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

To the Editor:

Sirvent et al[1] 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. Mechanistically, they found that nonoxidized mannan-coupled glutaraldehyde-polymerized grass pollen allergoids are captured and internalized by 2 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 T_H2-polarizing cytokines IL-4 and IL-13 and by the release of effector molecules such as histamines and leukotrienes upon FcεRI signaling by IgE-allergen complexes.[2] Basophils receive activation signals not only via allergen-IgE complexes[3] but also via Toll-like receptors[4] and possibly C-type lectin receptors. In fact, basophils express several lectin receptors such as C-type lectin domain family 12 member A and DC immunoreceptor.[5,6] Thus, it is likely that in addition to DCs, nonoxidized mannan-coupled allergoids might modulate basophil functions to exert immunotherapeutic benefits. Therefore, we analyzed 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 to avoid any loss of cells and consequently misinterpretation of data (see this article's Online Repository at www.jacionline.org). Furthermore, 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 $CD14^+$ PBMCs (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 1, *A* and *B*). Because basophils display enhanced expression of various receptors on receiving activation stimuli, we explored whether they express these lectin receptors on activation. However, irrespective of stimulation (IL-3 or degranulation stimuli), basophils remained negative for DC-SIGN and CD206 (Fig 1, *B* and *C*). Absence of DC-SIGN was also confirmed on isolated basophils. Furthermore, the absence of DC-SIGN and CD206 on basophils in our report is not due to nonreactivity of antibodies used in flow cytometry because monocyte-derived DCs, used as positive control, uniformly expressed CD206 and DC-SIGN (Fig 1, *A* and *B*). As expected, $CD14^+$ circulating monocytes, used as negative control, did not stain for both the markers, thus confirming lack of nonspecific binding of antibodies (Fig 1, *A* and *B*).

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

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REFERENCES

1. Sirvent S, Soria I, Cirauqui C, Cases B, Manzano AI, Diez-Rivero CM, et al. Novel vaccines targeting dendritic cells by coupling allergoids to nonoxidized mannan enhance allergen uptake and induce functional regulatory T cells through programmed death ligand 1. J Allergy Clin Immunol 2016;138:558-67.e11.
2. Voehringer D. Protective and pathological roles of mast cells and basophils. Nat Rev Immunol 2013;13:362-75.
3. Oettgen HC. Fifty years later: emerging functions of IgE antibodies in host defense, immune regulation, and allergic diseases. J Allergy Clin Immunol 2016;137:1631-45.
4. Suurmond J, Stoop JN, Rivellese F, Bakker AM, Huizinga TW, Toes RE. Activation of human basophils by combined Toll-like receptor- and FcepsilonRI-triggering can promote Th2 skewing of naive T helper cells. Eur J Immunol 2014;44:386-96.
5. Bates EE, Fournier N, Garcia E, Valladeau J, Durand I, Pin JJ, et al. APCs express DCIR, a novel C-type lectin surface receptor containing an immunoreceptor tyrosine-based inhibitory motif. J Immunol 1999;163:1973-83.
6. Divekar A, Conklin J, Yang X, Ransom J. Role of C-type lectin receptor CLEC12A in human basophil activation (48.6). J Immunol 2012;188: 48.6.
7. Maddur MS, Sharma M, Hegde P, Stephen-Victor E, Pulendran B, Kaveri SV, et al. Human B cells induce dendritic cell maturation and favour Th2 polarization by inducing OX-40 ligand. Nat Commun 2014;5:4092.

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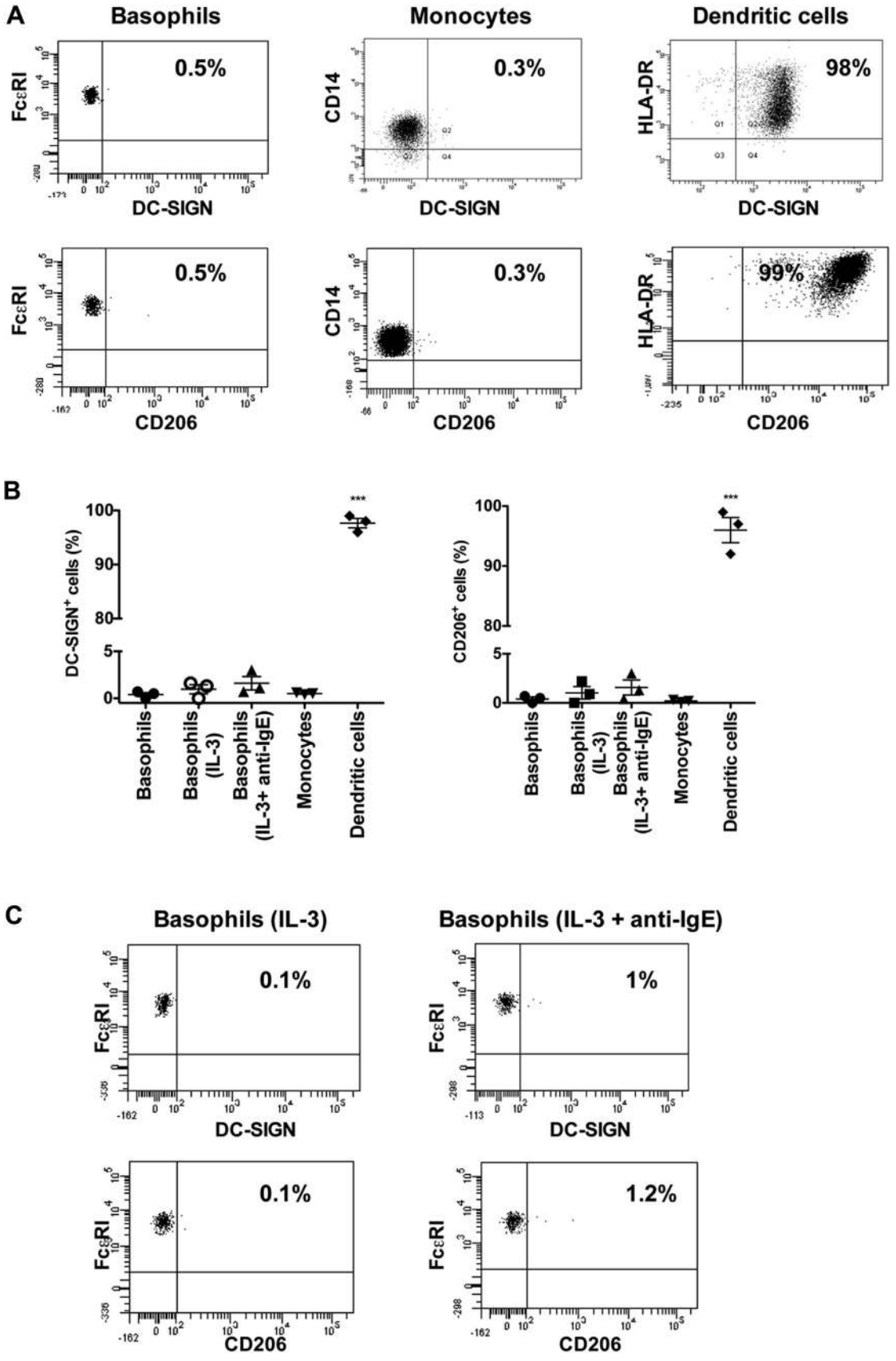

FIG 1. Human basophils are deficient for DC-SIGN and CD206. **A-C,** Flow cytomeric analysis of DC-SIGN and CD206 on steady-state basophils, stimulated basophils (IL-3 or IL-3 and anti-IgE), and monocytes and monocyte-derived DCs. Representative dot-plots and percentage of cells (mean ± SEM, n = 3) positive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

METHODS

Cells and stimulation

Buffy bags of healthy donors were obtained from Centre Necker-Cabanel (EFS, Paris, France), 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 Ammonium-Chloride-Potassium Lysing Buffer (Lonza, Levallois, France). Briefly, blood was span down and resuspended and incubated in Ammonium-Chloride-Potassium lysing buffer for 30 to 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, Friesoythe, Germany) for 24 hours. In addition, cells were cultured with IL-3 (100 ng/million cells) for up to 24 hours and during the last 30 minutes, cells were treated with anti-IgE antibodies (100 ng/million cells; Sigma-Aldrich, St Quentin Fallavier, France). Phenotype of basophils was analyzed in steady state and stimulated conditions by flow cytometry (LSR II, BD Biosciences, Le Pont De Claix, France) and the data were analyzed using FACSDiva software (BD Biosciences).

PBMCs were obtained from buffy bags of healthy donors by Ficoll density gradient centrifugation. Monocytes were isolated from PBMCs by using CD14 microbeads (Miltenyi Biotec, Paris, France) 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 flow cytometry

The following antibodies were used for flow cytometry. FcεRIα-BV510 (Clone AER37 [CRA-1]) was from BioLegend (London, United Kingdom) 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 1-way ANOVA (repeated measures with Tukey's multiple comparison test). A P value of less than .05 was considered significant. Statistical analysis was performed by using Prism 5 GraphPad Software (La Jolla, Calif). Data are presented as mean ± SEM.