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

3

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15 **Key words:** Basophils; DC-SIGN; CD206; dendritic cells; mannose receptor;
16 immunotherapy

17

18 **Abbreviations:**

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

20 DC: dendritic cells

21 DCIR: dendritic cell immunoreceptor

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

24 TLR-Toll-like receptor

25

26 **To the editor,**

27 Sirvent et al., recently showed that novel vaccines targeting dendritic cells (DCs) by
28 coupling glutaraldehyde-polymerized grass pollen allergoids to nonoxidized mannan
29 enhance allergen uptake and induce functional regulatory T cells through programmed
30 death ligand 1.¹ Mechanistically, they found that nonoxidized mannan-coupled
31 glutaraldehyde-polymerized grass pollen allergoids are captured and internalized by
32 two lectin receptors on DCs: mannose receptor (CD206) and DC-specific intercellular
33 adhesion molecule-3-grabbing nonintegrin (DC-SIGN or CD209). These data thus
34 indicated that DCs could be targeted by C-type lectin receptors for efficient allergen
35 immunotherapy.

36 Basophils are one of the key players of allergic responses. They mediate allergic
37 inflammation by secretion of Th2-polarizing cytokines IL-4, IL-13 and by the release
38 of effector molecules like histamines and leukotrienes upon FcεRI signalling by IgE-
39 allergen complexes.² Basophils receive activation signals not only via allergen-IgE
40 complexes³ but also via toll-like receptors (TLRs)⁴ and possibly C-type lectin
41 receptors. In fact, basophils express several lectin receptors like C-type lectin domain
42 family 12 member A (CLEC12A) and dendritic cell immunoreceptor (DCIR).^{5,6} Thus,
43 it is likely that in addition to DCs, nonoxidized mannan-coupled allergoids might also
44 modulate basophil functions to exert immunotherapeutic benefits. Therefore, we
45 analysed the expression of mannose receptor and DC-SIGN on steady state circulating
46 human basophils and on stimulated basophils.

47 We analyzed basophils in whole blood of healthy donors without their purification in
48 order to avoid any loss of cells and consequently misinterpretation of data (see Online
49 Repository at www.jacionline.org). Further, erythrocyte-lysed whole blood cells were
50 stimulated with IL-3 (100 ng/10⁶ cells) for 24 hours. IL-3-stimulated basophils were

51 also stimulated for degranulation with anti-IgE antibodies (100 ng/10⁶ cells) for 30
52 minutes. As controls for the expression of DC-SIGN and CD206, we used CD14⁺
53 peripheral blood monocytes (negative control), and rhIL-4 (500 IU/10⁶ cells) and
54 rhGM-CSF (1000 IU/10⁶ cells)-differentiated monocyte-derived DCs (positive
55 control).⁷

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

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

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129 polarization by inducing OX-40 ligand. *Nat Commun.* 2014;5:4092.

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131 **Figure Legend**

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

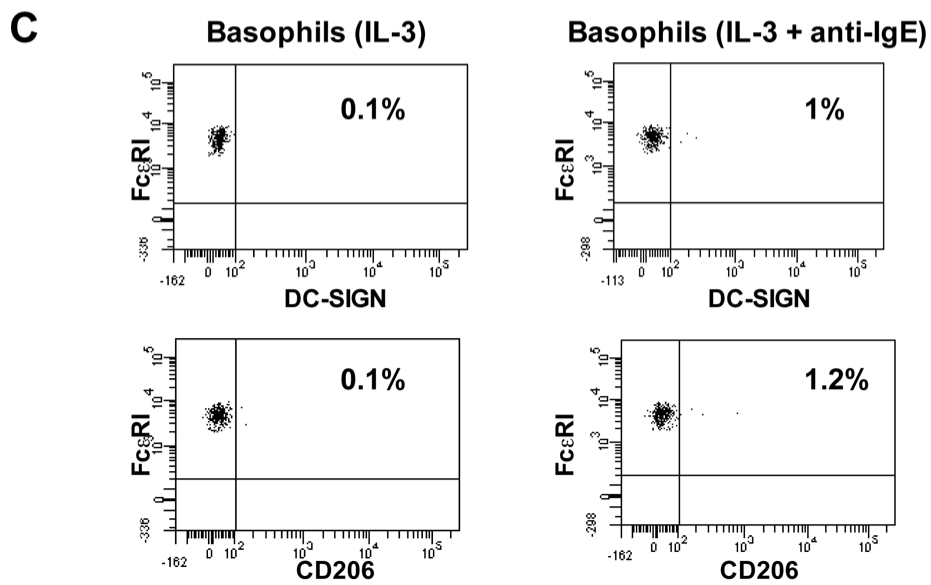
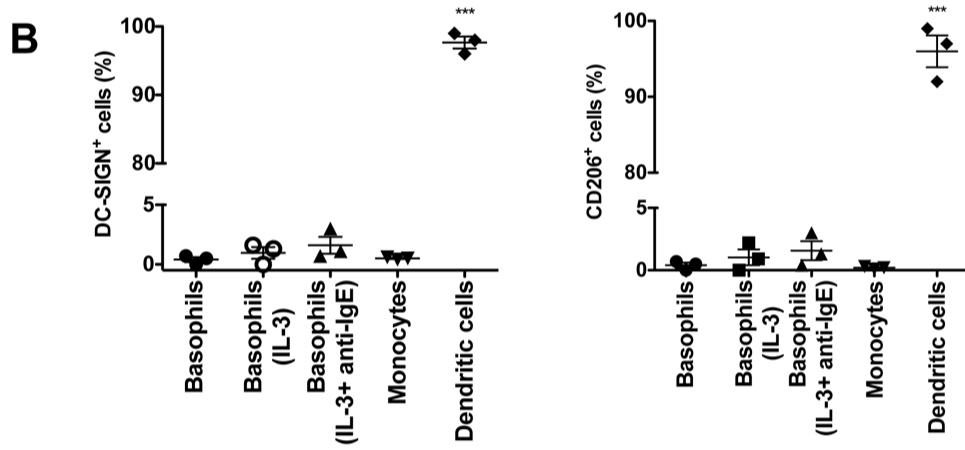
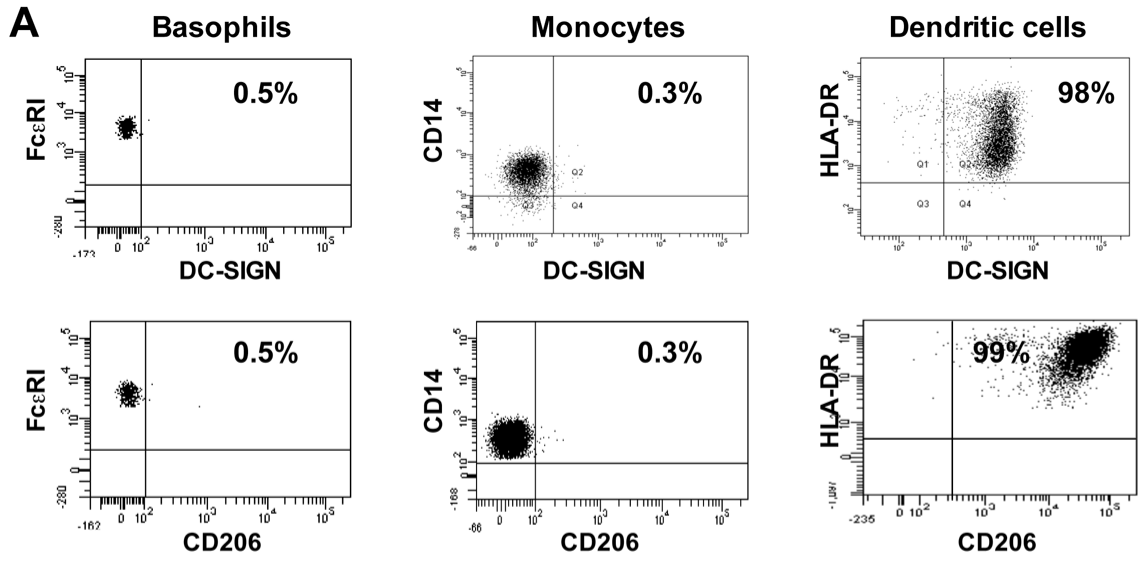
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Online Repository

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

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receptor

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169 **METHODS**

170 **Cells and stimulation**

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

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

185 Peripheral blood mononuclear cells (PBMCs) were obtained from buffy bags of
186 healthy donors by Ficoll density gradient centrifugation. Monocytes were isolated
187 from peripheral blood mononuclear cells by using CD14 microbeads (Miltenyi
188 Biotec) and were cultured for 5 days in rhIL-4 (500 IU/10⁶ cells) and rhGM-CSF
189 (1000 IU/10⁶ cells) (both from Miltenyi Biotec) to obtain monocyte-derived DCs.

190

191 **Antibodies for the flow cytometry**

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

194 17F6) was obtained from Miltenyi Biotec. CD123-BV421 (Clone: 9F5), CD209-FITC
195 (Clone: DCN46), CD206-PE (Clone: 19.2), HLA-DR-APC or PE (G46-6, BD
196 Biosciences) and CD14-APC (Clone: M5E2) antibodies were from BD Biosciences

197

198 **Statistical analysis**

199 Levels of significance for comparison between samples were determined by One-way
200 analysis of variance (repeated measures with Tukey's multiple comparison test).

201 $P < 0.05$ was considered significant. Statistical analysis was performed by Prism 5

202 GraphPad Software. Data are presented as mean \pm SEM.

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