Human basophils may not undergo modulation by DC-SIGN and mannose receptor–targeting immunotherapies due to absence of receptors

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Human basophils may not undergo modulation by DC-SIGN and mannose receptor-targeting immunotherapies due to absence of receptors

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Abbreviations:

CLEC12A: C-type lectin domain family 12 member A (CLEC12A)

DC: dendritic cells

DCIR: dendritic cell immunoreceptor

DC-SIGN: dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin

TLR-Toll-like receptor

To the editor,

Sirvent et al., recently showed that novel vaccines targeting dendritic cells (DCs) by coupling glutaraldehyde-polymerized grass pollen allergoids to nonoxidized mannan enhance allergen uptake and induce functional regulatory T cells through programmed death ligand 1.[1] Mechanistically, they found that nonoxidized mannan-coupled glutaraldehyde-polymerized grass pollen allergoids are captured and internalized by two lectin receptors on DCs: mannose receptor (CD206) and DC-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN or CD209). These data thus indicated that DCs could be targeted by C-type lectin receptors for efficient allergen immunotherapy.

Basophils are one of the key players of allergic responses. They mediate allergic inflammation by secretion of Th2-polarizing cytokines IL-4, IL-13 and by the release of effector molecules like histamines and leukotrienes upon FcεRI signalling by IgE-allergen complexes.[2] Basophils receive activation signals not only via allergen-IgE complexes[3] but also via toll-like receptors (TLRs)[4] and possibly C-type lectin receptors. In fact, basophils express several lectin receptors like C-type lectin domain family 12 member A (CLEC12A) and dendritic cell immunoreceptor (DCIR).[5,6] Thus, it is likely that in addition to DCs, nonoxidized mannan-coupled allergoids might also modulate basophil functions to exert immunotherapeutic benefits. Therefore, we analysed the expression of mannose receptor and DC-SIGN on steady state circulating human basophils and on stimulated basophils.

We analyzed basophils in whole blood of healthy donors without their purification in order to avoid any loss of cells and consequently misinterpretation of data (see Online Repository at www.jacionline.org). Further, erythrocyte-lysed whole blood cells were stimulated with IL-3 (100 ng/10^6 cells) for 24 hours. IL-3-stimulated basophils were

also stimulated for degranulation with anti-IgE antibodies (100 ng/10^6 cells) for 30 minutes. As controls for the expression of DC-SIGN and CD206, we used $CD14^+$ peripheral blood monocytes (negative control), and rhIL-4 (500 IU/10^6 cells) and rhGM-CSF (1000 IU/10^6 cells)-differentiated monocyte-derived DCs (positive control).[7]

Circulating basophils were identified as positive for FcεRI and CD123 and negative for BDCA-4. We found that human basophils at steady state are negative for DC-SIGN and CD206 (Fig.*1A-B*). As basophils display enhanced expression of various receptors upon receiving activation stimuli, we explored if they express these lectin receptors upon activation. However, irrespective of stimulation (IL-3 or degranulation stimuli) basophils remained negative for DC-SIGN and CD206 (Fig.1*B-C*). Absence of DC-SIGN was also confirmed on isolated basophils. Further, the absence of DC-SIGN and CD206 on basophils in our report is not due to non-reactivity of antibodies used in the flow-cytometry as monocyte-derived DCs, used as positive control, uniformly expressed CD206 and DC-SIGN (Fig.1*A-B*). As expected, $CD14^+$ circulating monocytes, used as negative control, did not stain for both the markers, thus confirming lack of non-specific binding of antibodies (Fig.1*A-B*).

Our results thus indicate that human basophils lack DC-SIGN and mannose receptors and hence unlike DCs, they may not directly respond and modulated by DC-SIGN- and mannose receptor-binding nonoxidized mannan-coupled allergoids. In addition, our data also suggest that basophils do not get activated by DC-SIGN- and mannose receptor-binding allergens unless they are IgE-bound. Thus, expression pattern of DC-SIGN and mannose receptor among innate cells diversifies allergic as well as tolerogenic responses.

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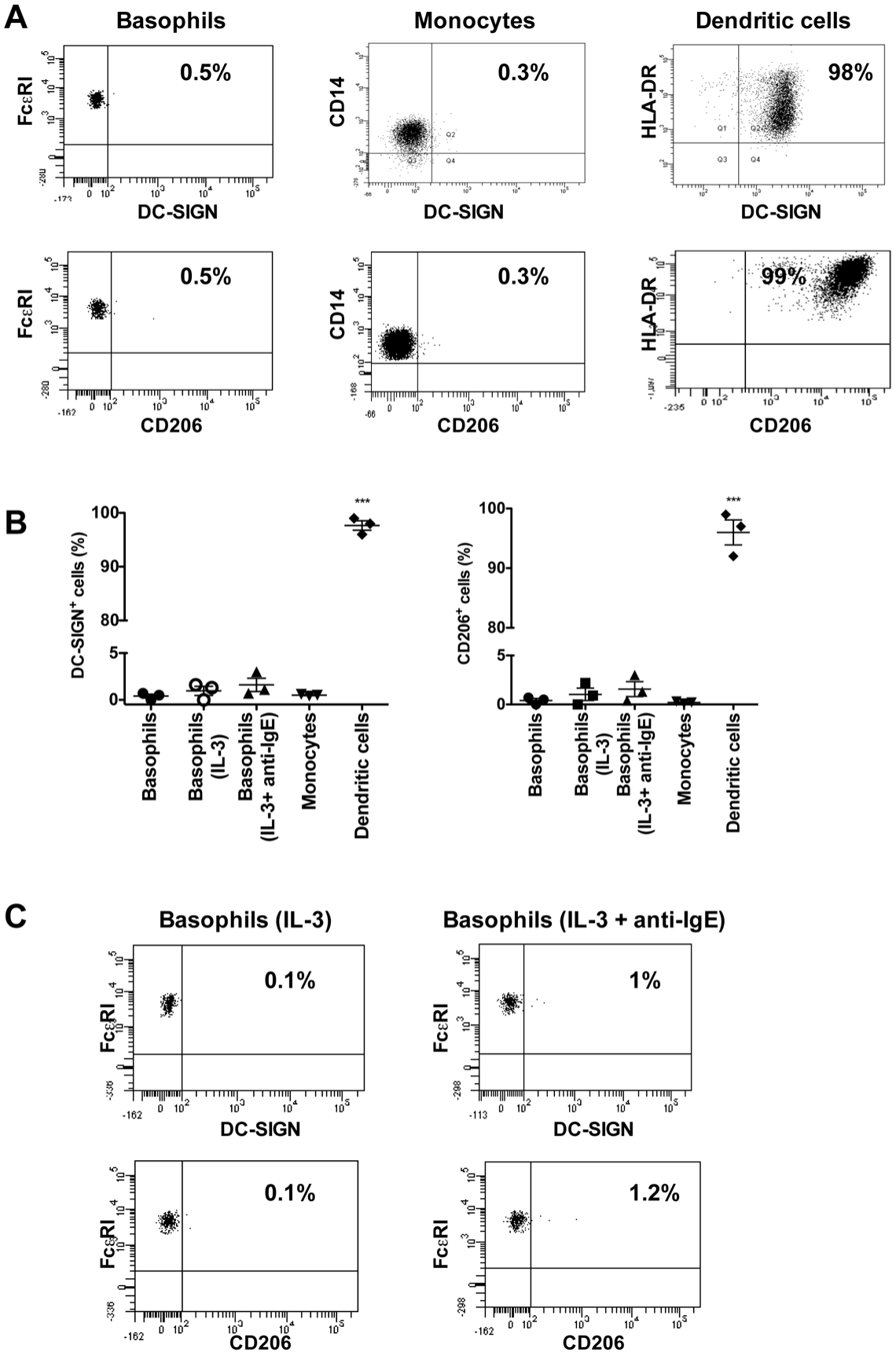
Figure Legend

FIG.1. Human basophils are deficient for DC-SIGN and CD206. **A, B**, **C**, Flow cytomeric analysis of DC-SIGN and CD206 on steady state basophils, stimulated basophils (IL-3 or IL-3 and anti-IgE), monocytes and monocyte-derived DCs. Representative dot-plots and percentage of cells (mean ± SEM, n= 3) positive for DC-SIGN and CD206. ***P < .001.


A
Basophils
Monocytes
Dendritic cells
FcεRI
CD14
HLA-DR
DC-SIGN
CD206
0.5%
0.3%
98%
99%
B
DC-SIGN+ cells (%)
CD206+ cells (%)
***
Basophils
Basophils (IL-3)
Basophils (IL-3+ anti-IgE)
Monocytes
Dendritic cells
C
Basophils (IL-3)
Basophils (IL-3 + anti-IgE)
0.1%
1%
1.2%

142

143

Online Repository

Human basophils are deficient for the expressions of DC-SIGN and mannose receptor

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METHODS

Cells and stimulation

Buffy bags of healthy donors were obtained from Centre Necker-Cabanel (EFS, Paris) and INSERM-EFS ethical committee approval (N°15/EFS/012) for the use of such material was obtained. Experiments were performed in accordance with the approved guidelines of INSERM.

Red blood cells were lysed using ACK (Ammonium-Chloride-Potassium) Lysing Buffer (Lonza). Briefly, blood was span down and resuspended and incubated in ACK lysing buffer for 30-60 seconds. Cells were washed with medium and resuspended in serum-free X-VIVO medium. Cells were stimulated with IL-3 (100 ng/million cells; ImmunoTools) for 24 hours. In addition, cells were also cultured with IL-3 (100 ng/million cells) for up to 24 hours and during last 30 minutes, cells were treated with anti-IgE antibodies (100 ng/million cells; Sigma-Aldrich). Phenotype of basophils was analysed in steady state and stimulated conditions by flow cytometry (LSR II, BD Biosciences) and the data was analyzed using FACSDiva™ software (BD Biosciences).

Peripheral blood mononuclear cells (PBMCs) were obtained from buffy bags of healthy donors by Ficoll density gradient centrifugation. Monocytes were isolated from peripheral blood mononuclear cells by using CD14 microbeads (Miltenyi Biotec) and were cultured for 5 days in rhIL-4 (500 IU/10^6 cells) and rhGM-CSF (1000 IU/10^6 cells) (both from Miltenyi Biotec) to obtain monocyte-derived DCs.

Antibodies for the flow cytometry

The following antibodies were used for the flow cytometry. FcεRIα-BV510 (Clone: AER37 (CRA-1)) was from BioLegand and BDCA-4 (CD304)-APC (Clone: AD5-

17F6) was obtained from Miltenyi Biotec. CD123-BV421 (Clone: 9F5), CD209-FITC (Clone: DCN46), CD206-PE (Clone: 19.2), HLA-DR-APC or PE (G46-6, BD Biosciences) and CD14-APC (Clone: M5E2) antibodies were from BD Biosciences

Statistical analysis

Levels of significance for comparison between samples were determined by One-way analysis of variance (repeated measures with Tukey's multiple comparison test). $P<0.05$ was considered significant. Statistical analysis was performed by Prism 5 GraphPad Software. Data are presented as mean ± SEM.