

Human IgE⁺ and IgE⁻ are not equivalent to mouse highly cytokinergic IgE

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Background: We have previously defined IgE⁺ as the IgE on basophils from a subset of highly allergic asthmatic subjects that release histamine after stimulation with histamine-releasing factor (HRF). The mechanism of IgE⁺ remains an enigma. Recently, there have been reports of monomeric highly cytokinergic IgEs causing mediator release, cytokine release, and phosphorylation events in cultured rodent and human mast cells in the absence of antigen.

Objective: We investigated whether human IgE⁺ might exist as highly cytokinergic IgE in the human system.

Methods: IgE⁺ was defined as causing greater than 10% histamine release by using HRF as a stimulus of human basophils. By definition, IgE⁻ did not support histamine release to HRF. Once defined, serum and various purified human IgEs were used to stimulate purified human basophils or cultured human mast cells. The cells were examined for histamine release, extracellular signal-regulated kinase (ERK) phosphorylation, and IL-13 secretion.

Results: We found that neither IgE⁺ nor IgE⁻ induced ERK phosphorylation, histamine release, and IL-13 release from freshly isolated basophils in the absence of a specific antigen. However, human IgE alone did stimulate ERK phosphorylation in cultured human mast cells and IL-3–primed human basophils. **Conclusion:** Human IgE⁺ is not an equivalent of the mouse highly cytokinergic IgE. However, human IgE did have effects on cultured mast cells and basophils. The effect of highly cytokinergic IgE on ERK phosphorylation and cytokine secretion might be due to the priming effect of human basophils and mast cells. (*J Allergy Clin Immunol* ■■■■;■■■:■■■-■■■.)

Key words: Asthma, allergy, histamine-releasing factor, highly cytokinergic IgE, IL-13, IL-3, phosphorylation, extracellular signal-regulated kinase, basophil, mast cell

Asthma and allergic diseases are markedly increasing in the United States. More than 50 million Americans have allergies, and 20 million have asthma. Additionally, approximately 50% of

Abbreviations used

BMMC: Bone marrow–derived mast cell
ERK: Extracellular signal-regulated kinase
HC IgE: Highly cytokinergic IgE
HRF: Histamine-releasing factor
HrHRF: Human recombinant histamine-releasing factor
LPR: Late-phase reaction

asthmatic patients experience a recrudescence of symptoms, termed the late-phase reaction (LPR), 6 to 24 hours after exposure to allergen. This LPR exacerbates symptoms and airway inflammation. The mechanism of the LPR involves many inflammatory mediators. Histamine-releasing factor (HRF), which is present in LPR skin blister fluids and nasal lavage fluid during the LPR, is believed to play a prominent role in chronic allergic processes.¹ Previous studies demonstrated that HRF stimulated histamine release from human basophils of only certain highly allergic donors.² Subsequently, this factor was cloned and purified by our laboratory.³ In deciphering the biology of human recombinant HRF (HrHRF), we uncovered a functional heterogeneity among human IgE molecules. IgE from HrHRF responders was termed IgE⁺, and IgE from HrHRF nonresponders was termed IgE⁻.⁴ Basophils from highly allergic asthmatic subjects released histamine not only to HrHRF but also to IL-3 and deuterium oxide (D₂O).^{3,5,6} Despite multiple attempts, including deglycosylation experiments, the exact molecular basis of IgE⁺ remains an enigma.

In 2000, Budde et al⁷ published that the reactivity of IgE⁺ to their mononuclear cell–derived HRF was due to monomeric, and not polymeric, IgE. Recently, several studies showed that certain types of mouse monomeric IgE molecules, termed highly cytokinergic IgE (HC IgE), activated mouse bone marrow–derived mast cells (BMMC) in the absence of a specific antigen. This activation was demonstrated by an upregulation of cell survival, phosphorylation of signaling molecules, and mediator release.^{8,9} In human cord blood–derived mast cells, Gilchrist et al¹⁰ reported that the chemokine I-309 RNA and protein levels were upregulated not only in response to IgE/anti-IgE activation but also by IgE alone in the presence of IL-4. Cruse et al¹¹ also reported that human monomeric IgE stimulated cultured human lung mast cells to release histamine and IL-8 in the absence of a specific antigen. Recently, a study by Matsuda et al¹² showed that human monomeric IgE in the absence of known specific antigen enhanced chemokine production in cultured human mast cells, and this response was augmented by preincubation of the cells in IL-4. However, it is important to note that all the mouse and human studies noted above were performed with

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cultured-derived cells that had been cultured for 8 to 12 weeks in medium containing growth factors (ie, stem cell factor). It is still unclear whether the effects of monomeric HC IgE exist in the human system using cells not subjected to the potential effects of *in vitro* culture. Because heterogeneity of human IgE has been reported, it is possible that some highly allergic patients might produce HC IgEs, which could activate mast cells or basophils in the absence of antigen. Finding human HC IgE might reveal that a subset of human IgE molecules from certain highly allergic donors could, when combined with specific cytokines *in vivo*, be able to activate mast cells and basophils in the absence of a specific antigen. In this study we tested the hypothesis that human HC IgE molecules might exist and possibly explain the previously mentioned heterogeneity of IgE.

METHODS

Human basophil purification and culture

Permission to conduct this study was given by the Johns Hopkins Medical Institutional Review Board. For all experiments using phosphorylation, human basophils were purified from peripheral blood by means of double Percoll density gradients and negative selection with magnetic beads (Stem Cell Technologies, Inc, Vancouver, British Columbia, Canada). The purity of basophils was determined by means of alcian blue staining, as previously described.¹³ Human basophils were treated with lactic acid briefly (14 seconds on ice) before any stimulation to obtain additional unoccupied IgE receptors.^{14,15} In some cases human basophils were cultured in 10 ng/mL IL-3 in Iscove's Modified Dulbecco's Medium (IMDM) with 5% FBS, essential amino acids, and 10 μ g/mL gentamicin (Gibco, Carlsbad, Calif) for approximately 24 to 72 hours. Cells were then washed twice and incubated in IL-3-free medium for 4 hours before any experiments were performed.

Mouse BMMC preparation

Mouse BMMCs were obtained, as previously published,¹⁶ by using wild-type C57B/6 mice provided by Dr Zhu at the Johns Hopkins Asthma and Allergy Center. BMMCs from 2 mice were combined, cultured in IMDM and 30% WEHI-conditioned media as a source of murine IL-3, and harvested, and experiments were performed on different days between weeks 5 and 10. BMMCs expressed 93.7% c-Kit, as determined by means of flow cytometry. All animal work was approved by the Johns Hopkins Animal Care and Use Committee, and all mice were handled according to the regulations of the American Association for the Accreditation of Laboratory Animal Care.

Human mast cell preparation

Human mast cells obtained from CD34⁺ cells were cultured in StemPro media (Invitrogen, Carlsbad, Calif) with stem cell factor (100 ng/mL) and IL-6 (100 ng/mL), both from Biosource International, Inc (Camarillo, Calif).¹⁷ After 6 weeks, cells were harvested, washed, and placed in cytokine-free medium for 4 hours. Subsequently, the cells were assessed for extracellular signal-regulated kinase (ERK) 1/2 phosphorylation, cytokine production, and histamine release.

IgE preparation

Human IgEs included 3 high-titer human IgE sera from allergic patients and 13 human myeloma IgEs. Ten of the 13 human myeloma IgEs were gifts from Dr Johansson. The other 3 myeloma IgEs included (1) PS myeloma,² (2) human myeloma IgE purchased from Calbiochem (San Diego, Calif), and (3) JK myeloma from Dr Hamilton at the Johns Hopkins Asthma and Allergy Center. In some cases human IgE sera from allergic donors were further purified by using anion exchange chromatography, followed by mAb-based positive and negative affinity chromatography.¹⁸ It should be noted that IgEs containing serum were chosen because they had

nearly matched concentrations of IgE (F1141, 12 μ g/mL; GG, 13.8 μ g/mL; 7267, 12.8 μ g/mL), and the range of dilutions of serum used for passive sensitization were 1:6 to 1:6.8. Additionally, to obtain a final concentration of 5 μ g/mL, sera were diluted 1:2.4 to 1:2.8. Therefore all dilutions of sera were comparable. Mouse HC IgEs were SPE-7 (Sigma, St Louis, Mo) and H1DNP- ϵ -26 IgE provided by Dr MacGlashan at the Johns Hopkins Asthma and Allergy Center.

Passive sensitization and stimulation with HrHRF

Dextran-sedimented human basophils were passively sensitized with various human IgEs at 2 μ g/mL, according to published methods, to define donor populations releasing HrHRF.² After sensitization, the cells were stimulated with buffer, HrHRF (4.2 μ mol/L), or anti-IgE (0.1 μ g/mL) for 45 minutes. The supernatants were collected, and histamine release was measured by means of automated fluorometry.¹⁹ Human IgEs were grouped into IgE⁺ and IgE⁻ based on their response to HrHRF. HrHRF-induced histamine release of greater than 10% over that of the buffer control was defined as IgE⁺. The same experiments were done with BMMCs by using mouse recombinant HRF.

Western blotting analysis of signaling molecules

Human basophils were stimulated with 5 μ g/mL human IgE⁺ or IgE⁻ (either serum or purified IgE) alone for 5 and 15 minutes. Cell pellets were lysed in SDS sample buffer and subjected to Western blotting. The phosphorylation of ERK1/2 was assessed by using anti-phospho-p44/42 mitogen-activated protein kinase (Cell Signaling Technology, Beverly, Mass). The same experiments were done with mouse BMMCs. The supernatants were stored at -20°C for histamine measurements.

Cytokine measurements

Human blood-enriched basophils were incubated with medium, human IgEs (5 μ g/mL), IL-3 (10 ng/mL), or IgE (5 μ g/mL) plus IL-3 (10 ng/mL) for 4 hours and 18 hours. The source of human IgE was either purified IgE or human serum, as described in the figure legends. The supernatants collected at 4 hours were for IL-4 and histamine measurements, and those harvested after 18 hours were assayed for IL-13 protein. IL-4 and IL-13 levels were measured by means of ELISA (eBioscience, San Diego, Calif).

Statistical analysis

The IL-13 data are expressed as means $\pm$ SEMs of 3 independent experiments with duplicates. The paired Student *t* test was used for mean comparisons. Differences were considered statistically significant at *P* values of less than .05.

RESULTS

SPE-7 does not induce histamine release in BMMCs on stimulation with mouse HRF

To determine whether mouse HC IgE, SP-7, has a similar functional property as human IgE⁺, mouse BMMCs were sensitized with either SPE-7 or H1DNP- ϵ -26 (both defined as HC IgEs) for 30 minutes and then stimulated with buffer, mouse recombinant HRF (10 μ mol/L), dinitrophenylated human serum albumin (0.1 μ g/mL, 1 μ g/mL), or anti-mouse IgE (0.1 μ g/mL, 1 μ g/mL). Both dinitrophenylated human serum albumin and anti-IgE were used as positive controls. Our data showed that neither SPE-7 nor H1DNP- ϵ -26 induced BMMCs to release more than 10% of total histamine over that seen with buffer control on stimulation with HRF, as performed by using classical passive sensitization methods (Fig 1, A). Thus by our definition, SPE-7 is not IgE⁺. On the other hand, similar to previously published data

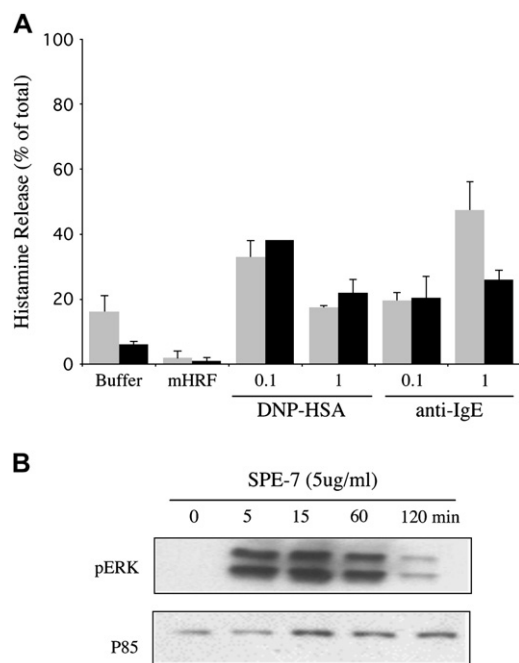


FIG 1. Effects of mouse HC IgEs on histamine release and ERK1/2 phosphorylation of mouse BMMCs. **A**, Neither SPE-7 (5 μg/mL, black bars) nor H1DNP-ε-26 IgE (5 μg/mL, gray bars) induced significant histamine release from BMMCs on stimulation with mouse HRF (n = 2). **B**, SPE-7 alone (5 μg/mL) induced phosphorylation of ERK1/2 in BMMCs (representative of 5 experiments). DNP-HSA, Dinitrophenylated human serum albumin. pERK, Phosphorylated ERK.

concerning ERK phosphorylation,⁹ SPE-7 alone (5 μg/mL) induced phosphorylation of ERK1/2 in BMMCs in the absence of a specific antigen (Fig 1, B). The blot was reprobed with anti-p85 to control for equal loading.

Neither human IgE⁺ nor IgE⁻ stimulates ERK1/2 phosphorylation in freshly isolated human basophils

We screened all human IgEs available to us using our previously published passive sensitization assay.² According to our criteria, only several human IgE sera were defined as IgE⁺, whereas all human myeloma IgEs were defined as IgE⁻ (myeloma IgE⁺ data not shown). As expected, anti-human IgE did not discriminate between IgE⁺ and IgE⁻ molecules. To investigate whether human IgE in the absence of the specific antigen activates human basophils through the mitogen-activated protein kinases, we studied the phosphorylation of ERK1/2. We found that several human IgE sera (IgE⁺: 7267 and GG; IgE⁻: F1491), but none of our human myeloma IgEs (MF, MV, MN, MB, MC, and JK), stimulated ERK1/2 phosphorylation in human basophils (Fig 2, A and B). Fig 2, C, shows basophil histamine release using IgE⁺ serum (GG), and IgE⁻ serum (F1491). Only IgE⁺ serum supports histamine release to HrHRF. Because human sera contain many non-IgE components (eg, cytokines or IgG complexes), it is possible that the phosphorylation of ERK1/2 might be induced by other components in sera other than human IgE. To rule out a potential serum effect, we purified IgEs from these sera and repeated the same experiments. After purification, human IgEs (both IgE⁺ from GG and IgE⁻ from

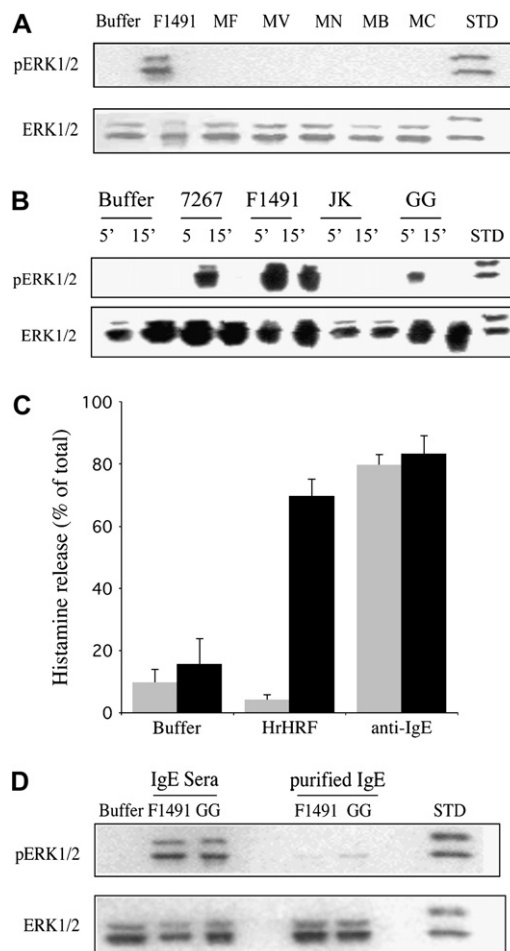


FIG 2. Phosphorylation of ERK1/2 in basophils stimulated with buffer or various human IgEs (5 μg/mL) for 5 minutes (**A**) and 5 minutes and 15 minutes (**B**) is shown (n = 1). **C**, Histamine release to HrHRF and anti-IgE on cells sensitized with IgE⁺ (GG, black bars) and IgE⁻ (F1491, gray bars; n = 3). **D**, ERK1/2 phosphorylation by serum or purified IgE. pERK, Phosphorylated ERK; STD, standard.

F1491) induced only minimal detectable ERK1/2 phosphorylation in human basophils (n = 2; Fig 2, D). Thus eliminating serum factors from human IgE⁺ and IgE⁻ sera possessing cytokinergic activity greatly reduced ERK phosphorylation in human basophils in the absence of specific antigens.

Neither human IgE⁺ nor IgE⁻ induces cytokine production in freshly isolated human basophils

Human basophils secrete both IL-4 and IL-13, which are important cytokines involved in the allergic process. To investigate the effects of human IgE on IL-4 and IL-13 secretion, we incubated human basophil-enriched suspensions with medium, IgE⁺ or IgE⁻ serum (5 μg/mL), purified IgE⁺ or IgE⁻ (5 μg/mL), IL-3 (10 ng/mL), and A23187 (500 ng/mL) for 4 hours and 18 hours. As shown in Fig 3, there were low but detectable levels of IL-13 secretion in basophils treated with IgE⁺ serum (23.47 ± 2.64 pg/10⁶ cells) and IgE⁻ serum (38.5 ± 8.63 pg/10⁶ cells) but no detectable IL-13 from basophils treated with purified IgEs. As the positive controls, IL-3 and the calcium ionophore A23187 induced significant IL-13 levels from the same donors

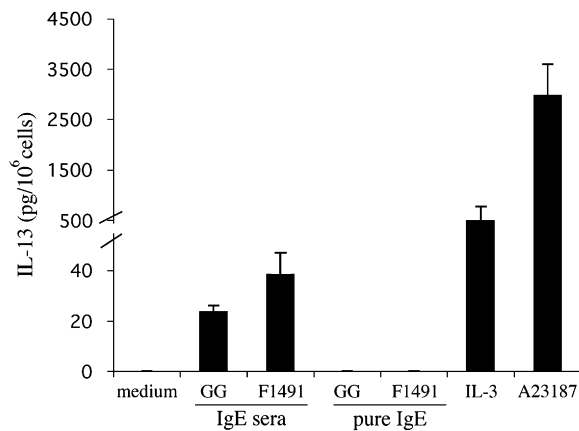


FIG 3. Effects of human IgEs on IL-13 secretion in the absence of specific antigens. Basophil-enriched suspensions were stimulated with medium alone, human IgE⁺ or IgE⁻ sera (5 μ g/mL), purified IgE molecules (5 μ g/mL), IL-3 (10 ng/mL), and A23187 (500 ng/mL) for 18 hours. Supernatants were harvested for IL-13 levels and measured by means of ELISA ($n = 3$ for all conditions, except $n = 1$ for pure IgE conditions).

(Fig 3). There was no histamine and minimal IL-4 detected in basophil cultures incubated with any IgE preparation or with IL-3 alone. As expected, A23487 did induce IL-4 secretion and histamine release, which was most evident after 4 hours' incubation (data not shown). The IL-13 production that was observed by using IgE serum samples was present whether the basophils had undergone lactic acid treatment to remove IgE or were left untreated. Thus although sera containing IgE induced IL-13 secretion, the purified IgEs did not.

Human IgE stimulates ERK phosphorylation in cultured human mast cells and IL-3-primed human basophils

Because all published mouse and human monomeric IgE studies to date have been done with cultured mast cells, it is possible that the HC IgE phenomenon might be due to a priming effect induced by the various cytokines or growth factors required in the culture medium. To test this hypothesis, we investigated whether culturing or priming human cells in a cytokine-rich medium would have an effect on the phosphorylation of ERK. As shown in Fig 4, A, human CB IgE (Calbiochem, San Diego, Calif) and serum 7267 induced ERK1/2 phosphorylation in cultured human mast cells in the absence of a specific antigen. Furthermore, we incubated the freshly purified basophils in IL-3 (10 ng/mL) containing medium. After 48 to 72 hours, cells were harvested and incubated with human IgE sera (7267) or purified human IgE (CB IgE) for 5 and 15 minutes. Interestingly, we found that the ERK1/2 phosphorylation induced by IgE serum 7267 was markedly enhanced in cultured human basophils compared with that seen in freshly isolated human basophils ($n = 3$; Fig 5, B). Human CB IgE induced weak ERK phosphorylation only once in cultured human basophils.

Human IgE augments IL-3-induced IL-13 secretion from human basophils

To further test the culture or priming effect involved in the monomeric HC IgE phenomenon, we investigated whether

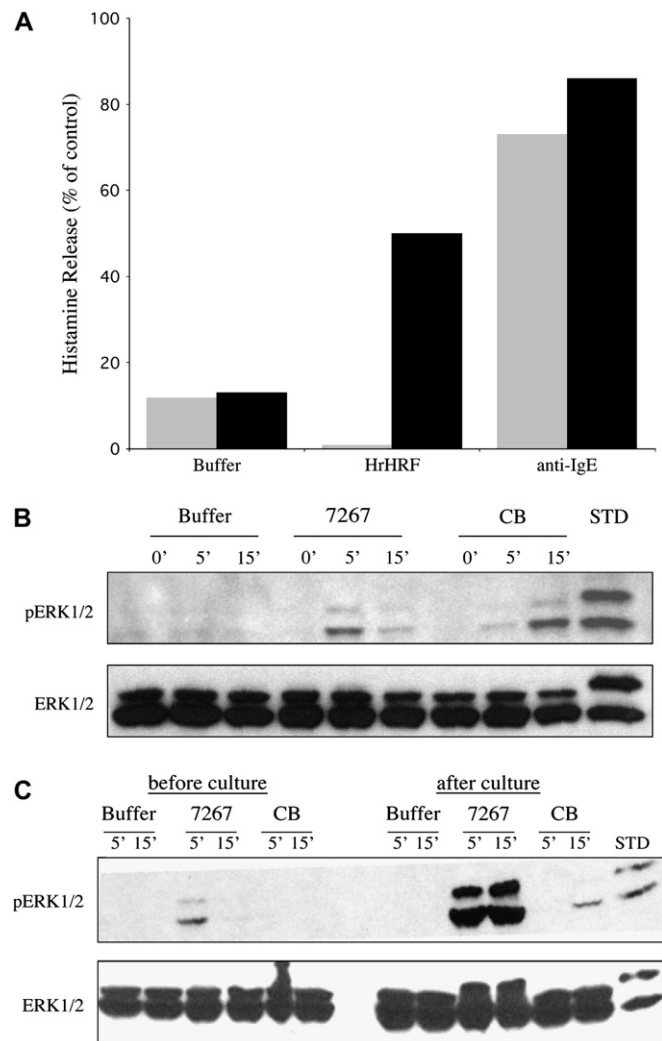


FIG 4. A, Histamine release from basophils sensitized with IgE⁺ (7267, black bars) and IgE⁻ (CB, gray bars; $n = 1$). B, ERK1/2 phosphorylation of human mast cells stimulated with buffer, 7267, or CB IgE (5 μ g/mL) for 0, 5, and 15 minutes (representative of 5 experiments). C, ERK1/2 phosphorylation induced by human IgE in freshly purified and IL-3-primed human basophils (representative of 3). pERK, Phosphorylated ERK; STD, standard.

purified IgE from JK sera alone can enhance basophil IL-13 secretion induced by IL-3 secretion from human basophils. Human basophil-enriched suspensions were incubated with human JK IgE (5 μ g/mL), IL-3 (10 ng/mL), and IL-3 (10 ng/mL) plus JK IgE (5 μ g/mL) for 18 to 20 hours. As shown in Fig 5, A, human JK IgE augmented IL-13 secretion induced by IL-3 but failed to induce detectable IL-13 from basophils when used without IL-3. This effect was dose dependent and most evident when the IgE concentration reached 5 μ g/mL. To rule out the possibility that some contaminating cells in the basophil-rich suspension might contribute to this synergistic effect, we repeated experiments with purified human basophils. Not surprisingly, the 5 μ g/mL JK IgE augmented the IL-3-induced IL-13 secretion from 277.73 ± 100.11 pg/10⁶ cells to 568.94 ± 172.61 pg/10⁶ cells. This phenomenon was observed whether the purified basophils had been treated with lactic acid or left untreated before incubation with IgE. Because IL-13 levels induced by IL-3 varied among different donors, we calculated the IL-13 secretion as

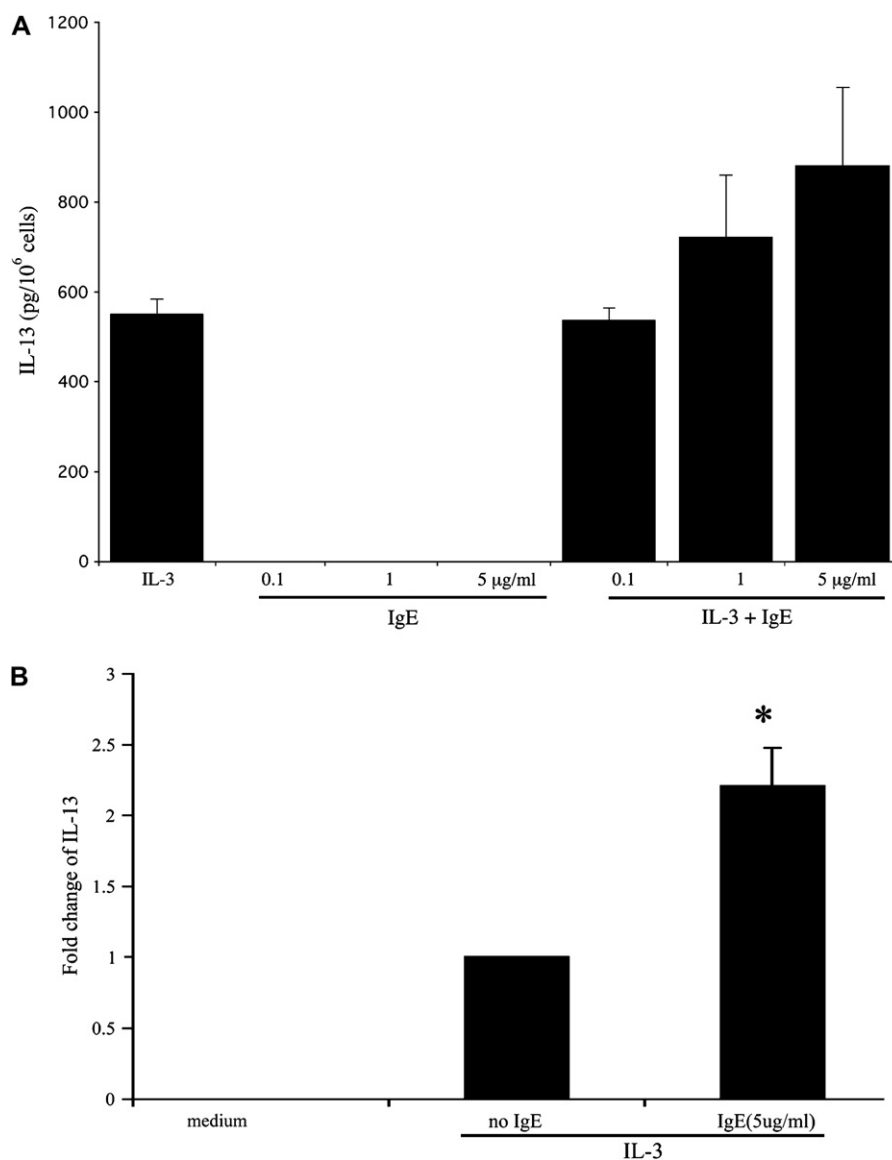


FIG 5. A, Human IgE alone augmented IL-3-induced IL-13 secretion in human basophils ($n = 2$). The synergistic effect of IgE on IL-3-induced IL-13 secretion only appeared when the dose of IgE reached 5 $\mu\text{g}/\text{mL}$. **B,** IgE (5 $\mu\text{g}/\text{mL}$) augmented the IL-3-induced IL-13 secretion approximately 2-fold compared with IL-3 alone ($n = 3$). * $P < .05$.

a fold increment of the IL-13 level induced by IL-3 only. Human IgE alone (5 $\mu\text{g}/\text{mL}$) augmented the IL-3-induced IL-13 secretion by approximately 2-fold (Fig 5, B).

DISCUSSION

We have previously defined human IgE⁺ as the IgEs on basophils from a subset of highly allergic asthmatic subjects that release histamine after stimulation with HRF, as well as IL-3 and D₂O.³⁻⁶ However, the exact mechanism of IgE⁺ remains unknown. It has been reported that several mouse monomeric HC IgEs activate mouse BMMCs in the absence of a specific antigen.^{8,9,11} We hypothesized that human IgE⁺ might exist as HC IgE, as recently suggested in the mouse system.

First, we investigated whether mouse HC IgE was equivalent to human IgE⁺. Our data showed that neither SPE-7 nor H1DNP-

ε-26 IgE induced mouse BMMCs to release histamine on stimulation with mouse HRF. However, in agreement with previous reports, our results did show that SPE-7 alone was able to stimulate strong ERK phosphorylation in mouse BMMCs. It should be noted that the genetic background of the mice might be important in eliciting degranulation, as evidenced by histamine release when using an HC IgE, such as SPE-7. For example, mice of various genetic backgrounds were used in the article by Kitaura et al.⁹ More recently, SPE-7 produced only minimal histamine release with BMMCs derived from C57B/6 mice (T. Kawakami, unpublished data). Nevertheless, the purpose of Fig 1 in this article was only to validate that mouse-derived BMMCs do respond to HC IgE (ie, SPE-7), as evidenced by ERK phosphorylation. Our intention was not to recapitulate data done in mice but to attempt to extend this observation into human cells. Nonetheless, the 2 mouse HC IgEs that we tested are IgE⁻ and not IgE⁺

according to our definition. In our study all human myeloma IgEs we had available were determined to be IgE⁻ by using our passive sensitization experiments. None of them stimulated ERK1/2 phosphorylation or cytokine secretion from human basophils. Only several IgE⁺ and IgE⁻ sera induced significant ERK phosphorylation in human basophils. However, histamine release accompanied by ERK phosphorylation was only detected in 1 of 4 experiments. To exclude a serum effect, we used purified IgEs from these sera. Purified IgEs induced very minimal and insignificant ERK1/2 phosphorylation. There was no significant difference between the ERK1/2 phosphorylation induced by purified IgEs and buffer control. Also, there was no significant difference between the minimal ERK1/2 phosphorylation induced by IgE⁺ and IgE⁻. We also investigated whether human IgE⁺ alone was able to activate human basophils to release T_H2 cytokines, such as IL-4 and IL-13. Kalesnikoff et al⁸ reported that mouse HC IgE alone was able to upregulate IL-6, IL-4, and IL-13 mRNA expression and to stimulate IL-6 secretion in mouse mast cells. In our study we observed that neither purified IgE⁺ nor IgE⁻ induced IL-13 secretion in freshly purified human basophils. The IgE concentration we used in our study was 5 µg/mL, which was the optimal concentration used in the mouse HC IgE studies. It has been reported by Kawakami and Kita²⁰ that HC IgE at 5 µg/mL was able to activate mouse BMMCs to release mediators and induce phosphorylation of signaling molecules. Thus neither human IgE⁺ nor human IgE⁻ alone induced mediator release or ERK1/2 phosphorylation in freshly isolated human basophils.

There have been several human studies reported in which human monoclonal IgE molecules activated cultured human mast cells in the absence of specific antigens.^{11,12} The difference between our data and the other mouse and human studies might be explained by the differences in cells and culture conditions. In their studies the main cell sources were cultured mast cells. All the BMMCs and cultured human mast cells in the literature had been cultured in growth factor (or cytokine)-rich medium for at least 4 to 8 weeks before experiments were performed.^{8,9,11,12} In our study we used freshly purified human basophils from peripheral blood. Unlike the cultured human mast cells, these basophils are end-stage cells. Thus it is possible that the HC IgE phenomenon might be due to a priming effect on cultured cells grown in cytokine-rich medium. For example, Lorentz et al²¹ reported that IL-4-induced priming of human intestinal mast cells is important for enhanced survival and cytokine generation and is associated with the phosphorylation of ERK1/2 and increased mRNA of c-Fos. This priming was reversible when the IL-4 was withdrawn. Also, Gilchrist et al¹⁰ showed that IgE alone induced chemokine I-309 secretion from human umbilical cord blood-derived mast cells in IL-4-containing medium. However, in the absence of IL-4, this effect of IgE alone was not observed.

IL-3 is known to prime human basophils for IgE-mediated release and to directly induce IL-13 production.²²⁻²⁴ We have previously shown that HrHRF was able to prime/enhance secretion of IL-4 and IL-13 from the basophils of IgE⁻ donors,²⁵ even though HrHRF does not directly induce histamine release from these donors. These data suggested that HC IgE might only be active in primed cells versus end-stage cells, such as primary blood basophils. To investigate this hypothesis, we cultured purified basophils in IL-3-containing medium and human mast cells in IL-6- and stem cell factor-containing medium. Our

results show that human basophils become more responsive to IgE after culturing in IL-3-containing medium. As shown in Fig 4, B, the ERK1/2 phosphorylation induced by IgE⁺ serum 7267 alone was markedly enhanced in cultured human basophils compared with that seen in freshly purified basophils. Interestingly, the commercial human myeloma IgE from Calbiochem was able to stimulate ERK phosphorylation in both cultured human mast cells and cultured human basophils in the absence of a specific antigen. We did not observe this effect in freshly purified human basophils, as shown in Fig 4. As shown in Fig 4, A, serum 7267 is IgE⁺ and supports histamine release to HRF, whereas myeloma CB IgE does not and is IgE⁻.

IL-4 and IL-13 are 2 major cytokines produced by human basophils and play important roles in asthma and allergic diseases.²⁵ Interestingly, in studies of murine allergic asthma, IL-13, but not IL-4, was found to be necessary and sufficient for generation of the asthma phenotype.^{26,27} Our data show that human IgE alone does not activate human basophils to secrete IL-13. However, we found that IgE alone augments the IL-13 secreted from human basophils in the presence of IL-3 (Fig 5). Of note, IgE enhanced the IL-3-induced IL-13 secretion by 2-fold compared with IL-3 alone. This was seen in basophils from both basophil-enriched suspensions and purified basophils, and this synergic effect was maintained at relatively the same level. Our findings are consistent with the previous study reported by Kawanishi,²⁸ which described that the presence of both IL-3 and IgE is required to induce murine mast cell development with higher frequency than that with IL-3 alone and that IgE alone failed to develop the growth of mast cells. The reason for IgE augmentation of IL-13 secretion in the presence of IL-3 is not clear.

Whether human HC IgE exists is still unclear. We studied the monomeric IgE effect by using freshly isolated human basophils and compared them with primed basophils rather than using cultured cells alone. Our data show that human IgE in the absence of a specific antigen stimulated ERK1/2 phosphorylation only in IL-3-primed human basophils and cultured human mast cells. Furthermore, IgE augmented the IL-3-induced IL-13 secretion in human basophils, whereas IgE alone did not stimulate IL-13 secretion. In highly allergic patients it is possible that basophils and mast cells might be primed by various cytokines *in vivo* and that IgEs from these patients might function as HC IgE *in vivo*. The effect of monomeric IgE on primed basophils and mast cells has great clinical relevance. A novel anti-IgE therapy (monoclonal anti-IgE antibody) has been shown to be efficacious in asthma and other allergic diseases.²⁹ If human HC IgE functions on primed cells *in vivo*, this might be an additional rationale for the use of this therapy.

In conclusion, our data show that human IgE⁺ is not equivalent to human HC IgE. However, HC IgE, as defined in rodents, might also exist *in vivo* in human subjects, with IgE molecules possibly activating primed basophils and mast cells in the absence of specific antigens similar to mouse HC IgE.

Clinical implications: Circulating monomeric IgE does not seem capable of causing anaphylaxis in human subjects.

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