 and IL-13 and to induce the secretion of IL-6 in murine bone marrow-derived mast cells (Kalesnikoff et al., 2001). That study also showed that IgE induced the phosphorylation, and presumably activation, of Erk kinase, p38 MAP kinase, Jun kinase and protein kinase B (Kalesnikoff et al., 2001).

IgE has also been shown to increase intracellular histamine levels in mouse bone marrow-derived mast cells through an increase in histidine decarboxylase expression (Tanaka et al., 2002).

In the case of I-309, the ability of IgE to induce secretion of the chemokine requires prior growth of the CBMCs in IL-4. Previous studies have examined the effects of IL-4 on human mast cells. IL-4 promotes maturation and survival of human mast cells, increases FcεRI expression and causes an increase in preformed mediators such as histamine and tryptase within the cells (Bischoff et al., 1999; Nilsson and Nilsson, 1995; Toru et al., 1998a; Xia et al., 1997; Yanagida et al., 1995). IL-4 also augments the expression of GM-CSF, IL-3, IL-5, IL-8, IL-13, MIP-1α and TNF-α (Lorentz et al., 2000; Ochi et al., 2000; Tachimoto et al., 2000; Toru et al., 1998b).

In summary, we have demonstrated that human umbilical cord blood-derived mast cells secrete the T-cell chemotactic chemokine I-309 in response to activation of high-affinity IgE receptors. In addition, we have shown that a preparation of IgE is sufficient to induce the secretion of I-309 as well as GM-CSF and MIP-1α in the absence of histamine release. These data in concert with a growing body of literature suggest that IgE itself may exert influences on mast cells in the absence of antigen. In most of these studies, including the present study, one can not completely exclude that there is a low level of IgE aggregation that these various cytokines and chemokines are sensitive to or that some other property of the particular IgE sample leads to stimulation of these proteins. Further detailed studies will need to be conducted and corroborated using isolated mast cells rather than cultured cells. If the effects of IgE stand the test they may have an important role in priming of the overall allergic response and may additionally contribute to low-level chronic inflammatory conditions.

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