Evaluation of The Correlation and Reproducibility between Histamine, IL-4, and IL-13 Release from Human Basophils

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ABSTRACT

Human basophils play a key role in allergic diseases such as asthma and in a variety of immunological disorders. The generation of IL-4 and IL-13 can be induced from basophil by IgE-mediated and non-IgE-mediated mechanisms. Time and stimulus-dependent differences in the regulation of these cytokines could have relevance to their biological effects. The aim of the present study was activation of basophils in order to evaluate the extent of histamine, IL-4, and IL-13 generations.

Basophil-enriched suspensions were prepared by Percoll gradients. The release of histamine and cytokines was assessed after activation with either anti-human IgE (1/1000 or 1/10000, 4 h or 24 h) or IL-3 (100 ng/ml, 24 h). Results were analysed statistically, using ANOVA test.

Using anti-IgE, there was no significant correlation between the extent of either IL-4 (r=0.24, \(p=0.35\)) or IL-13 (r=0.47, \(p=0.098\)) and histamine release. Using IL-3 as stimulator, results showed that the extent of IL-13 correlated with histamine release (r=0.44, \(p=0.036\)). There was no correlation between the extent of IL-4 and the degree of either histamine (r=0.077, \(p=0.72\)) or IL-13 (r=0.162, \(p=0.5\)). The reproducibility of cytokines isolated from the same donor (on different occasions) indicated that the ability of anti-IgE to induce cytokines was consistently similar for a given donor.

Our data showed that the pathways leading to IL-3-triggering histamine release and IL-13 generation show similarity. Donor-dependent differences may be responsible for this wide range in the extent of releasibility. The ability of IL-3 to release cytokines from basophils showed a wider range.

Keywords: Allergy; Basophil; Cytokine; Histamine; IL-4; IL-13
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INTRODUCTION

Human basophils play a key role in allergic diseases such as asthma and in a variety of immunological disorders. It is well established that basophils can release a wide variety of mediators that are responsible for the propagation of allergic diseases. 1-6 Allergic responses are dependent on the ability of antigen-specific IgE to bind to basophils via FcεRI receptors with high affinity on the cell surface. Upon appropriate IgE-dependent stimulation by antigen, basophils release and generate a variety of inflammatory mediators, such as histamine, sulfidoleukotrienes (sLTs), and the proallergic cytokines, IL-4 and IL-13. 1, 6-9 While the preformed histamine and de novo-generated sLTs are mainly responsible for the symptoms seen in immediate-type allergic reactions, IL-4 and IL-13 may participate in late phase reactions. 10,11

Studies have demonstrated that basophils are a rich source of IL-4 and IL-13. 12-14 These cytokines are released to a greater degree by basophils from asthmatics than non-asthmatic controls. In terms of IgE-dependent stimulation, the kinetics of IL-4 release are different from IL-13 generation from basophils. At present, the reasons for these differing kinetics are not known, but factors such as autocrine effects are now being investigated. 15 IL-4 generation occurs earlier, peaking at 4 hours after IgE-dependent stimulation. 12,13,16,17 On the other hand, IL-13 secretion is generally more sustained than IL-4. IL-13 is detectable at 3 h following stimulation and generation steadily increases up to 24 h. 18

Both IL-4 and IL-13 exert a vital role in the pathogenesis of allergic reactions. 19 IL-4 shares many properties with IL-13 which include the inhibition of cytokine secretion induced by lipopolysaccharide (LPS), down-regulation of monocyte functions (e.g. IL-1, IL-12 secretion), induction of surface antigen on human B cells including CD23 (the low affinity IgE-receptor), and synthesis of Ig class switching to IgE. 20 IL-13 further increases the allergic reaction by promoting the differentiation and survival of eosinophils. Unlike IL-4, IL-13 does not directly affect T cell functions. These cytokines have been shown to bind differently to various inflammatory cell types. Therefore, differences in IL-4 or IL-13 receptor expression on cells could exert different functions in the regulation of immune responses. 21

Whereas activation through the high-affinity IgE receptor induces the generation of both cytokines in basophils, there is evidence that IL-4 and IL-13 might be differentially regulated in response to IgE-independent stimuli. For example, studies have shown that the function of basophils is strongly regulated by certain cytokines such as IL-3, IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF) and nerve growth factor (NGF). 22,25 Recent studies have demonstrated that basophils express very prominent and much higher levels of IL-3 receptor, compared to receptors for IL-5, GM-CSF and NGF. 26,27 In addition, IL-3 not only promotes proliferation of immature basophils but also amplifies various aspects of the biological function of basophils such as causing increased generation of mediators and prolonging survival. 28,30

Thus, time and stimulus-dependent differences in the regulation of IL-4 and IL-13 generation from basophils could be of relevance to the biological effects of these cytokines. In the present study, therefore, we investigated the effects of IgE-dependent (anti-IgE) and IgE-independent (IL-3) activation of basophils and evaluated the extent of histamine release, IL-4, and IL-13 generations from human basophils.

MATERIALS AND METHODS

Participants

Healthy and normal volunteers participated in the study after giving written informed consent. People with diseases such as allergic or parasitic infections were excluded. The provisions of this study were approved by the Local Research Ethics’ Committee. The percentage of male and female donors was 59 and 41%, respectively, and age ranges were from 25 to 45 years with mean age of 35 ± 10.

Basophil Isolation

Basophil-enriched suspensions were obtained by Percoll density gradient centrifugation. Briefly, basophil-enriched preparations were isolated from whole fresh blood. Blood was layered over a two-step discontinuous Percoll gradient consisting of 15 ml of 62% Percoll overlaid with 15 ml of 53% Percoll prepared in 50 ml tubes and centrifuged. A basophil-rich layer (5–15% purity), which was located 1 cm above the 53/62% interface, was harvested. The contaminant cells were lymphocytes and monocytes. These cells were washed three times in PIPES buffer...
and basophils were investigated for the release of histamine, IL-4 and IL-13.

**Mediator Release**

The followings were purchased from the sources indicated: goat anti-human IgE, PIPES (free acid), Percoll, BSA; gentamicin and RPMI 1640 (Gibco BRL, Dundee, U.K.); IL-13 (Peprotech, Rocky Hill, NJ, U.S.A.); ELISA kits for human IL-4 and IL-13 (Mast Diagnostics, Amsterdam, Netherlands).

The release of histamine, IL-4 and IL-13 was assessed from basophil-enriched suspension activated with either anti-human IgE or IL-3. Mediator release experiments were performed in RPMI 1640 medium supplemented with BSA (1 mg/ml), gentamicin (10 mg/ml) and calcium chloride (made up to 1 mM). Basophils (80,000–300,000 basophils per sample) were incubated (15 or 30 min) with a RPMI before challenge with a stimulus, typically, in a total reaction volume of 220 ml. Cells incubated (supplemented with Co2+) in RPMI alone served as measures of spontaneous mediator release, and all values cited for stimulus-induced mediator generation were corrected by subtracting this spontaneous mediator release. In experiments monitoring IL-4 generation, basophils were activated for 4 h with an optimal releasing concentration of anti-IgE (1:100,000 or 1:10,000 dilutions). In experiments monitoring IL-13 generation, basophils were activated for longer (20 or 24 h). These conditions for optimal generation of IL-4 and IL-13 generation have been reported by others and confirmed by us in a series of preliminary experiments. In some experiments, cells were activated with IL-3 (100 ng/ml) for 24 h for the generation of mediators. After activation, the cells were centrifuged (450 x g, 4 min) and the supernatants saved and analysed for mediator release. For the analysis of histamine content, an aliquot (50 ml) of the supernatant, diluted in PBS buffer (950 ml), was analysed using a modification of the automated fluorometric technique of Siraganian. The remainder of the supernatant was assayed for IL-4 and IL-13 content by enzyme-linked immunosorbent assay (ELISA). The limit of sensitivity was 0.2 and 0.5 pg/ml for the IL-4 and IL-13 assays, respectively. The Optical Density (OD) of samples was measured at 450 nM using a Dynatech plate reader.

**Data Analysis**

Total mediators (histamine and cytokines) content was obtained by incubating cells in the presence of 1.6% perchloric whilst the spontaneous release was determined by incubating cells in buffer and control Mediators release was determined by stimulating cells with stimulators. Stimulated mediators release was calculated as a percentage of the total mediators and then corrected by subtracting the spontaneous release. All experiments were carried out in duplicate.

Data are expressed as means ± s.e.m. EC50 values were determined using GraphPad Prism software (version 2). In order to establish whether stimulator caused statistically significant effects, either paired t-tests or ANOVA, followed by Dunnett’s test, was performed. In all instances, the raw data were subjected to statistical analyses.

**RESULTS**

**Correlation and Reproducibility between Histamine IL-4, and IL-13 Release, from Basophils Induced with Anti-IgE**

In a further series of experiments, the effects of IL-3 on basophils were investigated. Cells were challenged with IL-3 (100 ng/ml) for 1, 4, 16 or 24 h to evaluate the kinetics of the release of histamine, IL-4 and IL-13 (not shown). Significant (p < 0.05) levels of histamine release and IL-13 generation were obtained after 16 h incubation of basophils with IL-3. IL-4 generation was also monitored in these experiments but in only two donors (out of four), IL-4 was generated to a considerable degree. Overall, basophils produced higher concentration of IL-13 than IL-4 upon stimulation with IL-3 over 24 h. Histamine was released in a dose-dependent fashion by IL-3 from basophils following 24 h of activation (not shown).

In further experiments, correlations between histamine release, IL-4, and IL-13 generation from basophils were evaluated. Basophils from different donors were exposed to IL-3 for 24 h and mediator generation was assessed.

These experiments showed that the extent of IL-13 generation correlated well with the degree of histamine release (Figure 1b, r=0.44, p=0.036). In contrast, there was no correlation between the extent of IL-4 generation and the degree of either histamine release (Figure 1a, r=0.077, p=0.72) or IL-13 generation (Figure 1c, r=0.162, p=0.5) in basophils.

These experiments showed that the extent of IL-13
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Figure 1. The relationship between histamine release, IL-4, and IL-13 generation in response to anti-IgE from basophils. Cells were incubated for 4 h (IL-4) or 24 h (IL-13) with anti-IgE (1:10000 or 1:100000) for optimal basophil secretion. Supernatants were harvested for determination of histamine, IL-4 and IL-13. Values were plotted as correlations comparing IL-4 against % histamine release ($r=0.24$, $p=0.35$) (a), and IL-13 against histamine release ($r=0.47$, $p=0.098$) (b). Each point represents data from a separate donor.

(A)      (B)

Figure 2. Reproducibility of IL-4 and IL-13 generation from basophils. Basophil-enriched preparations were incubated for 4 h (IL-4) or 24 h (IL-13) with anti-IgE (1:10000 or 1:100000). Supernatants were then harvested for determination of (a) IL-4 and (b) IL-13. For each donor, each point represents data generated from basophils isolated on different occasions. Horizontal broken lines represent mean cytokine generation. Each abbreviation represents data from a separate donor. Asterisks denote allergic donors.

Correlation and Reproducibility between Histamine IL-4, and IL-13 Release, from Basophils Induced with IL-3

In a further series of experiments, the effects of IL-3 on basophils were investigated. Cells were challenged with IL-3 (100 ng/ml) for 1, 4, 16 or 24 h to evaluate the kinetics of the release of histamine, IL-4 and IL-13 (Figures 3 & 4). Significant ($p<0.05$) levels of histamine release and IL-13 generation were obtained after 16 h incubation of basophils with IL-3. IL-4 generation was also monitored in these experiments but in only two donors out of four, IL-4 was generated to a considerable degree. Overall, basophils produced higher concentrations of IL-13 than IL-4 upon stimulation with IL-3 over 24 h. Histamine was released in a dose-dependent fashion by IL-3 from basophils following 24 h of activation (not shown).
Figure 3. The relationship between histamine, IL-4, and IL-13 generation in response to IL-3 from basophils. Cells were incubated for 24 h with IL-3 (100 ng/ml) for optimal basophil secretion. Supernatants were harvested for determination of histamine, IL-4 and IL-13. Values were plotted as correlations comparing IL-4 generation against histamine release (a), IL-13 generation against histamine release (b), and IL-13 against IL-4 generation (c). Each point represents data obtained from a separate donor.

Figure 4. Reproducibility of IL-4 and IL-13 generation from basophils. Basophil-enriched preparations were incubated for 24 h with IL-13 (100 ng/ml). Supernatants were then harvested for the determination of (a) IL-4 and (b) IL-13. For a given donor, each point represents data generated from basophils isolated on different occasions. Horizontal broken lines represent mean cytokine generation. Each abbreviation represents data from a separate donor. Asterisks denote allergic donors.
In further experiments, correlations between histamine release, IL-4, and IL-13 generation from basophils were evaluated. Basophils from different donors were exposed to IL-3 for 24 h and mediator generation was assessed. These experiments showed that the extent of IL-13 generation correlated well with the degree of histamine release (Figure 3b, r=0.44, \(p=0.036\)). In contrast, there was no correlation between the extent of IL-4 generation and the degree of either histamine release (Figure 3a, r=0.077, \(p=0.72\)) or IL-13 generation (Figure 3c, r=0.162, \(p=0.5\)) from basophils.

In additional experiments, the reproducibility of IL-3-dependent mediator generation from basophils from the same donors isolated on different occasions was evaluated (Figure 4). Cells were incubated with IL-3 (100 ng/ml) for 24 h and cytokine generation was assessed. In contrast to experiments with anti-IgE, the response of basophils to IL-3 showed greater variability in the extent to which cytokines were generated by a given donor but on different occasions.

**DISCUSSION**

The present study attempted to establish whether human basophils were capable of releasing cytokines in response to stimulators such as anti-IgE and IL-3. Initial studies employing different concentrations of anti-IgE indicated that optimal generation of either histamine or IL-4 occurs at different concentrations of anti-IgE. This finding is consistent with other studies which also showed that IL-4 secretion peaks at lower concentrations (ten-fold lower) of anti-IgE than that needed for histamine release.\(^{6,12}\) By contrast, it has been shown that anti-IgE-induced IL-13 generation parallels histamine release.\(^{31}\)

In general, we could not detect any significant correlations between the extent which basophils released histamine and generated cytokines in response to anti-IgE. These data suggest that the processes leading to histamine release and cytokine generation, when cells are challenged with anti-IgE, may differ. Similarly, when basophils were challenged with IL-3, there was no correlation between histamine release and IL-4 generation, but there was a significant correlation between the extent of IL-13 generation and histamine release. It is possible, therefore, that the pathways leading to IL-3-triggered histamine release and IL-13 generation show similarity.

Furthermore, in terms of cytokine generation induced by IL-3 or anti-IgE, our data showed that the ability of IL-3 to release cytokines from basophils showed a greater range of levels of generations. This is in agreement with other reports showing more variability in the response of basophils to IL-3.\(^{34}\) Donor-dependent differences may be responsible for this wide range in the extent of releasibility. Also the reproducibility of IL-3-dependent mediator generation from basophils showed considerable variability in the extent to which cytokines were generated by a given donor but on different occasions. This is in contrast with anti-IgE induced cytokine generation that was consistently similar for a given donor. This implies that the responsiveness of basophils to IL-3 is less strictly regulated. An individual’s status at the time when basophils are isolated, may influence cellular responsiveness to IL-3.

Previous studies have shown that stimulation of basophils with IL-3 induced large magnitude of IL-13 generation but modest levels of IL-4 and negligible levels of histamine release.\(^{26,31,35}\) Our studies indicated that IL-3 not only induced IL-13 and some modest levels of IL-4 generation from basophils, but also could induce substantial levels of histamine release. The discrepancy between our own data and those of others may relate to incubation time. While studies by others followed histamine release for only 2 h.\(^{36}\) Our findings showed that the kinetics of histamine release with IL-3 were very slow requiring more than 4 h. IL-3-dependent release differs from IgE-dependent histamine release since the latter is completed by 30-40 min following challenge. Interestingly, a similar slow onset of histamine release from basophils is also seen with LPS (unpublished data). This suggests that the mechanisms by which anti-IgE and IL-3 induce histamine release from basophils, differ. Given the slow kinetics of histamine release, it is possible that IL-3 acts through gene transcription to release histamine. It is known that IL-3 induces STATs (signal transducers and activators of transcription) to initiate transcription and the generation of cytokines.\(^{37,38}\) It is possible that a similar mechanism may be involved causing slow onset histamine release from basophils. Additional support for this comes from the finding that there was a significant correlation between the extent of histamine release and the degree of IL-13 generation from basophils activated with IL-3. Our studies, comparing the effect of IL-3 on mixed cells and purified basophil preparations (data not shown).
established that IL-3 induced histamine, IL-4 and IL-13 release from basophils and that IL-3 did not mediate effects by interacting with other cell populations. The later data indicated that the basophils could be the major source of releasing IL-4, IL-13 and histamine in mixed leukocyte preparations.

Overall, these findings indicated that basophils were capable of generating cytokines and histamine in response to both IgE- and non-IgE-dependent stimulators. The release of these mediators is likely to contribute to the elaboration of allergic diseases.

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