

Monomeric IgE enhances human mast cell chemokine production: IL-4 augments and dexamethasone suppresses the response

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Background: Mouse monoclonal IgE antibodies can promote the survival of mouse bone marrow–derived cultured mast cells and induce the cells to secrete mediators in the absence of known specific antigen.

Objective: To determine whether human IgE, in the absence of known specific antigen, had effects on the mediator secretion or survival of human mast cells.

Methods: We tested whether human IgE induced human cord blood–derived mast cells to secrete mediators or enhanced their survival on withdrawal of stem cell factor.

Results: Exposure to IgE, but not IgG, at concentrations as low as 2.5 µg/mL significantly enhanced the release of IL-8 and monocyte chemoattractant protein 1, but not histamine or cysteinyl leukotrienes. However, under the conditions tested, chemokine production in response to IgE alone was significantly less than that induced when aliquots of the same IgE-sensitized populations of human mast cells were stimulated with anti-IgE. The production of IL-8 and monocyte chemoattractant protein 1 in response to either IgE alone or IgE and anti-IgE was enhanced by preincubation of the cells in IL-4 and was inhibited by preincubation of the cells with dexamethasone. By contrast, we did not detect any ability of IgE to enhance mast cell survival on withdrawal of stem cell factor.

Conclusion: Exposure to human IgE *in vitro* in the absence of known specific antigen can enhance chemokine production by human mast cells, and this secretory response can be enhanced by preincubation of the mast cells with IL-4 and can be suppressed by dexamethasone. (J Allergy Clin Immunol 2005;116:1357–63.)

Key words: Apoptosis, chemokines, monomeric IgE, sodium azide, umbilical cord blood–derived mast cells

Abbreviations used

HUCBMC: Human umbilical cord blood–derived mast cell
LT: Leukotriene
MCP-1: Monocyte chemoattractant protein 1
SCF: Stem cell factor

IgE-dependent and antigen-dependent activation of mast cells is thought to contribute importantly to protective responses to certain parasites and to the pathogenesis of allergic disorders.^{1–3} In such settings, the cross-linking of IgE bound to its high-affinity receptor (FcεRI) by multivalent antigen results in aggregation of FcεRI and the initiation of signaling pathways leading to the secretion of preformed mediators such as histamine, newly synthesized lipid mediators such as leukotrienes, and an array of cytokines and chemokines.^{2–4}

Moreover, the binding of IgE to FcεRI in the absence of specific antigen can result in increased surface expression of FcεRI in mouse and human mast cells and basophils,^{5–8} reflecting stabilization of the expression of the receptor on the cell surface.^{9,10} Such IgE-dependent enhancement of FcεRI surface expression can enhance the cells' functional responses to activation by FcεRI aggregation.^{5–11} Enhanced surface expression of FcεRI also can be induced in certain mast cell populations by IL-4,^{8,12–14} and IgE and IL-4 can act synergistically to enhance both FcεRI surface expression and the functional responses of such cells on FcεRI aggregation.⁸

Exposure of mouse mast cells to IgE in the absence of known antigen also can enhance the survival of these cells under conditions of growth factor withdrawal^{15–17} and can induce the cells to release cytokines and other mediators.^{16,17} However, the relevance of these findings to human mast cell biology is unclear. Gilchrist et al¹⁸ showed that IgE induced human umbilical cord blood–derived mast cells (HUCBMCs), which were generated in IL-4–containing medium to release the chemokine I-309, but not histamine; however, the effects of IgE on mast cell survival were not reported, nor did IgE induce release of I-309 from mast cells that had been generated in the absence of exogenous IL-4. Moreover, the IgE preparations tested in that study were not HPLC-purified.

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In this study, we investigated whether exposure of HUCBMCs to human IgE, including HPLC-purified IgE that lacked any detectable IgE dimers or larger aggregates by mass spectrometry, induced the cells to secrete mediators or enhanced their survival. We also examined whether exposure of mast cells to IL-4 or dexamethasone influenced the cells' responses to IgE.

METHODS

HUCBMCs

Human umbilical cord blood–derived mast cells were obtained essentially as described in the study by Kempuraj et al.¹⁹ Heparinized cord blood was overlaid on Histopaque 1077 (Sigma, St Louis, Mo) and centrifuged at room temperature for 30 minutes at 400g. Mononuclear cells at the plasma-Histopaque interface were collected, and CD34⁺ cells were isolated by using a magnetic separation column and a CD133 cell isolation kit (Miltenyi Biotec, Auburn, Calif). Cells were maintained in culture medium (Isocove's modified Dulbecco medium [Gibco BRL, Life Technologies, Grand Island, NY] supplemented with 100 ng/mL recombinant human rSCF¹⁶⁴ [rhSCF; Amgen, Thousand Oaks, Calif]⁸; 1 ng/mL rhIL-3 [Pepro-tech, Rocky Hill, NJ], 50 ng/mL human rIL-6 [rhIL-6; Amgen]; 0.1% BSA [Sigma]; and, from Life Technologies, 10 mmol/L HEPES, 2 mmol/L L-glutamine, antibiotics [100 U penicillin/mL, 100 µg streptomycin/mL, and 10 µg gentamicin/mL], 1× MEM vitamin solution, 1× MEM amino acids [without L-glutamine], 1 mmol/L sodium pyruvate, and 50 µmol/L 2-mercaptoethanol), and half of the culture medium was changed weekly. At 8 weeks of culture, BSA was replaced with 10% FBS (Sigma). Cells were incubated with IL-3 during the first week of culture. Mast cell numbers and viability (which was always >90% according to trypan blue staining) were assessed immediately before the experiments. The preparations used in all of the experiments consisted of cell populations that were at least 10 weeks old and that expressed high levels of c-Kit as assessed by flow cytometry (data not shown). The purity of the mast cells at the time of individual experiments was 86 to >99% as determined by using Kimura stain²⁰ and/or tryptase immunostaining²¹; the 2 methods usually gave essentially identical results. Umbilical cord blood was obtained with the approval of the Stanford University Institutional Review Board.

Flow cytometry of HUCBMCs

Surface expression of FcεRI was assessed as previously described. Briefly, HUCBMCs were washed once in PBS (Gibco BRL) supplemented with 2% FCS (Sigma), preincubated with human IgG for 15 minutes, and then incubated for 1 hour at 4°C with 5 µg/mL of the mouse IgG2b antihuman FcεRI α-chain mAb, CRA-1, which can bind to the FcεRI α-chain whether or not it is occupied by IgE.¹³ After washing, cells were stained with FITC goat F(ab')₂ against mouse IgG (Jackson ImmunoResearch, West Grove, Pa) at 7.5 µg/mL for 30 minutes. An isotype-matched mouse IgG2b mAb with irrelevant specificity (MOPC195; Organon Teknica, Durham, NC) was used as negative control instead of CRA-1. Stained cells were analyzed by using a FACSCalibur (Becton Dickinson, San Jose, Calif); 10,000 events in each sample were analyzed, and ≥5000 viable mast cells (we gated on high forward and side scatter to detect viable mast cells) were studied to calculate the median fluorescence intensity ratio (experimental sample/negative control). Mast cell apoptosis was assessed by double staining with fluorescein isothiocyanate–conjugated annexin-V and propidium iodide (BD Pharmingen, San Jose, Calif) and flow cytometry.

Effects of monomeric IgE and IL-4 on HUCBMC apoptosis induced by stem cell factor deprivation

Human umbilical cord blood–derived mast cells (5×10^5 cells/mL) were maintained either in the usual culture medium or in stem cell factor (SCF)–free culture medium in the presence of increasing concentrations of purified human myeloma IgE (in concentrations as high as 20 µg/mL; catalog number, A12162H; Biodesign International, Kennebunk, Me), microcentrifuged at ~14,000g for 20 minutes at 20°C to remove possible large aggregates for as long as 96 hours. Cell viability and apoptosis were assessed at various points. On the basis of analysis by HPLC and mass spectrometry, such preparations consisted of >95% IgE monomers and <5% dimers, but without larger aggregates (see Fig E1 in the Online Repository in the online version of this article at www.jacionline.org). We therefore repeated some of our experiments by using preparations of IgE that had been purified by HPLC and then analyzed by mass spectrometry to confirm that these preparations of monomeric IgE lacked detectable dimers (see Fig E1, C, in the Online Repository in the online version of this article at www.jacionline.org).

In some experiments, HUCBMCs were incubated ± 2.5 µg/mL IgE and/or 10 ng/mL human rIL-4 (Pepro-tech) for 4 days to enhance FcεRI surface expression.⁸ At day 4, cells were washed and incubated for another 3 days in SCF-free culture medium $\pm$ as much as 20 µg/mL IgE. Analysis of mast cell FcεRI surface expression and apoptosis was performed at various time points.

Effects of monomeric IgE and IL-4 on human umbilical cord blood–derived mast cell stimulation and mediator release

Human umbilical cord blood–derived mast cells (5×10^5 cells/mL) were maintained either in usual culture medium or in culture medium supplemented with 10 ng/mL IL-4 for 4 days. At day 4, cells were incubated for 4 hours in usual culture medium ± 2.5 µg/mL IgE. After washings, cells maintained in culture medium alone were stimulated with vehicle, 10 µg/mL purified human IgG (catalog number I2511; Sigma), or 2.5 µg/mL human IgE; in addition, cells presensitized with IgE at 2.5 µg/mL for 4 hours were stimulated with 1 µg/mL anti-IgE (Sigma) (IgE/anti-IgE). In other experiments, cells were incubated overnight (before stimulation) and during activation with 1 µmol/L dexamethasone. Cells that had been treated with dexamethasone overnight were maintained in dexamethasone-containing medium throughout the period of exposure to IgE or IgE and anti-IgE. In a pilot study, IL-4 and dexamethasone treatments, under the conditions used in these experiments, did not affect HUCBMC viability when maintained in culture medium supplemented with SCF, as assessed by trypan blue exclusion staining. Cells were stimulated for 1 hour for histamine and cys-leukotriene (LT) release experiments, and for 6 hours for measurements of cytokine and chemokine production. Histamine was measured in the culture supernatants and in sonicated cells by using a RIA kit (Immunotech, Westbrook, Me). The percentage of histamine release was calculated as histamine in supernatant/(histamine in supernatant + histamine in cells) $\times$ 100. Cys-LT generation in supernatants was measured with an ELISA for LTC₄/D₄/E₄ (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom). For this ELISA, the cross-reactivity with LTC₄ is 100%; LTD₄, 100%; LTE₄, 70%; and LTB₄, is 0.3%; and the detection limit for cys-LT is 10 pg/mL. Cytokine and chemokine levels in the supernatants were assessed by ELISA (BD OptEIA ELISA [BD Biosciences, San Diego, Calif], for TNF-α, IL-5, IL-8, and monocyte chemoattractant protein [MCP-1]; R&D for SCF [R&D Systems Inc, Minneapolis, Minn]), and the detection limits

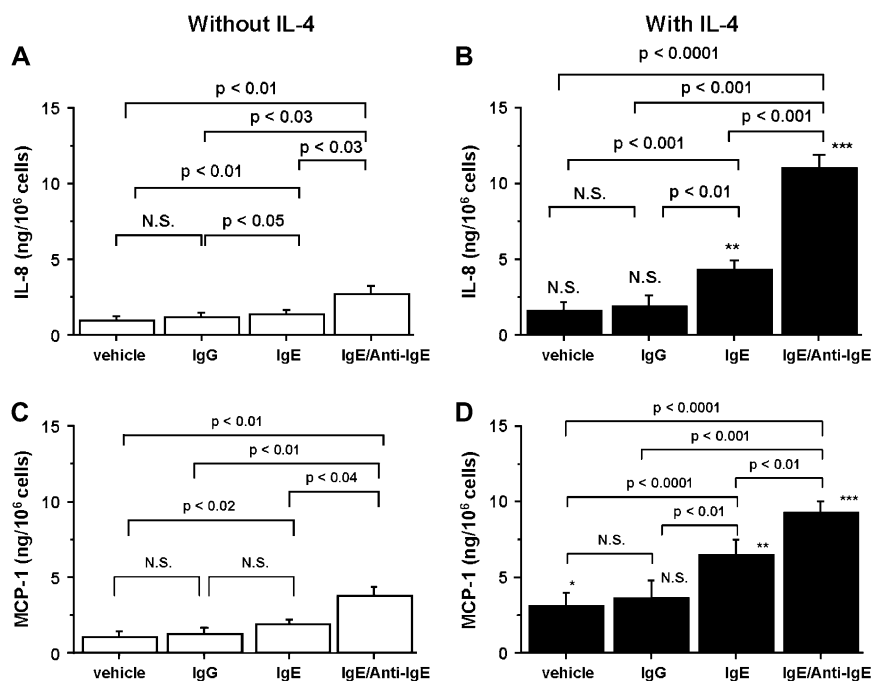


FIG 1. Effects of IgE and IL-4 on mast cell chemokine production. IL-8 (**A and B**) or MCP-1 (**C and D**) secretion by HUCBMCs maintained in the usual medium (SCF + IL-6) + (**B and D**) or – (**A and C**) IL-4 (10 ng/mL) for 4 days, washed $\times 3$, and then maintained in vehicle or 10 μ g/mL human IgG or 2.5 μ g/mL IgE for 6 hours, or in 2.5 μ g/mL IgE for 4 hours and then washed $\times 3$ and exposed to 1 μ g/mL anti-IgE (IgE/anti-IgE) for 6 hours. Results pooled from separate experiments with cells from 7 donors. * $P < .05$; ** $P < .01$; *** $P < .001$ vs corresponding values for HUCBMCs preincubated 4 days without exogenous IL-4; NS, not significant ($P > .05$).

are 2.0, 7.8, 3.1, 7.8, and 9.0 pg/mL for TNF- α , IL-5, IL-8, MCP-1, and SCF, respectively. Cys-LT, cytokine, and chemokine levels were calculated according to the manufacturer's instructions.

Statistical analysis

Data are expressed as means $\pm$ SEMs. For mean comparisons, we used the unpaired 2-tailed Student *t* test. Differences were considered statistically significant at *P* values $< .05$.

RESULTS

IgE induces cytokine/chemokine production by HUCBMCs, and this effect is enhanced by previous incubation of the cells in IL-4

We first confirmed our previously reported findings⁸ that preincubation with IgE alone or IL-4 (10 ng/mL) alone for 4 days can enhance surface expression of Fc ϵ RI on HUCBMCs, and that the highest levels of Fc ϵ RI surface expression are seen in cells incubated with both IgE and IL-4 (see Fig E2 in the Online Repository in the online version of this article at www.jacionline.org). We then found that exposure to IgE (2.5 μ g/mL) stimulated IL-8 secretion in HUCBMCs (Fig 1, A), an effect that was strongly enhanced (from 1.43 ± 0.29 to 4.25 ± 0.72 ng/10⁶ cells) in HUCBMCs that had been preincubated for 4 days with 10 ng/mL IL-4 (Fig 1, B). The even higher levels of IL-8 secretion that were induced by IgE and anti-IgE were also enhanced in cells that had

been preincubated with IL-4 (see, for example, Fig 1, A and B). Treatment with IL-4 alone for 4 days did not significantly enhance spontaneous IL-8 secretion by HUCBMCs, and treatment with human IgG (10 μ g/mL) had no significant effect on IL-8 secretion, either with or without previous incubation of the cells with IL-4. Treatment of HUCBMCs with microcentrifuged preparations of the chimeric IgE SE-44 at 2.5 μ g/mL also induced the release of IL-8 at levels similar to those induced by our standard IgE preparations (data not shown).

Exposure to IgE, but not to IgG, also induced HUCBMCs to secrete MCP-1 (2.06 ± 0.34 ng/10⁶ cells incubated with IgE at 2.5 μ g/mL, vs 1.44 ± 0.46 ng/10⁶ cells incubated with IgG at 10 μ g/mL, vs 1.16 ± 0.29 ng/10⁶ cells incubated with vehicle; Fig 1, C). As with IgE-induced secretion of IL-8, (1) levels of MCP-1 secretion in cells incubated with IgE and anti-IgE were significantly higher than those induced by incubation of the cells with IgE alone (3.71 ± 0.68 ng/10⁶ cells vs 2.06 ± 0.34 ng/10⁶; $P < .04$), and (2) preincubation of HUCBMCs with IL-4 enhanced the cells' secretion of MCP-1 in response to either IgE alone or IgE plus anti-IgE (Fig 1, D). However, in contrast with our findings for secretion of IL-8 (Fig 1, A and B), the increased levels of MCP-1 secretion observed in HUCBMCs preincubated with IL-4 and then placed in vehicle alone (3.41 ± 0.90 ng/10⁶ cells) were significantly higher than those observed in vehicle-challenged HUCBMCs that had not been preincubated with IL-4 (1.16 ± 0.29 ng/10⁶ cells; $P < .01$; Fig 1, C and D).

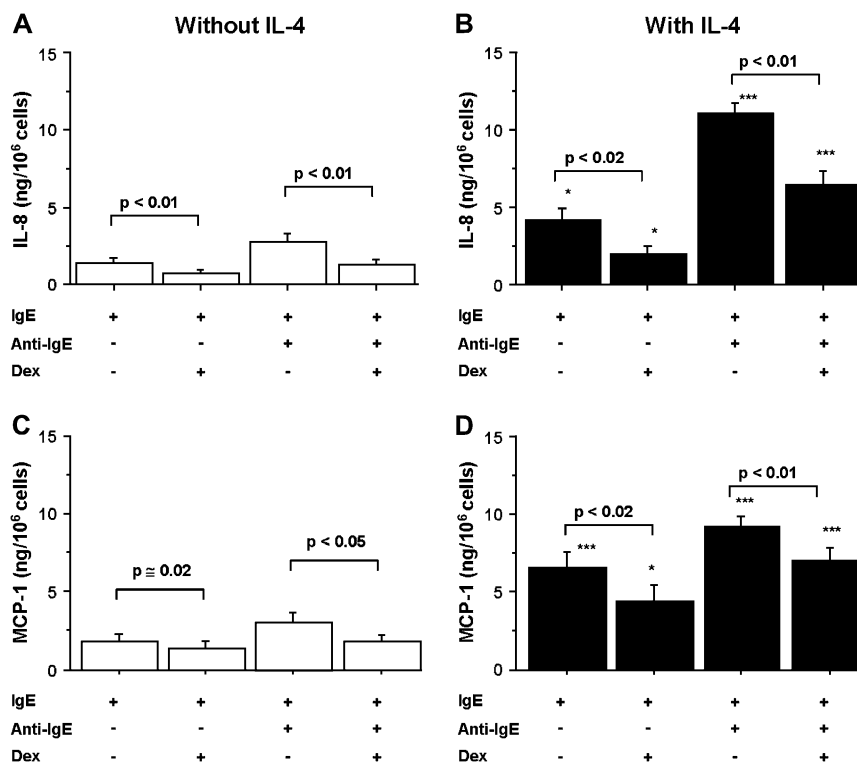


FIG 2. Dexamethasone can inhibit IgE-induced chemokine production by mast cells. IL-8 (**A and B**) and MCP-1 (**C and D**) secretion in HUCBMCs suspended in usual medium (SCF + IL-6) + (solid bars) or – (open bars) IL-4 (10 ng/mL) for 4 days. Aliquots of HUCBMCs were maintained o.n. on the last day of the 4-day period $\pm$ dexamethasone (Dex; 1 μ mol/L). Cells were then washed $\times 3$ and treated with 2.5 μ g/mL IgE for 6 hours or 2.5 μ g/mL IgE for 4 hours and then washed $\times 3$ and exposed to 1 μ g/mL anti-IgE (IgE/anti-IgE) for 6 hours. Results pooled from separate experiments with cells from 6 donors. * $P < .05$; ** $P < .01$; *** $P < .001$ vs corresponding values for HUCBMCs preincubated for 4 days without exogenous IL-4; NS, not significant ($P > .05$).

We also analyzed whether incubation with IgE induced the production of cytokines with mast cell prosurvival effects, such as SCF, TNF- α , and IL-5. Although SCF production was undetectable in cells stimulated with vehicle or IgE (with or without previous incubation with IL-4; $n = 2$), TNF- α and IL-5 levels secreted by such cells in the presence of IgE were close to the limit of detection of the assay (14.0 ± 4.1 pg/10⁶ cells and 18.6 ± 5.4 pg/10⁶ cells for TNF- α and IL-5, respectively; $n = 4$). Cells stimulated with IgE and anti-IgE in these experiments secreted 46.1 ± 9.4 and 21.3 ± 7.5 pg/10⁶ cells for TNF- α and IL-5, respectively. No TNF- α or IL-5 secretion was detected in cells stimulated with vehicle alone.

We repeated some of our experiments with HPLC-purified monomer fractions of IgE that lacked detectable IgE dimers by mass spectrometry (see Fig E1, C, in the Online Repository in the online version of this article at www.jacionline.org). As shown in Fig E3 (see the Online Repository in the online version of this article at www.jacionline.org), the HPLC-purified IgE preparations were at least as active as the microcentrifuged preparations of IgE in inducing IL-8 secretion from HUCBMCs. These results were obtained in 6 different experiments in which 4 different batches of HUCBMCs were tested with 3 different preparations of HPLC-purified IgE, all of which

lacked detectable IgE dimers by mass spectrometry. Similar results were obtained when we tested the ability of these HPLC-purified IgE preparations to induce secretion of MCP-1 from HUCBMCs (data not shown). These results indicate that the ability of the microcentrifuged IgE preparations to induce chemokine release from HUCBMCs cannot be attributed to the very small amounts of IgE dimers present in these preparations.

Dexamethasone can inhibit IgE-induced chemokine production by HUCBMCs

Incubation of HUCBMCs for 12 hours with 1 μ mol/L dexamethasone inhibited their ability to secrete IL-8 when stimulated by IgE (1.43 ± 0.29 ng/10⁶ in control cells vs 0.77 ± 0.20 ng/10⁶ cells in cells incubated with dexamethasone; $n = 6$ different donors; $P = .0062$; Fig 2, A). Similarly, dexamethasone inhibited IgE-induced activation of HUCBMCs that were maintained for 4 days in medium supplemented with IL-4 before stimulation (4.25 ± 0.72 ng/10⁶ cells vs 2.02 ± 0.47 ng/10⁶ cells; $P = .0109$; $n = 6$ different donors; Fig 2, B). The inhibitory effect of dexamethasone was also observed with IgE-induced MCP-1 production by HUCBMCs, with or without preincubation of the cells in IL-4 (Fig 2, C and D). Dexamethasone also inhibited IL-8 and MCP-1 production

by IgE-stimulated and anti-IgE-stimulated HUCBMCs (Fig 2, A-D). By contrast, overnight treatment with dexamethasone did not affect the viability of HUCBMCs or expression levels of surface FcεRI or IL-4 receptor ($n = 4$; data not shown).

IgE does not induce mast cell degranulation or leukotriene synthesis

Human umbilical cord blood–derived mast cells were incubated in the presence or absence of IL-4 (10 ng/mL) for 4 days, washed, and stimulated with 2.5 μg/mL IgE alone for 1 hour, or with IgE for 4 hours and then goat antihuman IgE Ab (IgE/anti-IgE) for 1 hour. IgE alone did not induce significant HUCBMC degranulation in these experiments, as assessed by percentage of histamine release, in cells with or without preincubation with IL-4 (see Fig E4, A, in the Online Repository in the online version of this article at www.jacionline.org). By contrast, activation with IgE and anti-IgE resulted in significantly increased percentage of histamine release compared with levels in cells treated with vehicle or monomeric IgE alone (percentage of histamine release with IgE and anti-IgE: in the absence of IL-4 preincubation, $6.70\% \pm 1.28\%$; with IL-4 preincubation, $14.93\% \pm 1.54\%$; $P = .0062$; $n = 4$ different donors). Similarly, we detected no significant effect of exposure to IgE alone on cys-LT synthesis (see Fig E4, B, in the Online Repository in the online version of this article at www.jacionline.org), either with or without incubation of cells with IL-4 (cys-LT levels were always approximately at or below detection limits, 5 pg/ 10^6 cells), although IgE/anti-IgE stimulation significantly increased cys-LT production compared with values for cells incubated with vehicle or IgE alone (cys-LT production stimulated by IgE/anti-IgE: in the absence of IL-4, $32.50 \pm 4.92/10^6$ cells; in the presence of IL-4, $100.51 \pm 20.50/10^6$ cells; $P = .018$; $n = 4$ different donors).

IgE does not enhance survival of HUCBMCs after SCF withdrawal

In initial experiments, we found that preparations of microcentrifuged IgE consistently enhanced HUCBMC survival after SCF withdrawal, with significant effects observed at concentrations of IgE of 2.5 μg/mL. However, in 9 experiments conducted with 6 batches of HUCBMCs and using 3 preparations of HPLC-purified IgE, we found that HPLC-purified preparations of IgE failed to exhibit survival-enhancing effects on HUCBMCs under the same conditions of testing, even when used in concentrations as high as 20 μg/mL.

The commercial preparations of IgE used in our experiments contained a small amount of sodium azide (ie, preparations that were diluted to 2.5 μg of IgE/mL contained 0.0003% sodium azide). Wilhelm et al²² reported that very low concentrations of sodium azide had the paradoxical effect of inhibiting apoptosis under certain conditions *in vitro*. We also found that low concentrations of sodium azide, in the absence of IgE, could enhance survival of HUCBMCs on withdrawal of SCF (see Fig E5 in the Online Repository in the online version of this

article at www.jacionline.org). Moreover, HPLC-purified IgE to which sodium azide was added to a concentration of 0.0003% enhanced HUCBMC survival on SCF withdrawal, whereas this effect was not observed when HPLC-purified IgE that lacked sodium azide was tested at either 2.5 or 20 μg IgE/mL (see Fig E6 in the Online Repository in the online version of this article at www.jacionline.org). We also found that microcentrifuged preparations of the chimeric IgE SE-44 (which contained no sodium azide), when tested at 2.5 μg/mL, lacked any detectable ability to enhance survival of HUCBMCs on withdrawal of SCF (see Fig E7 in the Online Repository in the online version of this article at www.jacionline.org).

DISCUSSION

We found that exposure of HUCBMCs to human myeloma IgE, but not IgG, in the absence of known antigen resulted in enhanced secretion of the chemokines IL-8 and MCP-1. This effect was substantial in cells that had been incubated for 4 days in IL-4 (at 10 ng/mL) before challenge with IgE at 2.5 μg/mL, a treatment that increased levels of FcεRI surface expression in these cells (see Fig E2 in the Online Repository in the online version of this article at www.jacionline.org). This result is consistent with those reported by Gilchrist et al,¹⁸ who observed that HUCBMCs maintained in IL-4-containing medium secreted the chemokine I-309 in response to IgE. However, in contrast with their results, we found that IgE also induced chemokine release from HUCBMCs that had never been exposed to exogenous IL-4 (Fig 1, A and C). Notably, the IgE preparations we tested retained their ability to induce chemokine secretion by HUCBMCs even after HPLC purification to remove all detectable dimers (see Fig E3 in the Online Repository in the online version of this article at www.jacionline.org); HPLC-purified IgE was not tested in the study by Gilchrist et al.¹⁸

The IgE concentration that induced mast cell chemokine secretion in our studies (ie, 2.5 μg/mL) is in the range observed in a small subset of subjects with allergic disorders.²³⁻²⁶ However, we have tested only HUCBMCs that have been generated *in vitro* by using a single culture protocol, and in most of our experiments, these cells were challenged with IgE derived from a single commercial source. Phenotypic characteristics of mast cells derived from different sources *in vitro* or *in vivo* can vary considerably,²⁷ and different mouse IgE antibodies can vary greatly in their ability to activate mediator and cytokine release from mouse mast cells *in vitro*.¹⁷

Indeed, while this manuscript was under review, Cruse et al²⁸ reported that preparations of human IgE at 3.0 μg/mL were able to induce release of histamine, LTC₄, and IL-8 from purified human lung mast cells *in vitro*. Although the IgE preparations used in that study were not purified by HPLC to remove IgE dimers, these findings suggest that purified human lung mast cells may be more sensitive to the effects of IgE on mediator release than are HUCBMCs. In addition, we and Gilchrist

et al¹⁸ found that previous incubation of HUCBMCs with IL-4 can greatly enhance the cells' ability to release chemokines in response to challenge with IgE *in vitro*. Accordingly, it is possible that mast cells in particular anatomical locations *in vivo*, or during certain immune responses associated with IL-4 production, may exhibit responses to monomeric IgE at concentrations even lower than those required to generate detectable responses in umbilical cord blood-derived mast cells *in vitro*. The relatively low levels of expression of FcεRI in our HUCBMCs, which is a characteristic of HUCBMCs maintained under the conditions used in our study,⁸ also may have contributed to the finding that, in these cells, IgE induced the release of chemokines but neither histamine nor cys-LT.

Although the *in vivo* relevance of our findings remains to be determined, the possibility that IgE itself may induce mast cell chemokine release has several implications. Chemokines are thought to contribute to the pathogenesis of allergic disease by regulating the migration of the cells that orchestrate the inflammation associated with these disorders, including mast cells,²⁹⁻³² and it has been proposed that mast cell migration at such sites may be regulated, in part, by RANTES and IL-8.^{33,34} We have confirmed that our HUCBMCs express receptors that can bind IL-8—that is, the chemokine receptors, CXCR1 and CXCR2 (data not shown). Moreover, Inamura et al³⁴ reported the dose-dependent migration of HUCBMCs in response to as little as 1 ng/mL IL-8.

Although MCP-1 is not a potent chemoattractant for mast cells, it has been shown to be a potentially important mediator of eosinophil, monocyte, and CD4⁺/CD8⁺ lymphocyte recruitment.^{29,33,35} MCP-1^{-/-} mice exhibit impaired development of certain T_H2 responses,³⁶ and MCP-1 can enhance airway inflammation in a mouse model by inducing the release of LTC₄ and histamine.³⁷ Finally, Szalai et al³⁸ reported that a polymorphism in the regulatory region of the gene encoding MCP-1 is associated with childhood asthma susceptibility and severity.

Notably, treatment of HUCBMCs with dexamethasone at 1 μmol/L (overnight before challenge and during the 6-hour period of challenge with IgE or IgE and anti-IgE) had no effect on levels of FcεRI surface expression, but significantly reduced levels of IL-8 or MCP-1 secreted by these cells on challenge with either IgE alone or IgE plus anti-IgE (Fig 2).

In the mouse, different monoclonal IgE antibodies varied markedly in their ability to induce *in vitro*-derived mast cells to undergo activation and secretion of the various classes of mediators.¹⁷ Yet even the most weakly cytokinergic of the mouse monoclonal antibodies enhanced mouse mast cell survival on growth factor withdrawal *in vitro*.¹⁷ By contrast, we so far have not been able to detect an effect of human myeloma IgE, or a chimeric human IgE antibody (SE-44; Tanox Inc, Houston, Tex), on the survival of HUCBMCs on withdrawal of SCF. Whether testing other preparations of human IgE and/or other populations of human mast cells may reveal an ability of human IgE to influence the survival of such cells

remains to be determined. However, our results certainly suggest that HUCBMCs are less sensitive than mouse mast cells to any survival-promoting effects of IgE *in vitro*.

The mechanisms by which IgE can induce the effects observed, whether in mouse or human mast cells, remains to be defined. In the mouse system, several lines of evidence indicate that the mechanism may reflect the ability of certain IgE preparations to induce aggregation of the FcεRI in the absence of known antigen.¹⁷ Given that 1 highly cytokinergic mouse IgE (SPE-7)¹⁷ can exist in at least 2 different isomeric forms, each of which can bind to a structurally distinct antigen,³⁹ it is possible that IgE antibodies that can induce mast cell activation responses do so by recognizing unknown exogenous antigens (or even autoantigens) and then inducing activation responses that are otherwise identical to those observed when these IgEs bind to their known antigens. Alternatively, it has been proposed that certain IgEs may spontaneously self-aggregate on the mast cell surface.¹⁶

Regardless of how IgE might enhance mast cell survival (in the mouse) and/or mediator secretion (in mouse and human mast cells) in the absence of antigens for which that IgE is known to have specificity, our findings offer a new perspective on the highly positive correlation between serum levels of IgE and the prevalence of asthma and other allergic diseases.²³ In addition to enhancing antigen-dependent mast cell activation and mediator/cytokine secretion by increasing surface expression of FcεRI,⁵⁻¹¹ high levels of IgE may also promote human mast cell activation independently of the presence of antigens for which the IgE is known to have specificity.¹⁵⁻¹⁷ Moreover, our findings raise the possibility that the effects of IgE on mast cell function may be especially pronounced in subjects experiencing active T_H2-associated responses linked with ongoing production of IL-4. One may even speculate, on the basis of our findings with dexamethasone, that one benefit of corticosteroid treatment in allergic disorders is that it can suppress an ongoing low level secretion of certain mast cell mediators in response to ambient levels of IgE, even in the absence of exposure to those exogenous allergens to which that subject is known to harbor sensitivity.

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