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Histamine releasibility and expression of Lyn and Syk kinases in Indian subjects and role of less potent IL-3 in non-releaser basophils

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Abstract

Background. Allergen-mediated activation of the IgE signal pathway in basophils and mast cells leads to release of mediators in-vitro and in-vivo systems. However, 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. Interestingly, when these non-releaser basophils are incubated with the IL-3, it leads to the recovery of the histamine releasibility. *Objective.* To investigate histamine releasibility in the Indian population and to evaluate the role of IL-3 with reference to non-releaser phenotypes. *Methods.* Peripheral blood basophils from healthy adults were purified by density gradient centrifugation and negative immuno-selection. Histamine release assay was performed fluorometrically. Assessment of Lyn and Syk expression were carried out by flow-cytometry. SNP analysis in the IL-3 gene was carried by sequencing analysis. *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 Lyn and Syk kinases in basophils ($p < 0.05$). Histamine release also significantly correlated with expression of Lyn and Syk kinase ($p < 0.05$). Non-releasers showed the presence of SNP at +79 (T–C), which leads to the one amino acid change at 8th position in the mature IL-3 from serine to proline. *Conclusions.* About 18% of the Indian subjects studied showed non-releaser phenotype and also had reduced Lyn and Syk kinase expression. Non-releasers have also shown the presence of less potent isoform of IL-3/P8, which is suspected to be responsible for the non-releaser phenotype. This needs to be extended to a larger sample size and could be a potential target for the development of therapeutics for allergic patients.

Keywords: Basophils; Histamine non-releasers; IgE; IL-3; Lyn; Syk

1. Introduction

Basophils are 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 (20%) from USA 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 as reported by Lichtenstein and Mac Glashan [1] and Marone et al. [2]. Such information is lacking from other ethnic populations. Basophils possessed statistically similar densities of cell-surface IgE antibodies per basophil for both releaser and non-releasers [3]. IgE on non-releaser basophils are reported to be cross-linked by the polyclonal anti-IgE antibody by Ishizaka et al. [4]. Nguyan et al. [3] also reported that these basophils are unresponsive to any IgE-mediated stimulation but, in contrast, respond to non-IgE-mediated stimuli, such as phorbol ester, 12-*o*-tetradecanoyl phorbol-13 acetate, calcium ionophore, A23187, and to formyl-methionyl-leucyl-phenylalanine peptide.

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Interestingly, interleukin-3 (IL-3) has been demonstrated to markedly enhance IgE-mediated histamine and leukotriene release in human basophils. However, IL-3 equivalently enhanced formyl methionine peptide-induced release in both releaser and non-releaser basophils, suggesting that lack of an effect on anti-IgE-induced release, is not due to a lack of IL-3 receptors [5,6]. However, it has been demonstrated by Yamaguchi et al. [7] that culturing the non-releaser basophils with IL-3 containing media for 72 h resulted in the acquisition of the responsiveness to IgE-mediated stimulation [7]. Interestingly, Lyn and Syk kinases, which are the important kinases of the IgE-mediated signal transduction pathway are reported to be absent in the non-releaser basophils and were thought to be the reason for the non-releasibility of histamine. However, m-RNA expression of these kinases was found similar in both releasers and non-releasers. Possibility of any mutation leading to premature termination of translation of Lyn and Syk was ruled out as no mutation or SNP has been observed [8,9].

In view of lack of such studies in Indian subcontinent which are ethnically different group than the US population, the present work was undertaken to investigate the histamine releasibility and kinase expression with reference to the role of IL-3 in Indian population for the first time.

2. Materials and methods

2.1. Subjects for histamine releasibility studies

Blood donors consisted of healthy adult subjects of 19–51 years age, visiting the Blood Bank of a Hospital in Delhi. Randomly 83 donors (79 male, four females) were included for the study. Subjects, who were on any kind of medication, were excluded from the study. About 50 ml of heparinized blood was collected from donors with their informed consent after approval from institutional ethics committee.

2.2. 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 Biotech GmBH, Germany) according to manufacturer's instructions [10]. Briefly, Peripheral Blood Mononuclear Cells (PBMC)[1] 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 10^8 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-through. Purity of the cells ranged from 40% to 60% in our samples.

2.3. Histamine release assay

Total leukocytes were used for the assay of histamine release from the basophils as also used by Jee-Woong Son et al. [11]. The assay was carried out fluorometrically as per the method outlined by Siraganian [12]. 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 250*g* for 10 min. Cells were pelleted and resuspended in 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 [13]. 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 500*g* 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 using fluorescence spectrophotometer (Jobin Yuon, Edison, NY).

Histamine releasibility was calculated as follows:

$$\begin{aligned}&\text{Percent histamine release}\\&= (100 \times \text{stimulated histamine release}\\&\quad - \text{spontaneous histamine release})\\&\quad /(\text{total histamine content})\end{aligned}$$

2.4. Flow-cytometry for kinase expression

Expression of the protein kinases in basophils was carried out using the method outlined by Schmid et al. [14]. Briefly, basophils obtained after MACS were suspended in the PBS with 0.1% sodium azide. Approximately 10^4 cells were fixed in the 200 μl of the fixation buffer containing 4% formaldehyde for 30 min. Cells were centrifuged for 5 min at 250*g* and supernatant was aspirated. Cells were then resuspended in the Permeabilization Buffer with 0.1% saponin for 20 min at 37 °C and washed twice in the Permeabilization Buffer. Cells incubated with the Rabbit anti-Lyn and Rabbit anti-Syk (Santacruz Biotech, USA) antibodies and washed thrice in the Permeabilization

[1] *Abbreviations used:* CD, cluster of differentiation; FACS, florescence activated cell sorting; IL, interleukin; OPT, *ortho*-phthaldehyde; PCR, polymerase chain reaction; PIPES, piperazine-1,4-bis(2-ethanesulfonic acid); TNF-α, tumor necrosis factor-alpha.