

Association between reduced levels of total serum IgE and FcεRI expression in non-releaser basophils

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Abstract

Background: FcεRI-mediated signal pathway in basophils and mast cells leads to release of histamine and other mediators. Interestingly, basophils from 10% to 20% of the population do not release histamine and other mediators on activation of the IgE signal transduction pathway and this has been attributed to the absence of tyrosine kinases Lyn and Syk.

Objective: To investigate the association between histamine releasability, total serum IgE and expression of IgE receptor in releaser and non-releaser phenotypes in Indian population.

Methods: Basophils from peripheral blood of healthy adults were purified by density gradient centrifugation and negative immuno-selection. Histamine release assay was performed fluorometrically. Total serum IgE was estimated by ELISA and assessment of IgE receptor expression was carried out by flow cytometry.

Results: Histamine release after ConA challenge varied greatly from 0% to 100% in Indian subjects. Eighteen percent subjects showed less than 5% histamine release (non-releasers). Flow-cytometric analysis revealed a significantly reduced expression of FcεRI in non-releaser basophils ($p < 0.05$). Total serum IgE levels were also significantly low ($p < 0.05$) in non-releasers as compared to releasers.

Conclusions: About 18% of the Indian subjects studied showed non-releaser phenotype and also had reduced serum IgE levels and FcεRI expression. As many components like, histamine releasability, serum IgE and IgE receptors, were found to be reduced in non-releasers, suggesting a common regulators of the phenotype. These needs to be further evaluated and could lead to identification of a potential target for the development of therapeutics for allergic patients.

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Introduction

Basophils are the most important cells involved in the allergic reactions by releasing mediators of inflammation after IgE-mediated activation. However, basophils from approximately one-fifth of the human population

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(20%) are reported to be unresponsive (non-releasers), in terms of both histamine and leukotriene release, to an IgE cross-linking stimulus, such as anti-IgE antibody (Lichtenstein and MacGlashan, 1986; Marone et al., 1986). As compared to releaser basophils, non-releaser basophils possess statistically similar densities of cell-surface IgE antibody (400,000 versus 287,000) with low levels of IgE molecules per basophils in healthy non-allergic volunteers (Nguyen et al., 1990). IgE on non-releaser basophils were found to be cross-linked by the poly clonal anti-IgE antibody (Ishizaka et al., 1973). These basophils are unresponsive to any IgE-mediated stimulation, but in contrast, respond to non-IgE-mediated stimuli, such as the phorbol ester, 12-*o*-tetradecanoyl phorbol-13 acetate, the calcium ionophore, A23187 (Nguyen et al., 1990). Interestingly, Lyn and Syk kinases, which are the important kinases of the IgE-mediated signal transduction pathway were reported to be absent in the non-releaser basophils and are thought to be the reason for the non-releasability (Kempley et al., 1999; Lavens-Phillips and Mac Glashan, 2000).

The critical role of IgE in both the early and late phases, of allergic inflammation, is well established. IgE influences the allergic inflammatory response by interacting with the high-affinity IgE receptor (FcεRI) on mast cells and basophils, binding to the low-affinity IgE receptor (CD23 or FcεRII) to augment cellular and humoral immune responses. In addition to its ability to activate mast cells, IgE can bind to the low-affinity IgE receptor CD23 on B cells, thus influencing the function of these cells (Richards and Katz, 1991; Corominas et al., 1993). Passive sensitization of B cells with IgE substantially enhances B-cell immune responses, such as the presentation of antigen. In addition, the presence of antigen-specific IgE has been shown to amplify the *in vitro* production of IgE in a CD23-dependent manner (Konig et al., 1988; Pirron et al., 1990; Kisselgof and Oettgen, 1998).

An interesting relationship exists between serum IgE levels and its receptors. Higher IgE levels are associated with increased numbers of IgE receptors expressed on mast cells and basophils. Thus, IgE affects by positive feedback mechanisms that enhances FcεRI receptor density (Yamaguchi et al., 1997; Mac Glashan et al., 1997; Kubo et al., 2001).

IgE-mediated upregulation of FcεRI substantially enhances the ability of mast cells or basophils sensitized with IgE to degranulate in response to allergen challenge and increased release of mast-cell or basophil cytokines such as IL-4, which leads to increased IgE levels and IgE receptor density and vice versa. Yamaguchi et al. (1997, 1999) observed 4-fold to 5-fold less FcεRI receptors on mast cells in IgE-deficient mice as compared with wild-type mice. The administration of anti-IgE (omalizumab) to human subjects has been found to inhibit mast-cell activation and IgE-mediated antigen presentation,

thus suppressing the allergic inflammatory response (Zhu et al., 2002; Corren et al., 2004; Lin et al., 2004).

In view of lack of such studies in human population in Indian subcontinent, the present work was undertaken to investigate the relationship between histamine releasability, total serum IgE and FcεRI expression in Indian subcontinent for the first time in the country.

Materials and methods

Subjects for histamine releasability studies

Donors of blood consisted of healthy adult subjects of 19–51 years of age, visiting the Blood Bank of a Hospital in Delhi. Randomly 83 donors were selected for the study (Kumar et al., 2007). Subjects, who were on any kind of medication, were excluded from the study. About 50 ml of blood was drawn from donors with their informed consent after clearance from institutional ethics committee.

Purification of basophils

After transportation of blood in cold conditions (Ice containers) to the laboratory, basophils were single step separated using Basophils Isolation Kit (Miltenyi Biotec GmbH, Germany) as reported by us (Kumar et al., 2007). Briefly, peripheral blood mononuclear cells (PBMCs) were obtained by centrifugation over the histopaque (1.077 g/ml). Interface between the plasma and histopaque was collected. Purity of the basophils ranged between 1% and 4%. Leukocytes were then resuspended in PIPES buffer (PIPES 25 mM, 110 mM NaCl, 5 mM KCl, 40 mM NaOH pH 7.4 without Ca⁺⁺ and Mg⁺⁺) and washed twice in the PIPES buffer. Cells were then incubated with the FcR blocking reagent. Cells were incubated with 100 μl of hapten-conjugated antibody cocktail containing CD-3, CD-7, CD-14, CD-15, CD-16, CD-36, CD-45 RA and human anti-HLA-DR antibodies (per 10⁸ total cells) for 15 min and 100 μl of anti-hapten micro-beads at 4 °C. Cells were then loaded on the LS column and enriched basophils were collected as the negatively selected cells in the flow-thorough. Purity of the cells ranged from 40% and 60% in our samples in spite of repeated efforts.

Histamine release assay

Histamine release assay in total leukocytes was carried out fluorimetrically using the method outlined by Siraganian (1975).

Total leukocytes were used for the assay of histamine release from the basophils. Histamine assay was carried out fluorimetrically as reported by us earlier (Kumar et al., 2007). Briefly, 10 ml of blood was mixed with the

6% dextran, 3% dextrose and physiological saline. This was allowed to sediment for 90 min at room temperature (30 °C). Leukocyte-rich plasma were removed from the solution and centrifuged at 250g for 10 min. Cells were pelleted and re-suspended in the 2 ml PIPES-ACMD. Cell suspension (900 µl) was incubated with 100 µl of 10 µg/ml ConA as it has been reported to function by cross-linking cell surface IgE (Windelborg et al., 1993). In parallel, cells were incubated without any activator to correct for the spontaneous histamine release. Total histamine of the cells was measured by adding 0.4 N perchloric acid to the cell suspension. Tubes were centrifuged at 500g and supernatant-containing histamine was kept at –70 °C until assayed. Histamine was condensed with the OPT (0.1%) in methanol after extraction in butanol. Fluorescence of the samples was measured by 355 nm excitation and read at 444 nm (Jobin Yuon, Edison, NY).

Histamine releasability was calculated as follows:

$$\text{Percent histamine release} = \frac{100 \times (\text{stimulated histamine release} - \text{spontaneous histamine release})}{\text{Total histamine content}}$$

Flow cytometry for IgE expression

Expression of the surface IgE on basophils was analyzed as per the method outlined by Schmid et al. (1991) as reported by us earlier (Kumar et al., 2007). In brief, basophils obtained after magnetic activated cell sorting were suspended in the PBS with 0.1% sodium azide. Approximately 104 cells were fixed in the 200 µl of the fixation buffer containing 4% formaldehyde for 30 min. Cells were centrifuged for 5 min at 250g and supernatant was aspirated. Cells were incubated with rabbit anti-human IgE PE (Sigma, USA) labeled at 4 °C for 15 min. Cells were washed with PBS twice again and pellet was then suspended in 500 µl of sheath fluid.

Flow cytometry was performed on FACSvantage flow cytometer (Becton Dickinson, USA). Basophils (10,000 events) were gated on the basis of bright IgE staining. Analysis was carried out by WinMDI. Data for the expression was based on the mean fluorescence intensity of the PE signal produced by different samples and was considered as IgE expression on the basophils. For flow-cytometric analysis of surface IgE expression on basophils 5 and 9 samples only were analyzed from non-releaser and releasers subjects, respectively, depending on the availability of the samples.

Estimation of total serum IgE in individual sera

Total IgE in the sera were measured by using total IgE estimation kit (Bethyl Laboratories, USA) Briefly, 96-well polystyrene microtiter plates were first washed

with sodium carbonate buffer (pH 9.6) and then coated with 100 µl of goat anti-human IgE in 0.1 M sodium carbonate buffer (pH 9.6) and incubated overnight at 40 °C.

The plates were then washed thrice, with wash buffer (pH 8.0). After washings, free sites were blocked with 1% BSA in wash buffer (100 µl in each well) at room temperature (25 °C) for 2 h. After incubation plate was washed again with the wash buffer. One hundred microliter of sera diluted to 1:10 in 1% BSA in wash buffer) was placed in the wells in duplicate and incubated at 40 °C overnight. Human IgE (Lot RC 80-108-5) from Bethyl Laboratories USA was used as calibrator (3.5–500 ng/ml). Plate was again washed. After thorough washings 100 µl of goat anti-human-HRP-labeled diluted to 1:10,000 with BSA in wash buffer was added to each well and incubated for 4 h at room temperature. For color development, 100 µl of substrate solution (citrate phosphate buffer + 4 mg/10 ml

ABTS) was added to each well. The reaction was stopped after 8 min using 100 µl of 1% SDS. Optical density was measured by Immuno reader at 405 nm (Molecular Devices, USA).

Optical density of the calibrators was plotted against the concentration of the calibrators. IgE values were deduced from the standard curve.

Results

Demographic profile of the subjects studied for the histamine releasability

A total of 83 subjects were investigated for the histamine releasability studies, visiting Blood Bank of Guru Teg Bahadur Hospital, Delhi. Out of 83 individuals, 79 were males and only 4 were females. The average age of the subjects was 30.77 ± 8.07 years ranging from 19 to 51 years. Fifty-nine percent of the subjects were in the age range of 20–30 years.

Histamine releasability in general population

Histamine releasability experiments after activation of basophils with IgE activator (Con A), were carried out in all the 83 Indian subjects as reported by us (Kumar et al., 2007). Variations in the amount of histamine release were observed from 0% to 100% and were categorized in to four groups depending on the percent histamine release (Fig. 1) 18.0% (15/83) showed less

than 5% of histamine release and were thus considered *non-releasers*. While 19.2% (16/83) showed maximal histamine releasability ranging from 5% to 20%. Mean histamine release in this group was $13.4 \pm 4.5\%$. Mean maximal histamine releasability in releasers with 20–45% histamine release was observed to be $29.43 \pm 6.25\%$ ($n = 26$) and mean histamine releasability in releasers with more than 45% (26/83) histamine release was found to be $69.42 \pm 15.7\%$ (Fig. 1).

Total serum IgE and histamine releasability

The correlation between the histamine releasability and total serum IgE was studied in 61 individuals, whose serum samples were available with us, from 83 subjects studied for histamine release. Total serum IgE values ranged from 62.2 to 2030.4 IU/ml. The statistically significant correlation between the total serum IgE levels and histamine releasability was recorded ($r = 0.234$, $p < 0.05$) indicating a moderate linear correlation between total serum IgE, and histamine releasability (Fig. 2).

Total serum IgE levels were further analyzed in the various classes categorized on the basis of histamine release in 61 individuals as mentioned above (Fig. 3). Mean serum IgE values in the non-releasers (<5% histamine release) categorized as Class I was found to be 273.5 ± 305.5 IU/ml ($N = 13$) ranging from 66.4 to 966.9 IU/ml. While in Class II with 5–20% of histamine release showed higher mean serum IgE levels as 316.21 ± 670 IU/ml ranging from 82.6 to 2035.5 IU/ml ($N = 18$). However, mean serum IgE in Class III with 20–45% ($n = 20$) histamine release was 533.4 ± 487 IU/ml ranging from 64.2 to 1638.7 IU/ml, while it was 617 ± 701.3 IU/ml ranging from 104 to 2282.3 IU/ml in Class IV with histamine release of more than 45% ($N = 10$). Thus, an interesting observation was recorded that in the class with more than 20% histamine release (Classes II and III) had significantly higher serum IgE

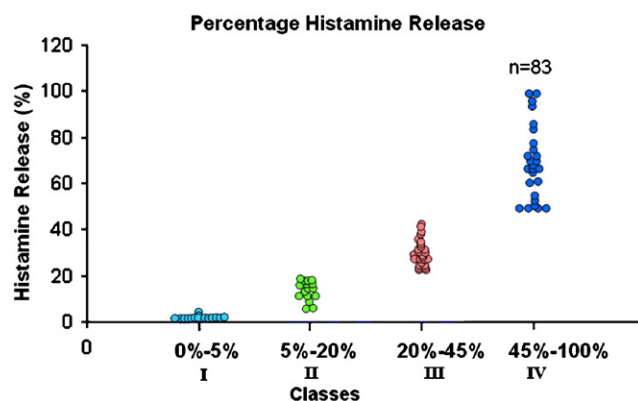


Fig. 1. Graph showing the histamine release in 83 subjects depicted in four groups as Class I 0–5%, Class II 5–20%, Class III 20–45% and Class IV >45%.

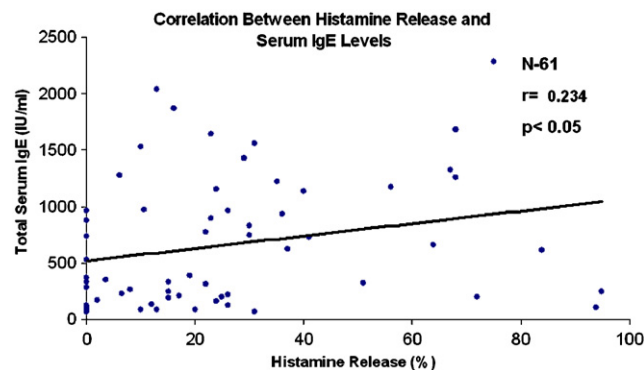


Fig. 2. Scatter diagram showing correlation between % histamine release and total serum IgE (IU/ml) in 61 individuals. A linear correlation seems evident between histamine release and mean serum IgE concentration, $r = 0.242$ ($p < 0.05$).

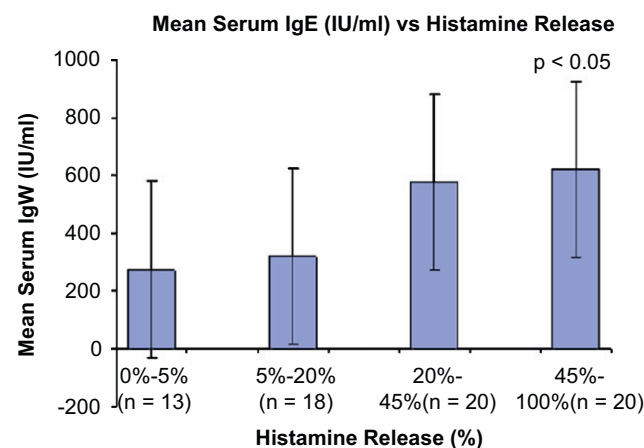


Fig. 3. Histogram showing comparison between mean serum IgE levels in different classes of releasers. Releasers with more than 20% histamine releasers ($n = 30$) and non-releasers ($n = 13$) with less than 5% histamine release showed significant differences in serum IgE levels ($p < 0.05$).

levels as compared to the non-releasers (Class I) ($p < 0.05$). However, a statistically significant correlation between non-releasers (Class I) and releasers (Class II) was found lacking ($p > 0.05$) (Fig. 3).

Expression of surface IgE on the basophils

IgE expression on the basophils was studied using flow cytometry. The cells were labeled with anti-IgE PE antibodies. Bright IgE-stained cells (10,000 events) were gated for non-releasers ($N = 5$) and releasers ($N = 9$) as shown for two representative non-releasers and non-releasers, respectively (Fig. 4). The expression of surface IgE (FcεRI) in the non-releaser basophils (<5% histamine release) was found to be 1614.7 ± 1517 ($n = 5$) MFU (mean fluorescence units) ranging from

Cell surface IgE staining differences in non-releasers (A,B) and releasers (C,D)

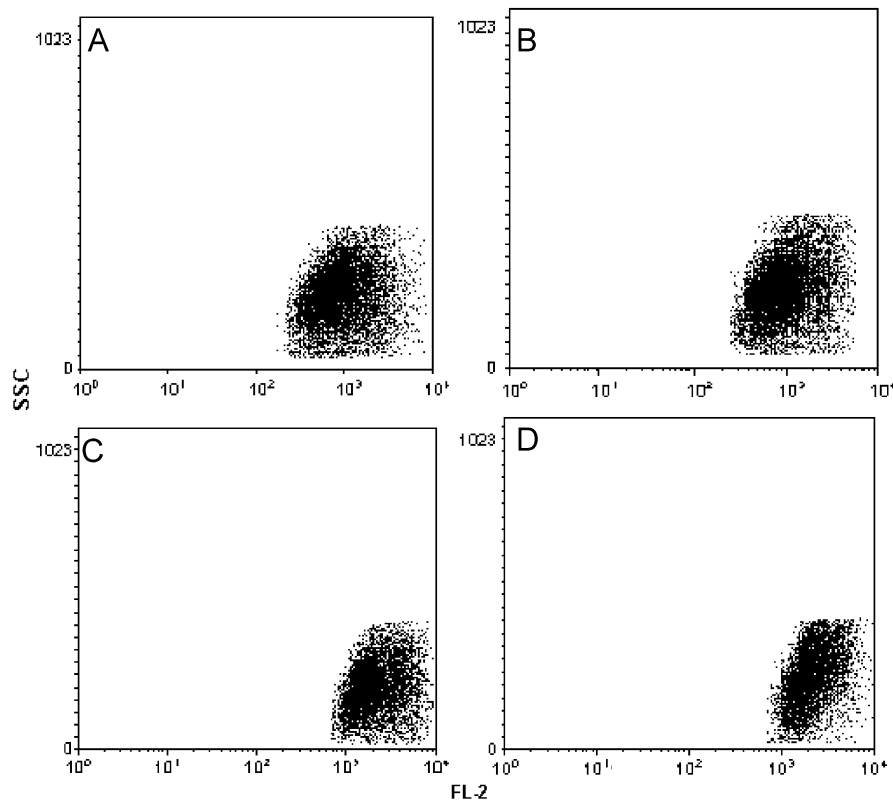


Fig. 4. Dot plots showing IgE staining on basophils of non-releasers (A, B) and releasers (C, D). 10,000 brightly stained events were recorded for the gated basophils.

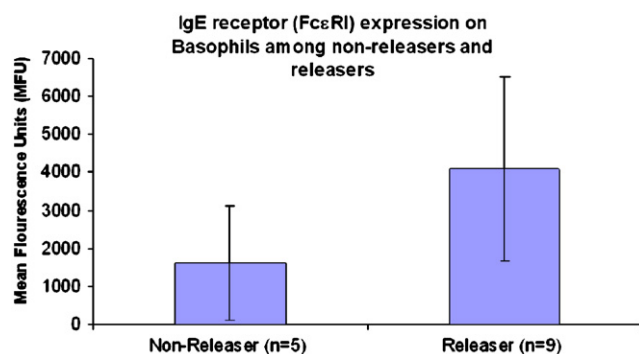


Fig. 5. Histogram showing mean IgE receptor expression in the non-releaser ($n = 5$, $<5\%$ histamine release) and releasers ($n = 9$, $>20\%$ histamine release). Significantly reduced expression of IgE receptor on basophils in non-releasers as compared to releasers was observed ($p < 0.05$).

440 to 4468 (MFU) as compared to the 4084.3 ± 2440.9 ($n = 9$) mean fluorescence units in the releaser group ($>5\%$ histamine release), ranging from 2023 to 8682 MFU (Fig. 5). Thus, IgE receptor expression in releasers was significantly higher as compared to the non-releasers ($p < 0.05$).

Discussion

Basophils from about 20% of population are known to be non-responder of the IgE-mediated stimuli (Nguyen et al., 1990). These basophils are known as non-releasers as they do not release histamine and other mediators on IgE-mediated activation. These basophils were reported to be deficient in IgE (Kepley et al., 1999). Later, Kepley et al. (2000) reported an important role of IL-3 in turning on the histamine release and kinase expression. In the absence of any such information with respect to Indian population, which is ethnically different from the west, we investigated histamine releasability in Indian subjects. Our interest was to validate the findings in different ethnic groups. So we evaluated relationship between histamine releasability, total serum IgE and FcεRI expression. These observations are important with respect to allergic patients as more than 20% of the population in India suffers from one or other allergic ailments (Anonymous, 2000).

Studies on the histamine releasability in Indian population revealed, that it varies from 0% to 100%. However, 18.0% individuals are unresponsive towards the IgE-mediated activation and do not release

histamine or released less than 5% (Kumar et al., 2007). This is in confirmation with the studies by Nguyen et al. (1990), who also reported that, 10–20% of the normal individuals do not release histamine after activation with the anti-IgE. Histamine releasability is also an important criterion determining the atopic status of the individuals. Atopic individuals shows significantly more IgE-mediated histamine release as compared to the non-atopic and healthy control subjects as observed by Son et al. (1999). The severity of the symptoms in the allergic patients also correlated with the histamine releasability (Winther et al., 1999).

It is generally accepted that the density of the cell surface IgE controls the extent of reaction, as the density determines the magnitude of the signal transmitted to the interior of the cells. So, we compared the IgE receptor expression in different individuals. Expression of high affinity IgE receptor on basophils is found to be significantly reduced ($p < 0.05$) in the non-releaser individuals as compared to releasers. These observations are in variance with the observations made by Nguyen et al. (1990) who observed that non-releaser basophils possessed statistically similar density of cell surface IgE.

Studies comparing the total serum IgE in releasers and non-releasers demonstrated, significantly less serum IgE levels in the non-releasers as compared to the releasers ($p < 0.05$). These observations are in conformity with the observations made by Son et al. (1999) who observed a significant correlation between the serum IgE levels and anti-IgE-mediated maximal histamine release. A significant correlation between the total serum IgE, and IgE receptor expression has also been observed in 14 cases ($p < 0.05$). Malveaux et al. (1978) also reported a significant correlation between IgE receptors on basophils and serum IgE levels.

Basophils and mast cells are the important sources of IL-4, which provide the important signal for the class switching from IgG to IgE as reported by Punnonen et al. (1994). Non-releaser basophils have been observed to be poor releasers of other products of the IgE-mediated pathway, including IL-4 which provide class switching signal for IgG to IgE. Non-releaser basophils have been observed to be poor releasers of other products of IgE-mediated signaling including IL-4. Thus, poor release of these cytokines from the basophils could reduce the serum IgE levels. Serum IgE has been reported to regulate IgE receptor expression (Lantz et al., 1997). Our observations are in conformity with these reports that IgE-mediated signal transduction pathway may regulate the expression of serum IgE and surface IgE expression on basophils.

To summarize we observed that IgE-mediated histamine releasability in Indian subjects, their serum IgE levels and surface IgE expression on basophils are interdependent on each other.

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