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1       **CHARACTERIZATION OF THE PHENOTYPE AND FUNCTION OF MONOCYTE-**  
2       **DERIVED DENDRITIC CELLS IN ALLERGIC CONJUNCTIVA**

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21       assay.

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25 **ABSTRACT**

26

27 **Background:** Dendritic cells (DCs) are the most potent antigen-presenting cells involved in  
28 initiating the immune response, presenting antigens to T cells and leading to T cell proliferation.

29 In an immature state, DCs lack accessory signals required for T cell stimulation but are highly  
30 specialized to capture antigens. Full DC maturation changes the cell surface phenotype and  
31 facilitates stimulation of T cell proliferative responses. To examine the degree of DC maturity  
32 associated with vernal keratoconjunctivitis (VKC), we examined the phenotype and antigen-  
33 presentation capability of blood derived DCs from VKC patients and from normal controls.

34 **Methods:** Flow cytometry was used to identify the cell surface expression of markers of DC  
35 maturity (CD83, CD86, MHC class II) and mixed leukocyte reactions to assess DC induction of  
36 T cell proliferation.

37 **Results:** DCs derived from VKC patients were of more mature phenotype than those from  
38 normal controls. However, these VKC DCs had reduced capability for induction of T cell  
39 proliferation compared to DCs from controls.

40 **Conclusion:** The increased maturity of DCs in VKC patients correlates with the heightened  
41 immune responsiveness associated with this disorder. A number of mechanisms may underlie  
42 the impaired ability of DCs in atopy to stimulate T cell proliferation. This impairment of DC  
43 induction of T cell activation is likely to be one factor which contributes to the modified  
44 inflammatory response seen in VKC patients and the recognised susceptibility of these patients  
45 to viral infection.

46

## 47 INTRODUCTION

48           Approximately 20% of the Western world suffers from allergic diseases, such as allergic  
49 rhinoconjunctivitis, allergic asthma and the atopic asthma / dermatitis syndrome.[1] DCs are the  
50 sentinel cells that ensure that an immune response is appropriate in specificity, intensity, and  
51 type to the nature of the threat posed by the antigen bearing entity. DCs are divided into 2 major  
52 subtypes in human: the myeloid DCs (including Langerhan's cells (LCs)) and plasmacytoid DCs.  
53 DCs are located in most tissues where they capture and process antigens, and where they exist  
54 in an 'immature' state: they lack the signals required for T cell stimulation but they are highly  
55 specialized to capture antigens. Following encounter with and uptake of an antigen, DCs  
56 process this antigen into smaller fragments, couple it to the major histocompatibility complex  
57 (MHC) class II molecule and present it on their surface to T cells. Additionally, a second co-  
58 stimulatory signal is also required to initiate T cell proliferation.[2] DCs that display the MHC  
59 class II molecule and the co-stimulatory molecules (CD83, CD86) on their surface are  
60 considered to be mature.

61           KleinJan *et al.* [3] studied nasal mucosal biopsy specimens taken from patients with  
62 perennial allergic rhinitis (AR) and found that the number of DCs in the nasal mucosa was  
63 higher and displayed a more mature phenotype in symptomatic patients than in healthy controls.  
64 This increase in LCs of the nasal mucosa was also observed during the pollen season in work  
65 done by Till *et al.* [4]. Allam *et al.* [5] performed detailed phenotypical comparison of nasal and  
66 oral myeloid DC (mDC) from atopic and non-atopic donors. In atopic donors, these mDCs  
67 showed elevated expression of the high affinity receptor for IgE (FcεRI) with receptor occupation  
68 by IgE but FcεRI was present only in low amounts in these cells in non-atopic donors. Semper  
69 *et al.* [6] studied the phenotype of DCs on bronchial biopsy specimens from normal and  
70 asthmatic airways and demonstrated the presence of FcεRI-bearing CD1a<sup>+</sup> LCs in both types of  
71 specimens. Total expression of FcεRI on all cells was slightly higher in the airways of  
72 asthmatics compared to normal controls, but this failed to reach statistical significance. They did

73 find—in keeping with other studies [7, 8]—that the numbers of CD1a<sup>+</sup> DCs was higher in  
74 asthmatics when compared to normal controls.

75         We have previously demonstrated that the number of DCs expressing FcεRI in the  
76 substantia propria of the conjunctiva in the atopic disorder vernal keratoconjunctivitis (VKC)  
77 patients is increased, indicating an increased ability of DCs to capture and process specific  
78 antigens for presentation to CD4<sup>+</sup> T cells, thereby initiating the immune response.[9] In this  
79 study, we sought to further define the phenotypic and functional characteristics of DCs  
80 associated with VKC. We examined DCs derived from the peripheral blood of VKC patients and  
81 normal patients, testing the hypothesis that DCs in VKC are more mature, and have increased  
82 capacity for T cell proliferation.

83

## 84 **METHODS**

### 85 **Selection of donors**

86         Donors had a clinical history and signs of VKC, and were selected from patients  
87 attending clinic follow-up appointments. The control group donors did not have any history of  
88 atopy and did not display any signs of ocular allergy (table 1). A difference did exist between  
89 the mean age of the donors in the VKC group compared to the control group but this was  
90 unavoidable and was consequent to the difficulties in obtaining blood from children for the  
91 control group and in adequate volume quantities to perform the experiments. Local ethics  
92 committee approval was obtained for the research and each patient gave written informed  
93 consent. All research procedures adhered to the guidelines established in the Declaration of  
94 Helsinki.

95

96

97

98

99 **Table 1: Background patient information.**

Patients for flow cytometry study							
Control subjects				VKC patients			
Patient number	Age (yr)	Sex	IgE level (kU/l)	Patient number	Age (yr)	Sex	IgE level (kU/l)
1	47	F	18	1	17	F	911
2	27	F	11	2	8	M	87
3	47	F	17	3	12	M	210
4	28	F	21	4	31	M	60
5	46	F	21	5	10	M	150
6	39	F	32	6	14	M	96
7	29	F	15	7	37	F	81
8	49	M	44	8	7	M	120
9	47	F	334	9	11	M	170
10	28	M	257	10	8	M	375
11	23	F	254	11	13	M	62
12	28	M	129	12	12	M	153
13	30	M	203				
Patients for MLR assays							
Control subjects				VKC patients			
Patient number	Age (yr)	Sex	IgE level (kU/l)	Patient number	Age (yr)	Sex	IgE level (kU/l)
1	41	M	11	1	10	M	80
2	21	F	31	2	29	M	16
3	50	M	13	3	13	M	69
4	42	F	18	4	9	M	216
5	49	F	37	5	31	M	10
6	38	F	9	6	14	F	42
7	21	F	212	7	11	F	48
8	25	F	55				

100

101 **Measurement of IgE concentration**

102 Serum (6 ml) was collected for measurement of IgE levels by a normal sandwich  
 103 enzyme-linked immunosorbent assay (ELISA; ImmunoCAP 100, Phadia, Uppsala, Sweden).

104 **MoDC isolation and culture**

105           Peripheral blood mononuclear cells were isolated from EDTA-treated blood using  
106 Lymphoprep (Sigma-Aldrich, Poole, United Kingdom) centrifugation. Isolation of CD14<sup>+</sup>  
107 monocytes from the resultant buffy coat layer was performed using anti-CD14 mAb conjugated  
108 to magnetic MicroBeads and the autoMACS system as described in the manufacturer's  
109 instructions (Miltenyi Biotech, Bergisch Gladbach, Germany). The derived pellet of purified  
110 monocytes was cultured into dendritic cells as per the method described by Sallusto and  
111 Lanzavecchia.[10] To generate monocyte-derived DCs (MoDCs), IL-4 (4 µg/ml, R & D systems,  
112 Abingdon, United Kingdom) and GM-CSF (6 µg/ml, GlaxoSmithKline Research Institute,  
113 Stevenage, United Kingdom) were added to each well of purified monocytes.

114 **Flow cytometry**

115           On day 6 or 7 the MoDC cultures from each well were combined for fluorescent staining.  
116 The cells were blocked for 10 minutes with human Fcγ fragments (Miltenyi Biotech) and then  
117 incubated on ice with the appropriate fluorescein isothiocyanate (FITC)- or phycoerythrin (PE) -  
118 conjugated antibody or with the respective isotype-matched control immunoglobulins.

119           Monoclonal antibodies directed against CD83 (FITC, clone HB15e), CD11c (PE, clone  
120 B-ly6), CD86 (FITC, clone 2331), HLA-DR (FITC, clone Tü39), CD23 (PE, clone M-L233), CD58  
121 (PE, clone 1C3) and CD11c (PE, clone D12) were purchased from Becton Dickinson (Cowley,  
122 United Kingdom). The antibody against FcεRI-α (PE, clone AER-37) was purchased from  
123 eBioscience (Insight Biotechnology Ltd, Wembley, United Kingdom). FITC- and PE-conjugated  
124 isotype control mouse immunoglobulins (Igs; IgG<sub>1</sub>, IgG<sub>2a</sub>, IgG<sub>2b</sub>,) were obtained from Becton  
125 Dickinson and eBioscience. Additional FACS staining of T cells was performed using CD3  
126 (FITC, clone HIT3a) (BD Pharmingen) and CD209 (DC-SIGN, PE, clone eB-h209, eBioscience).  
127 The αCD28/αCD3 (GlaxoSmithKline, Stevenage, United Kingdom) was used as a positive  
128 control.

129 Events were acquired using the Beckman Coulter flow cytometry machine and analyzed  
130 with Expo32 (Beckman Coulter Ltd, High Wycombe, United Kingdom). Cells were acquired and  
131 manually gated on the physical properties of the cell by FSC versus SSC. The measurements  
132 taken were the mean fluorescence intensity (MFI) and the percentage of positive cells as  
133 defined by the proportion expressing higher levels of fluorescence than those of the isotype  
134 controls.

### 135 **T cell isolation and culture**

136 T cells were isolated and purified from the buffy coat following layering of blood over  
137 Lymphoprep (Sigma-Aldrich) and centrifugation. The T cells were isolated from the resultant  
138 PBMC cell pellet using the pan T cell isolation kit II from Miltenyi Biotech (negative selection)  
139 according to the manufacturer's instructions. T cell purity was always greater than 99%.

### 140 **Mixed leukocyte reaction (MLR) assays**

141 MoDCs were harvested on day 6 and purity of population assessed with flow cytometry  
142 for CD11c +/- CD209. The MoDCs were washed and resuspended in T cell medium at a  
143 concentration of  $1 \times 10^4$  DCs/100  $\mu$ l T cell medium. In all allogenic MLR assays, T cells from the  
144 same donor were used, thereby maintaining consistency between the MLR results obtained  
145 from the normal control MoDCs and the VKC-derived MoDCs. Each of the MLRs for each  
146 patient was performed in triplicate at five different concentrations of MoDC to T cells (1:10, 1:20,  
147 1:40, 1:80 and 1:160). Graded doses of MoDC were added to T cells in 96-well U-bottom tissue  
148 culture plates (200  $\mu$ l/well)—the T cell number remained constant whilst serial dilution of MoDCs  
149 was performed to obtain the appropriate DC:T cell ratios for the MLR assays. The control wells,  
150 consisted of two sets of negative controls ( $1 \times 10^4$  MoDC only in T cell medium and  $1 \times 10^5$  T  
151 cells in T cell medium) and one positive control ( $1 \times 10^5$  T cells only in T cell medium in wells  
152 lined with  $\alpha$ CD28/ $\alpha$ CD3). The plates were incubated for 5 days at 37°C in 5% CO<sub>2</sub>. T cell

153 proliferation was quantified by incubating the cells with 1  $\mu$ Ci of methyl-titrated thymidine  
154 deoxyriboside (10  $\mu$ l; Amersham Life Sciences, Buckinghamshire, United Kingdom) during the  
155 last 16 hours of the 5-day culture. Cells were harvested on a filter and radioactivity measured in  
156 a beta-plate liquid scintillation counter.

## 157 **Statistical analysis**

158 The Mann Whitney U test was used to determine if there was significant up/down  
159 regulation of the measured cell surface markers when comparing VKC MoDC to those derived  
160 from control subjects and to determine if the proliferation of T cells observed between VKC and  
161 control subjects at the different ratios tested was significant. A value of  $p \leq 0.05$  was considered  
162 to be significant.

163

## 164 **RESULTS**

### 165 **Flow cytometric analysis of circulating DC phenotype**

166 MoDCs were isolated from the peripheral blood of 12 VKC patients and 13 normal  
167 controls. For each of the control group donors, a yield of 3 – 16.2 x 10<sup>6</sup> MoDCs was obtained  
168 after culture as compared with the VKC donors where a yield of 0.5 – 13.2 x 10<sup>6</sup> MoDCs was  
169 obtained. No morphological differences were readily identifiable between the two groups. DCs  
170 were identified as staining brightly for CD11c, the widely-used marker for human myeloid DCs.  
171 The cells were gated on their FSC and SSC properties with the MoDCs within the gate being >  
172 95% CD11c<sup>+</sup> (figure 1, (a)).

173 MoDCs of the control group individuals displayed constitutive cell surface expression of  
174 MHC class II, low expression of CD86, and no expression of CD83, a typical profile for DCs in  
175 the immature state. In MoDCs from VKC patients in comparison, there was a statistically  
176 significant upregulation of MHC class II, CD83 and CD86 (figure 1 (b) and table 2), an  
177 expression profile consistent with a more-mature state of development.

178 The cell surface adhesion proteins CD58, CD11b, and CD11c were constitutively  
 179 expressed in the control MoDCs (figure 1 (c) and table 2). Of the two IgE receptors, only CD23  
 180 was constitutively expressed on control MoDCs, and little or no expression of FcεRI was  
 181 detected on these cells. There was statistically insignificant upregulation of surface FcεRI on  
 182 VKC-derived MoDCs, but significant upregulation of CD11b, CD11c and CD58. CD23 was  
 183 down-regulated in VKC-derived MoDCs (p = 0.02).

184

185 **Table 2: The mean fluorescent intensity (MFI) recorded for each marker measured during flow**  
 186 **cytometric analysis. \* Denotes a statistically significant result.**

	Control subject MFI			VKC patient MFI			Fold change	p value
	Mean	SD	Range	Mean	SD	Range		
<b>MHC class II</b>	40.97	14.01	20.8-66.9	71.41	29.70	14.4-122	1.74	<0.01 *
<b>CD86</b>	2.42	1.85	0.45-5.68	5.98	3.40	1.71-11.7	2.47	<0.01 *
<b>CD83</b>	0.34	0.09	0.2-0.48	0.68	0.15	0.46-0.98	2.00	<0.001 *
<b>CD58</b>	4.18	2.59	1.97-12	9.28	3.17	4.77-15.6	2.22	<0.001 *
<b>CD11c</b>	7.86	4.29	3.63-18	12.09	6.77	5.07-30.7	1.54	0.05 *
<b>CD11b</b>	76.92	33.56	38.7-152	111.05	48.28	40.4-192	1.44	0.05 *
<b>CD23</b>	6.26	3.78	3.03-15.4	5.55	11.37	0.94-41.5	-1.13	0.02 *
<b>FcεRI</b>	0.52	0.24	0.27-0.92	0.54	0.18	0.23-0.91	1.04	0.77

187

188 **Functional analysis of circulating DCs in VKC patients**

189 Having determined that VKC patient-derived MoDCs had altered cell surface marker  
190 profiles compared to the controls, we then examined these cells for altered function by testing T  
191 cell stimulatory capacity. The responder T cells used were from a subject without any history of  
192 allergy or medication, and an IgE level of 61 kU/l (table 1).

193 For each of the control group donors, a yield of  $2.4 - 7.5 \times 10^6$  MoDCs was obtained  
194 after culture as compared with a yield of  $8 - 11 \times 10^5$  MoDCs for the VKC donors. No  
195 morphological differences were observed between the MoDCs from the VKC and the control  
196 donors. The MoDCs were labelled with anti-CD11c-PE and the T cells with anti-CD3-FITC. The  
197 yield of CD11c<sup>+</sup> cells from both normal and VKC cultured MoDC populations was >90% (figure 2  
198 (a)), whereas the yield of T cells from the T cell donor was consistently >99% pure (figure 2 (b)).

199 As expected, MoDCs from both the control group and VKC patients stimulated T cell  
200 proliferation. The mitogenic effect was statistically significantly greater with MoDCs from control  
201 group individuals than with MoDCs from VKC patients (figure 2 (c)) and was observed for all  
202 ratios of MoDC to T cells that were tested. Since all T cells used were from the same donor, the  
203 observed differences are likely to be explained by differing stimulatory capacity of MoDCs in the  
204 two groups. In a trial MLR using MoDCs derived from a non-atopic donor outside of our group of  
205 experimental subjects and T cells from the same donor as for the experiments described in this  
206 study, adequate proliferative responses were observed (data not shown). This effect on  
207 stimulation is therefore not limited to the patients in our control group.

208

209 **DISCUSSION**

210 **Phenotypic alterations in MoDCs derived from VKC patients**

211 The MoDC derived from VKC patients are a more mature phenotype than those from  
212 control subjects as indicated in the statistically significant upregulation of MHC class II and the

213 co-stimulatory molecules CD86 and CD83. This finding is comparable to those of McCarthy *et*  
214 *al.* [11] who found that DCs in atopic asthma patients expressed higher levels of CD80 and  
215 CD86. The upregulation of MHC class II and co-stimulatory molecules is likely to increase the  
216 antigen presenting capacity of DCs in VKC patients. CD83 expression on the cell surface is a  
217 marker of DC maturation.[12] Zhou and Tedder have demonstrated that most CD83<sup>+</sup> cells  
218 express high levels of CD86;[13] the VKC MoDCs in this study displayed upregulation of both  
219 molecules. As CD86 is upregulated in respiratory tract DCs and in circulating blood B cells in  
220 allergic asthma [11, 14] and on LC's in the conjunctiva of patients with VKC,[15] it is clear that  
221 this co-stimulatory molecule may contribute to the T-cell driven pathology of VKC.

222 Adhesion proteins upregulated on VKC MoDCs included CD58, CD11c and CD11b.  
223 These molecules facilitate the interaction of DCs with T cells and influence the ability of DCs to  
224 migrate to regional lymph nodes upon maturation.

### 225 **Expression of FcεRI on MoDCs**

226 DCs can express the high-affinity IgE receptor FcεRI, which, in the presence of specific  
227 IgE, facilitates the uptake of allergen, leading to increased activation of allergen-specific T cells.  
228 We found that MoDCs from VKC patients and normal subjects displayed similar levels of FcεRI  
229 expression. These results are consistent with those of Holloway *et al.*,[16] who found that the  
230 total expression of FcεRI on the surface of DCs isolated from the peripheral blood of healthy  
231 and asthmatic subjects were not significantly different. Other studies have associated elevated  
232 levels of DC FcεRI with atopic conditions.[17, 18] There are at least three possible explanations  
233 for the results in our study. First, the mature state of the VKC MoDCs may have led to  
234 downregulation of the FcεRI receptor: upon *in vitro* maturation, LCs completely and irreversibly  
235 downregulate their transcripts for FcεRI subunits, leading to a rapid loss of intracellular and  
236 surface receptor expression.[19] Second, although a direct correlation between serum IgE  
237 levels and expression of FcεRI on human basophils from allergic subjects has been shown,[20]

238 this control of FcεRI expression may not apply to DCs, since FcεRI on these cells exists as a  
239 trimeric complex lacking the β subunit, in contrast to the tetrameric form expressed by mast  
240 cells and basophils . Finally, DCs in different tissues may have different phenotypes which may  
241 be influenced by the microenvironment. Indeed we have found increased FcεRI expression in  
242 conjunctiva in allergic conjunctivitis.[9]

### 243 **Mechanisms underlying reduced T cell proliferation in response to VKC MoDCs**

244 Mature DCs are better able to prime T cells than immature DCs, an important early step  
245 in cell-mediated immunopathology. We found the expected potent T cell proliferation in  
246 response to MoDCs derived from control subjects, but it was surprising to find that our mature  
247 VKC MoDCs were *less* effective at stimulating T cell proliferation. This, however, is not the first  
248 report of a reduction in T cell function in atopic disease—the literature in support of this finding  
249 is well summarized in the review article by Strannegård and Strannegård.[21] In the setting of  
250 allergic disorders, this reduced capacity to cause T cell proliferation might be one reason why  
251 those with allergic disease are so prone to viral and fungal infections, in particular herpes  
252 simplex virus infection. Upon presentation of viral antigen from virus-infected cells it may be that  
253 the ineffective ability of DCs to stimulate T cell proliferation leads to impaired clearance of viral  
254 infection in atopic patients.

255 This is the first study to show a functional difference between the MoDCs derived from  
256 VKC patients and normal control subjects who had no known history of allergy. There are  
257 several possible explanations for reduced T cell-stimulating activity. The proliferative signal  
258 presented by the MoDCs to the T cells might be defective or insufficient. Indeed, the ‘strength of  
259 stimulation’ model of T cell activation and differentiation is based on the fact that naïve T cells  
260 interacting with DCs receive stimulatory signals of differing strengths, depending on the degree  
261 of DC maturation, the level of peptide-MHC and co-stimulatory molecule interaction, and the  
262 duration of T cell-DC interaction.[22] According to this model, T cells receiving a signal of

263 excessive strength undergo activation-induced cell death. The MoDCs from VKC patients, being  
264 in a more mature state of differentiation, may deliver a signal of excessive strength to the T  
265 cells, thereby causing cell death and a reduced proliferative response. Alternatively, inhibitory  
266 signals (DC-derived immunoglobulin receptor 2 (DigR2), cytotoxic T lymphocyte-associated  
267 receptor 4 (CTLA-4), programmed death-1 (PD-1), and DC-associated heparan sulfate  
268 proteoglycan-dependent integrin (DC-HIL)) presented or secreted by the VKC MoDCs hinder an  
269 adequate proliferative response from T cells. Finally, the difference in MoDC stimulatory  
270 capacity might be due to the differences in age and blood volumes between the donor groups,  
271 unavoidable limitations of this study. The normal control patients were older, on average, than  
272 the VKC patients, and the blood volumes withdrawn from the normal patients were higher than  
273 for the VKC patients.

274 In conclusion, we have shown that MoDCs derived from circulating monocytes in VKC  
275 individuals display a more mature phenotype than their normal counterparts, as evidenced by  
276 enhanced expression of MHC II, CD86, CD83, CD58, CD11c and CD11b. Despite this  
277 phenotypic maturity, the VKC MoDCs are less-potent T cell stimulators - and potentially less-  
278 effective APCs - than their normal counterparts. The impaired T cell proliferation may contribute  
279 to the abnormal inflammatory response, not least to viral or fungal infection, seen in patients  
280 with VKC and other allergic disorders.

281

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295 **REFERENCE LIST**

296

297 1. von BD, Koch S, Bieber T. Dendritic cells and atopic eczema/dermatitis syndrome.

298 *Curr.Opin.Allergy Clin.Immunol.* 2003;**3**:353-8.

299 2. Kleijmeer MJ, Ossevoort MA, van Veen CJ, van Hellemond JJ, Neefjes JJ, Kast WM et

300 al. MHC class II compartments and the kinetics of antigen presentation in activated

301 mouse spleen dendritic cells. *J.Immunol.* 1995;**154**:5715-24.

302 3. KleinJan A, Willart M, van Rijt LS, Braunstahl GJ, Leman K, Jung S et al. An essential

303 role for dendritic cells in human and experimental allergic rhinitis. *J.Allergy Clin.Immunol.*

304 2006;**118**:1117-25.

305 4. Till SJ, Jacobson MR, O'Brien F, Durham SR, KleinJan A, Fokkens WJ et al.

306 Recruitment of CD1a+ Langerhans cells to the nasal mucosa in seasonal allergic rhinitis

307 and effects of topical corticosteroid therapy. *Allergy* 2001;**56**:126-31.

308 5. Allam JP, Niederhagen B, Bucheler M, Appel T, Betten H, Bieber T et al. Comparative

309 analysis of nasal and oral mucosa dendritic cells. *Allergy* 2006;**61**:166-72.

310 6. Semper AE, Hartley JA, Tunon-De-Lara JM, Bradding P, Redington AE, Church MK et

311 al. Expression of the high affinity receptor for immunoglobulin E (IgE) by dendritic cells in

312 normals and asthmatics. *Adv.Exp.Med.Biol.* 1995;**378**:135-8.

313 7. Bellini A, Vittori E, Marini M, Ackerman V, Mattoli S. Intraepithelial dendritic cells and

314 selective activation of Th2-like lymphocytes in patients with atopic asthma. *Chest*

315 1993;**103**:997-1005.

- 316 8. Fokkens WJ, Bruijnzeel-Koomen CA, Vroom TM, Rijntjes E, Hoefsmit EC, Mudde GC et  
317 al. The Langerhans cell: an underestimated cell in atopic disease. *Clin.Exp.Allergy*  
318 1990;**20**:627-38.
- 319 9. Manzouri B, Ohbayashi M, Leonardi A, Larkin DF, Ono SJ. Characterization of dendritic  
320 cell phenotype in allergic conjunctiva: increased expression of Fc $\epsilon$ RI, the high-  
321 affinity receptor for immunoglobulin E. *Eye* 2008.
- 322 10. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human  
323 dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus  
324 interleukin 4 and downregulated by tumor necrosis factor alpha. *J.Exp.Med.*  
325 1994;**179**:1109-18.
- 326 11. McCarthy NE, Jones HA, Marks NA, Shiner RJ, Ind PW, Al-Hassi HO et al. Inhaled  
327 allergen-driven CD1c up-regulation and enhanced antigen uptake by activated human  
328 respiratory-tract dendritic cells in atopic asthma. *Clin.Exp.Allergy* 2007;**37**:72-82.
- 329 12. Zhou LJ, Tedder TF. CD14+ blood monocytes can differentiate into functionally mature  
330 CD83+ dendritic cells. *Proc.Natl.Acad.Sci.U.S.A* 1996;**93**:2588-92.
- 331 13. Zhou LJ, Tedder TF. Human blood dendritic cells selectively express CD83, a member  
332 of the immunoglobulin superfamily. *J.Immunol.* 1995;**154**:3821-35.
- 333 14. Hofer MF, Jirapongsananuruk O, Trumble AE, Leung DY. Upregulation of B7.2, but not  
334 B7.1, on B cells from patients with allergic asthma. *J.Allergy Clin.Immunol.* 1998;**101**:96-  
335 102.

- 336 15. Abu-El-Asrar AM, Al-Kharashi SA, Al-Mansouri S, Missotten L, Geboes K. Langerhans'  
337 cells in vernal keratoconjunctivitis express the costimulatory molecule B7-2 (CD86), but  
338 not B7-1 (CD80). *Eye* 2001;**15**:648-54.
- 339 16. Holloway JA, Holgate ST, Semper AE. Expression of the high-affinity IgE receptor on  
340 peripheral blood dendritic cells: differential binding of IgE in atopic asthma. *J.Allergy*  
341 *Clin.Immunol.* 2001;**107**:1009-18.
- 342 17. Novak N, Bieber T, Kraft S. Immunoglobulin E-bearing antigen-presenting cells in atopic  
343 dermatitis. *Curr.Allergy Asthma Rep.* 2004;**4**:263-9.
- 344 18. Semper AE, Heron K, Woollard AC, Kochan JP, Friedmann PS, Church MK et al.  
345 Surface expression of Fc epsilon RI on Langerhans' cells of clinically uninvolved skin is  
346 associated with disease activity in atopic dermatitis, allergic asthma, and rhinitis.  
347 *J.Allergy Clin.Immunol.* 2003;**112**:411-9.
- 348 19. Bieber T. Fc epsilon RI on antigen-presenting cells. *Curr.Opin.Immunol.* 1996;**8**:773-7.
- 349 20. MacGlashan D, Jr., McKenzie-White J, Chichester K, Bochner BS, Davis FM, Schroeder  
350 JT et al. In vitro regulation of Fc epsilon RI alpha expression on human basophils by IgE  
351 antibody. *Blood* 1998;**91**:1633-43.
- 352 21. Strannegard O, Strannegard IL. T lymphocyte numbers and function in human IgE-  
353 mediated allergy. *Immunol.Rev.* 1978;**41**:149-70.
- 354 22. Singh NJ, Schwartz RH. The strength of persistent antigenic stimulation modulates  
355 adaptive tolerance in peripheral CD4+ T cells. *J.Exp.Med.* 2003;**198**:1107-17.

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## 358 **Figure & Table Legends**

359 **Figure 1:** Flow cytometric analysis of **(a)** MoDCs cultured for analysis of CD11c surface marker.  
360 The FACS pattern was generated based on cells that were gated ('A'), and confirms their  
361 phenotype as CD11c+. The same methodology was used for characterization of normal MoDCs  
362 and VKC MoDCs. **(b)** and **(c)** Staining for surface markers labelled with FITC and PE  
363 respectively. Expression of each of these markers was determined by flow cytometry and is  
364 represented in the form of histograms. A shift of the curve to the right in comparison with the  
365 isotype control indicates surface expression of this marker. In **(b)** MoDCs derived from normal  
366 control and VKC patients were labelled with FITC-labelled antibodies against MHC class II,  
367 CD83 and CD86. The histograms show significant upregulation of the DC surface markers MHC  
368 class II, CD86 and CD83 in VKC subjects compared to normal control subjects; in **(c)** MoDCs  
369 derived from normal control and VKC patients were labelled with PE-labelled antibodies against  
370 CD11b, CD11c, CD23, CD58 and FcεRI. Significantly higher expression of CD11c, CD11b and  
371 CD58 was found on VKC-derived cells with significantly lower expression of the CD23 receptor  
372 but not of FceRI. (\* denotes  $p < 0.05$ .)

373

374 **Figure 2:** Flow cytometric analysis of MoDCs and T cells for MLRs. CD11c labelling **(a)** of cells  
375 derived from normal control and VKC patients that were gated ('A') indicated DC phenotype.  
376 CD3 labelling **(b)** of isolated lymphocytes that were gated ('A') confirmed T cell phenotype. **(c)**  
377 Allogeneic T cell proliferation stimulated by MoDCs derived from normal control and VKC  
378 patients. Donor T cells tested were identical for the MLRs involving control MoDCs and VKC  
379 MoDCs. T cells were co-cultured with MoDC for 5 days. Triplicate assays were performed at  
380 each MoDC:T cell ratio. Results shown indicate mean of triplicate assays for each patient and  
381 then mean of all patients at that ratio. Error bars indicate standard deviation. At all cell ratios

382 tested, higher T cell proliferation, as determined by thymidine incorporation, was found in  
383 response to incubation with MoDCs from normal control subjects than from VKC patients.

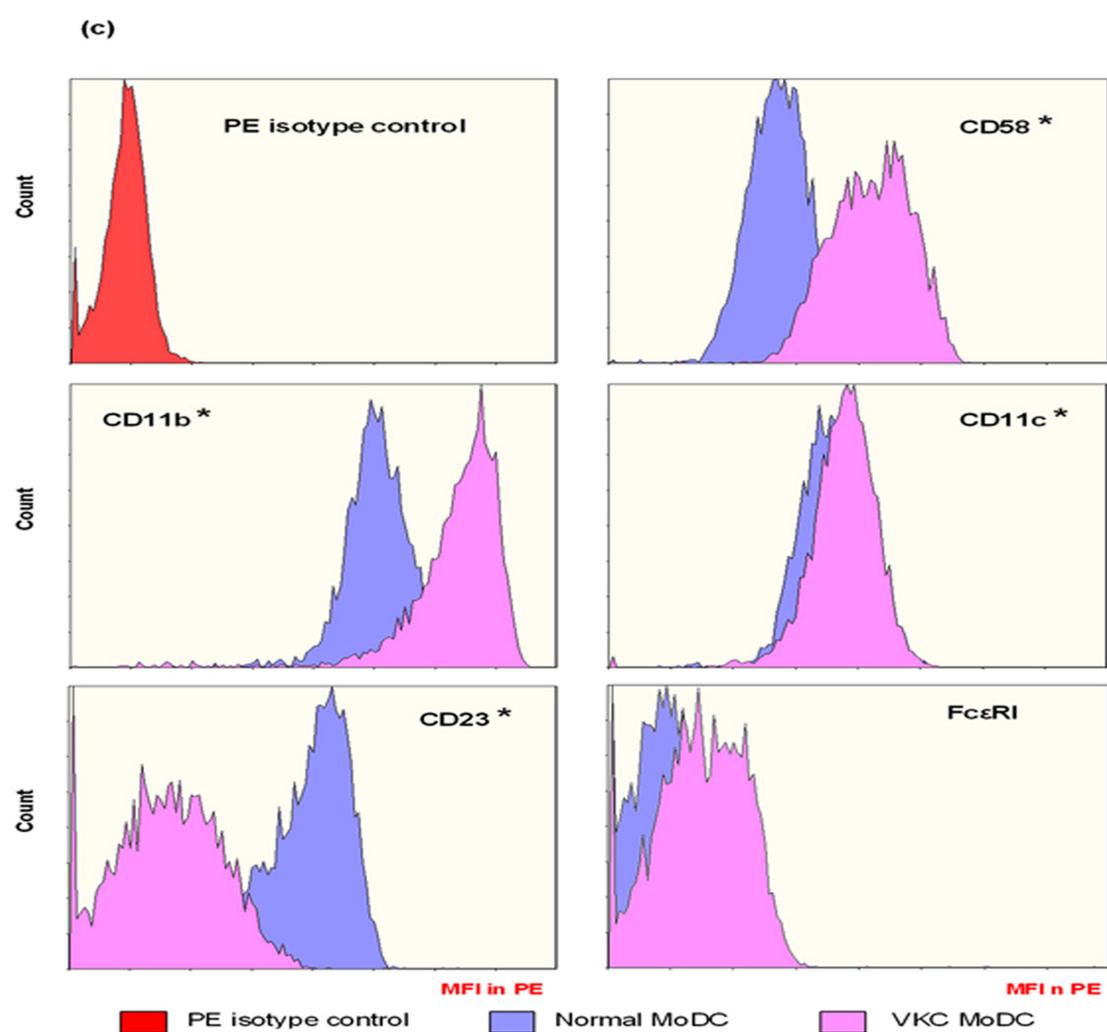
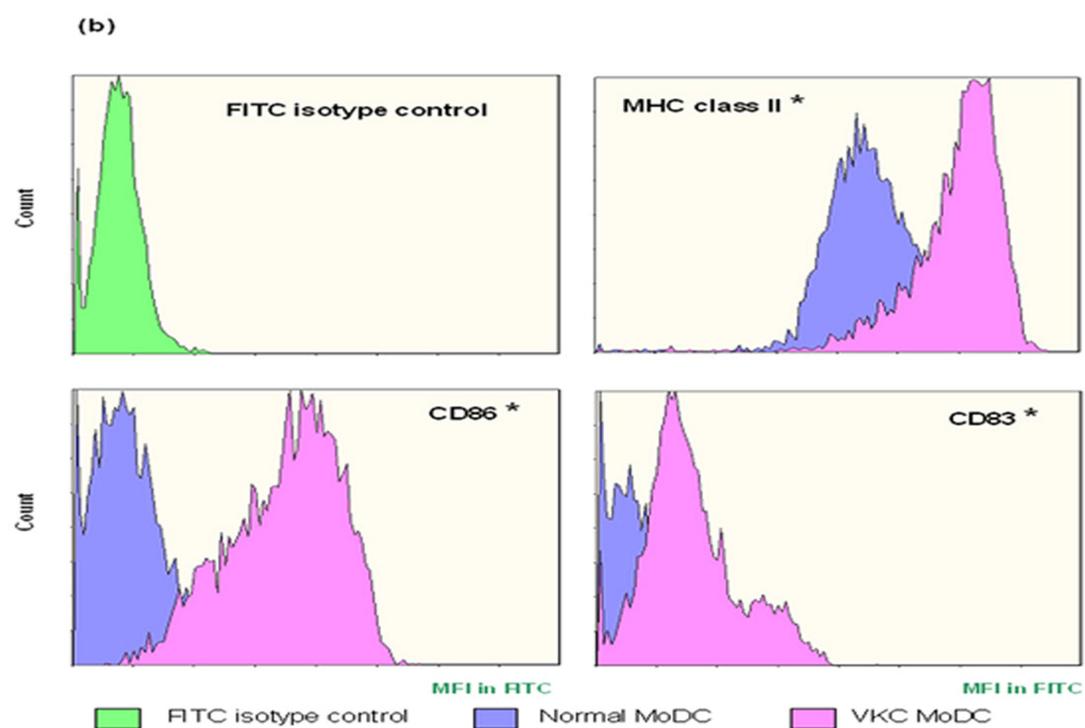
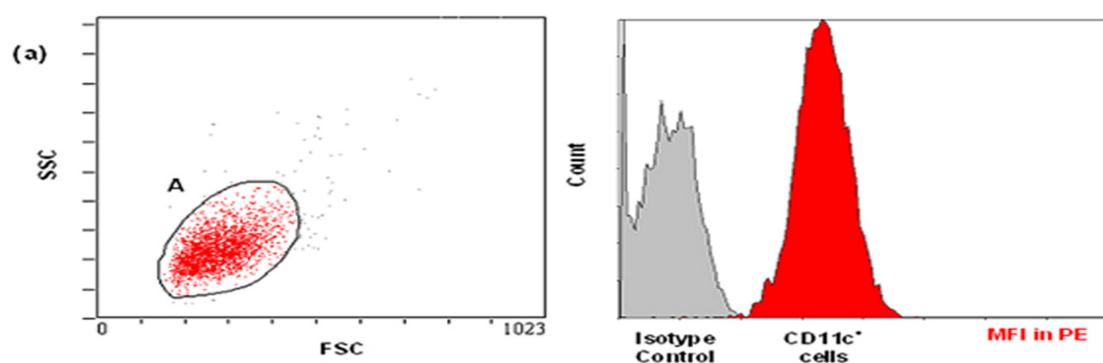
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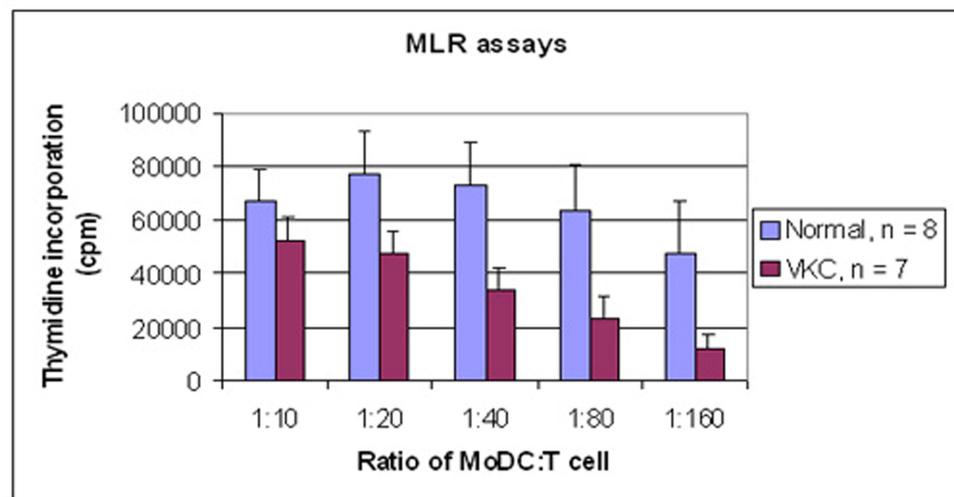
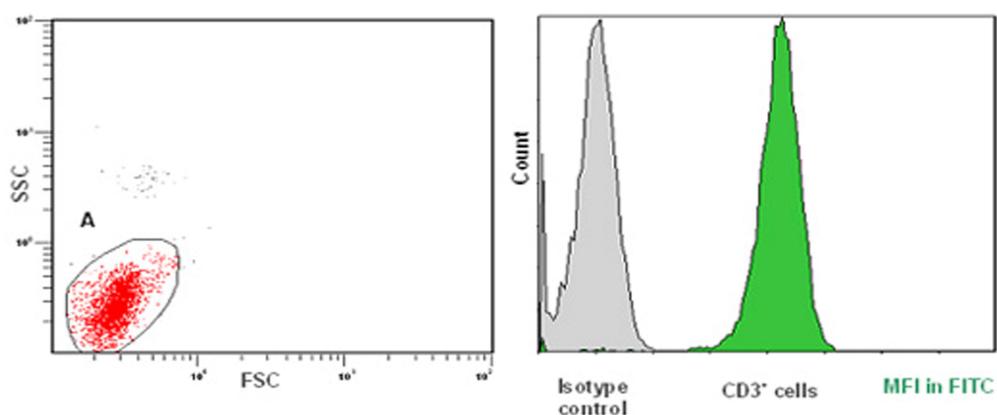
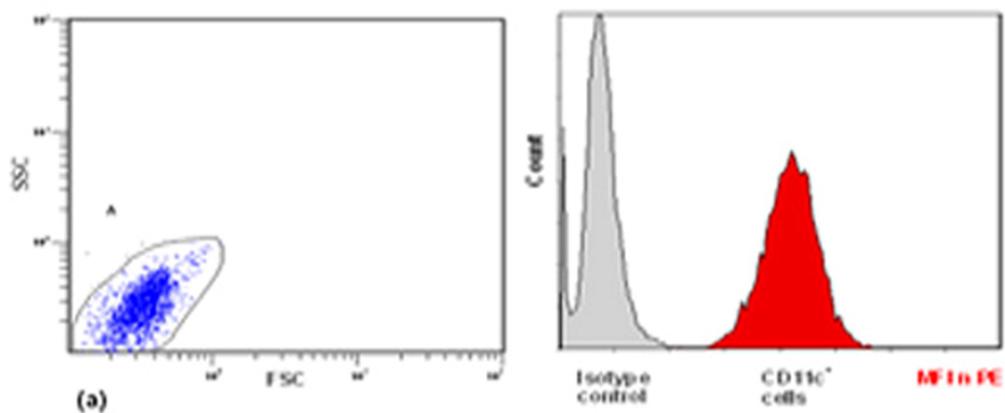
385 **Table 1:** Background patient information.

386

387 **Table 2:** The mean fluorescent intensity (MFI) recorded for each marker measured during flow  
388 cytometric analysis. \* Denotes a statistically significant result.

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(c)