Human Basophil Function Testing in Whole Blood using CD123

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Human basophils are hypersensitive effector cells, playing an important role in host defense mechanisms and allergic reations. If allergens bind to specific IgE fixed on the basophil membrane via FcγRI receptor, this event triggers cellular activation, degranulation, and the release of potent mediators, *e.g.* histamine and leukotriene C4.

Basophils are present in peripheral blood as a low frequency population, and usually do not exceed 1% of circulating leucocytes. J. Olweus *et al.* described a novel method for identifying dendritic cells using lineage markers along with CD123 and HLA-DR[3]. Basophils appear as a well separated population in the same whole blood flow assay as the $CD123^+$, $HLA\text{-}DR^-$ cells[4,5].

CD123 (IL-3Rα) is a useful marker for basophil identification, its expression is less variable than surface IgE[5]. Combination of CD123 and HLA-DR determines the basophil population in blood. If these two markers are combined with CD63[1], a basophil degranulation assay can be formulated.

A simple lyse-no wash 3-color flow cytometric assay can be constructed with CD63-FITC, CD123-PE and HLA-DR-PerCP. This formulation is capable to identify basophils and to quantify basophil degranulation upon chemotactic or allergen activation.

The gating strategy of basophil phenotyping is shown on Figure 1. Gate R2 isolates the low scatter, $CD123^+$ basophil population, while Gate 3 excludes any HLA^-DR^+ cells. The combined R2+R3 Gates define the basophils. (Gate R1 defines the optional absolute count bead region).

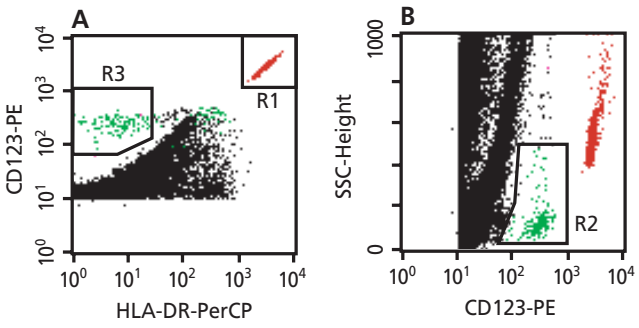

Figure 1. The basophil degranulation assay is very fast. 100 µl heparin anticoagulated blood sample is combined with 20 µl allergen, which is dissolved in a stimulation buffer, containing IL3 (2). This mixture is incubated at 37°C for 10 minutes. The activation is stopped on an ice bath. 20 µl staining reagent (CD63-FITC, CD123-PE, HLA-DR-PerCP) is added to the tube, and incubated on ice for 15-20'. The cells are fixed and lysed with 1 ml BD FACSLyse™ (Cat. No. 349202) at room temperature for 10', and analyzed by flow cytometry.

References:

1. Sainte-Laudy J, Vallon C, Guerin JC. Diagnosis of latex allergy: comparison of histamine release and flow cytometric analysis of basophil activation. Inflamm Res, Supplement 1: S35-S36. 1996.

2. Sainte-Laudy J, Vallon C, Guerin JC. Enhanced human basophil activation and histamine release by IL3 priming: application to sulfite allergy diagnosis. Inflamm Res. 1995 Apr;44 Suppl 1:S3-4.

3, Olweus J, BitMansour A, Warnke R, et al. Dendritic cell ontogeny: a human dendritic cell lineage of myeloid origin. Proc Natl Acad Sci USA 94: 12551-12556, 1997.

4.. Willmann K, Olweus J. Peripheral blood dendritic cells revealed by flow cytometry. Reagents Application Notes 3 (23-3580-00). 1998 Becton Dickinson Immunocytometry Systems, San Jose, CA USA.

5. Varro R , Chen C-H. A no-wash 3-color basophil degranulation flow assay using the CD123+HLA-DR- phenotype for basophil classification. Cytometry , Supplement 10: 116, 2000

Table 1. Basophil Related Products from BD Biosciences

Cat.No	Specificity	Clone	Isotype	Format	Size
557285	Hu CD63	H5C6	Mouse IgG1,k	FITC	100 tests
340545	Hu CD123	9F5	Mouse IgG1,k	PE	50 tests
347364	Hu HLA-DR	L243	Mouse IgG2a,k	PerCP	100 tests
554604	Hu IL3, recombinant			Recombinant	10 µg
inquire	3-color Basophil Assay CD63-FITC/CD123-PE/HLA-DR-PerCP				

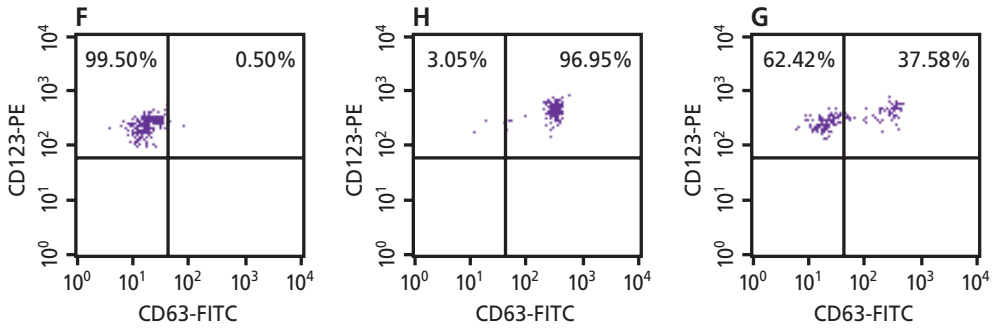

Figure 2. Degranulation of basophils of a blood donor allergic to pollen. Each dot-plot represents the CD123⁺ HLA-DR⁻ basophil population. Panel F was challenged with activation buffer only (negative control, no degranulation), panel H with the pollen mixture (>95 % of basophils degranulated). Panel G is a positive control, treated with the chemotactic peptide, N-formylmethionylleucyl phenylalanine (FMLP). This treatment causes partial degranulation of basophils, originated from either allergic or non-allergic individual.

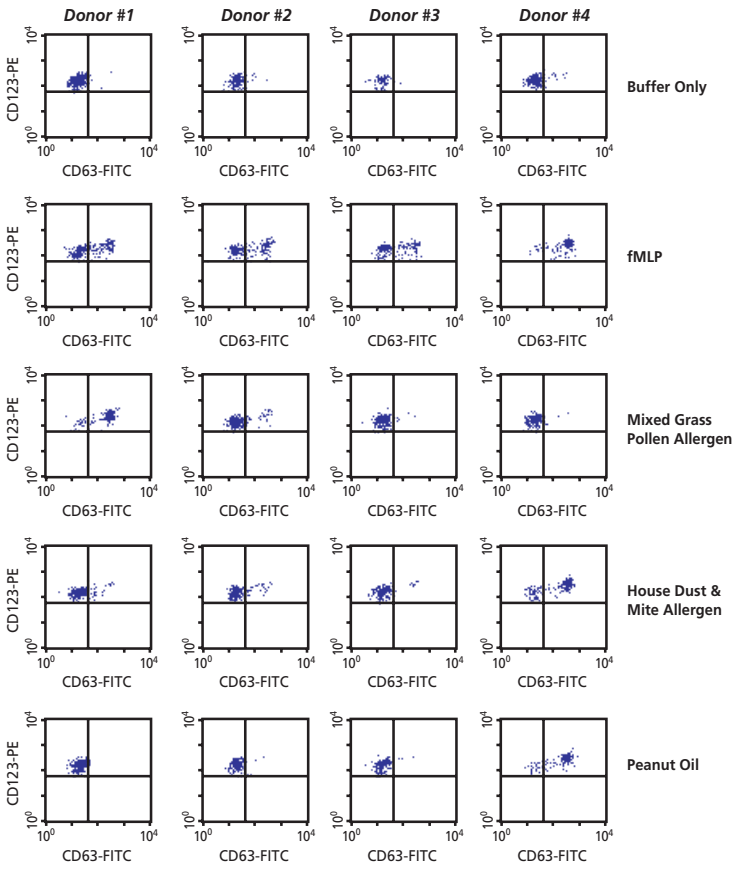

Figure 3. Individual responses to a panel of allergens. Donor #1 and Donor #2 have seasonal pollen allergies, and are not taking any allergy medication, Donor #3 has severe allergies year-round, Donor #4 is allergic to peanuts. Both of them taking medication to suppress the allergic symptoms. The individual degranulation patterns may provide additional information on the sensitization of the basophil population in these donors.