

Use of lipid rafting for the analysis of human basophil activation by flow cytometry

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Abstract

Background Human leukocyte activation induced by specific and non-specific stimuli is characterized by the formation of lipid rafts defined as lipid-ordered domains that are more tightly packed than the surrounding non-raft phase of the bilayer. These lipid rafts are formed in parallel with profound membrane reorganization.

Objectives Analyse the rafting and non-rafting proteins present on the activated and resting basophil membrane and study their interest for the flow cytometric analysis of basophil activation.

Methods Human basophils obtained from samples used for diagnostic cellular tests such as basophil or lymphocyte activation tests were stimulated either by the formyl-methionyl-leucyl-phenylalanine peptide (fMLP), by an anti-IgE or by an allergen. After 40 min at 37°C, they were labelled by different antibodies conjugated to fluorescent dyes as an anti-IgE FITC, an anti-CCR3 PE, an anti-CD63, an anti-CD203c PE, an anti-11b, annexin V FITC or by cholera toxin FITC. Moreover, several experiments were analysed using an Amnis cytometer, allowing one to obtain the picture of the analysed cells.

Results Anti-IgE or specific allergen elicits a membrane neo expression of CD63 at a high density and is poorly represented on resting basophil membrane. Upon an IgE-dependant activation some of the markers already present on resting basophil membrane, as CD203c, are up

regulated and others, such as the IgE/IgE FcεRI receptor and CCR3 are down regulated and submitted to the formation of clusters demonstrated by the pictures taken with the Amnis cytometer. For non-IgE dependant activators, such as fMLP, the picture was different since IgE was not down regulated, whereas CCR3 was down regulated. As demonstrated using annexin V or the cholera toxin used for analysing apoptosis, these phenomenon were paralleled by the formation of lipid rafts, gangliosides domains, such as GM1, which is accessible from the extra cellular medium. **Conclusions** Basophil activation leads to membrane events close to the apoptosis phenomenon. The flow cytometric analysis of these membrane events may lead to protocols for allergen-induced activation and, may significantly increase cellular test sensitivity, particularly for drugs allergy diagnosis for which the usual protocols, such as those using CD63 alone, are insufficiently sensitive.

Keywords Basophil activation · Lipid raft · Apoptosis · Membrane clusters · Flow cytometry · Image stream IS 100

Introduction

Allergic hypersensitivities, defined as adverse reactions involving the immune system, are characterized by the release of potent mediators by activated cells. Among the different attempts for reproducing in vitro the IgE mediated basophil activation, the most encouraging results have been obtained by flow cytometry [1]. This method is based on the analysis of membrane markers immune-modulation which are either up-regulated (CD63, CD203c, CD107, CD164) or down-regulated (Bsp1, CCR3, FcεRI, IgE) [2]. Recently, we have shown [3] that the combination within an algorithm of CD63 up-regulation and IgE down-regulation resulted in

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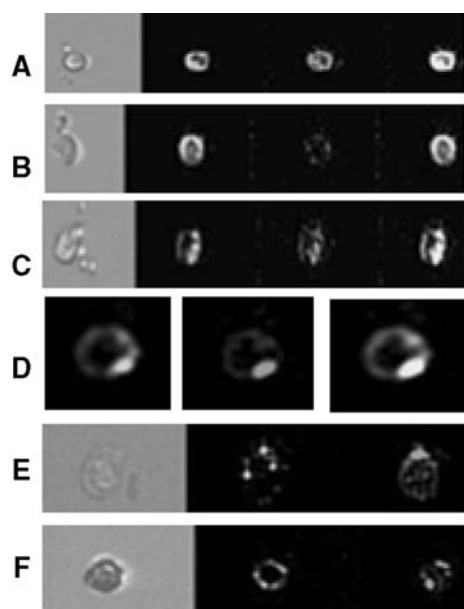


Fig. 1 Fluorescence aspects of human basophil using image stream IS 100 with the extended depth of field (EDF). **a, b, c, d** basophil observed in visible light, labelled with an anti-IgE FITC, labelled with an anti-CCR3 PE and the 2 images superimposed, **a** resting basophils, **b** fMLP induced activation, **c** anti-FcεRI induced activation, **d** at a higher magnification, wasp venom induced basophil activation. **e** and **f** basophils observed in visible light and in fluorescence after labelling with an annexin V FITC and an anti-CCR3, **e** anti-FcεRI induced activation and **f** fMLP induced activation

an increase of the sensitivity (especially for drugs) without decreasing specificity.

One of the main deep changes of membrane structure is the formation of the so-called lipid rafts. Membrane fragments isolated by cold detergents seemed to float like rafts [4]. Lipid rafts may be defined as local sub-microscopic organizations which are capable of floating freely on the bilayer of the cell membrane and which may coalesce upon clustering [5].

Studies of the high-affinity IgE receptor (FcεRI) demonstrated its translocation to lipid rafts upon crosslinking [6].

In order to visualize these phenomenon, we used, in parallel with a classical flow cytometer a cytometer combining a flow cytometer and a numeric camera (image stream IS100, Amnis) [7, 8] leading to the possibility of taking pictures in visible light or in fluorescence light of the cells of interest.

Materials and methods

Human basophils were tested either on an enriched leukocyte suspension prepared by buffy coat for the Amnis cytometer or on total blood for the classical flow cytometry. After dilution to 1/3 in a calcium buffer, basophils were

incubated (37°C, 30 min) in the presence of a target concentration of an anti-FcεRI (Buhlmann laboratories) or of *N*-formyl-methionyl-leucyl-phenylalanine (fMLP, Sigma, 10^{-7} M) and labelled by various conjugates comprising anti-CD45 ECD (Coulter Beckman), anti-CCR3 PE (Beckton Bickinson), anti-IgE FITC (Bethyl) or anti-annexin V FITC (Invitrogen). Basophil activation was stopped by addition of an EDTA buffer.

After a 15 min incubation in the dark, erythrocytes were lysed and leukocyte suspensions were resuspended in the cytometer's sheath buffer (Isoflow Coulter) and analysed within a maximum delay of 2 h, either by the image stream IS 100 or by the FC500.

Results and discussion

Results obtained with the image stream IS 100

fMLP induced activation

Resting basophils (Fig. 1) were CCR3, IgE bright and annexinV dim with an homogenous membrane labelling. Activated basophils were CCR3 dim and annexin V bright formation of fluorescent clusters. IgE marker distribution was not modified.

IgE dependant induced activation

Anti-FcεRI and allergen activated basophils were CCR3 and IgE dim (Fig. 1) and annexin V bright with formation of clusters.

Results obtained with the FC500

Results expressed in MFI showed that, for the IgE marker as compared to the controls (22 ± 15 , $n = 46$), the anti-FcεRI induced a deep MFI decrease for anti-FcεRI induced activation (16 ± 10 , $p < 0.001$) and no effect for fMLP induced activation (20 ± 11 , $p = \text{NS}$). For the CCR3 marker and as compared to the controls (20.8 ± 15 , $n = 36$) both anti-FcεRI and fMLP induced a deep down-regulation, respectively 16.1 ± 12.2 , $p < 0.001$ and 17.8 ± 13.5 , $p < 0.001$.

Immuno-modulation of human basophil surface markers depend on the stimulus and on the marker analysed. The IgE dependant immuno-modulation lead to co-located CCR3 and IgE clusters whereas CCR3 and GM1 clusters were not co-located. These results lead to the hypothesis that lipid rafts may involve these two markers independently of membrane reorganisation leading to GM1 expression.

These findings confirmed the potential interest of an early marker as IgE or CCR3 and a late marker as CD63 for the analysis of human basophil activation.

References

1. Ebo DG, Sainte-Laudy J, Bridts CH, et al. Flow-assisted allergy diagnosis. Application and future perspectives. *Allergy*. 2006;61:1039–208.
2. Hennersdorf F, Florian S, Jakob A, Baumgartner K, Sonneck K, Nordheim A, et al. Identification of CD13, CD107a, and CD164 as novel basophil-activation markers and dissection of two response patterns in time kinetics of IgE-dependent upregulation. *Cell Res*. 2005;15:325–35.
3. Sainte-Laudy J, Boumediene A, Touraine F, Orsel I, Brianchon C, Bonnaud F, et al. Use of both CD63 upregulation and IgE downregulation for the flow cytometric of allergen induced basophil activation. Definition of an activation index. *Inflamm Res*. 2007;56:1–6.
4. Brown DA, Rose JK. Sorting of GPI-anchored proteins to glycolipids-enriched membrane subdomains during transport to the apical surface. *Cell*. 1992;68:533–44.
5. Rajendran L, Simons K. Lipid rafts and membrane dynamics. *J Cell Sci*. 2005;118:10099–1102.
6. Field KA, Holowka D, Baird B. FcεRI-mediated recruitment of p53/56lyn to detergent-resistant membrane domains accompanies cellular signalling. *Proc Natl Acad Sci USA*. 1995;92:9201–5.
7. Ortyn WE, Perry DJ, et al. Extended depth of field imaging for high speed cell analysis. *Cytometry*. 2007;71:215–31.
8. George TC, Basiji DA, et al. Distinguishing modes of cell death using the Image Stream multispectral imaging flow cytometer. *Cytometry*. 2004;59:237–45.