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

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19
20 **Key words:** dendritic cells, allergic conjunctivitis, flow cytometry, mixed leukocyte reaction
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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⁺ 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⁺ 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⁺ 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⁺
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; IgG₁, IgG_{2a}, IgG_{2b},) 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 1×10^4 DCs/100 μ 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 μ 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 (1×10^4 MoDC only in T cell medium and 1×10^5 T
151 cells in T cell medium) and one positive control (1×10^5 T cells only in T cell medium in wells
152 lined with α CD28/ α CD3). The plates were incubated for 5 days at 37°C in 5% CO₂. T cell

153 proliferation was quantified by incubating the cells with 1 μ Ci of methyl-titrated thymidine
154 deoxyriboside (10 μ 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 10⁶ MoDCs was obtained
168 after culture as compared with the VKC donors where a yield of 0.5 – 13.2 x 10⁶ 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⁺ (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 | | |
| MHC class II | 40.97 | 14.01 | 20.8-66.9 | 71.41 | 29.70 | 14.4-122 | 1.74 | <0.01 * |
| CD86 | 2.42 | 1.85 | 0.45-5.68 | 5.98 | 3.40 | 1.71-11.7 | 2.47 | <0.01 * |
| CD83 | 0.34 | 0.09 | 0.2-0.48 | 0.68 | 0.15 | 0.46-0.98 | 2.00 | <0.001 * |
| CD58 | 4.18 | 2.59 | 1.97-12 | 9.28 | 3.17 | 4.77-15.6 | 2.22 | <0.001 * |
| CD11c | 7.86 | 4.29 | 3.63-18 | 12.09 | 6.77 | 5.07-30.7 | 1.54 | 0.05 * |
| CD11b | 76.92 | 33.56 | 38.7-152 | 111.05 | 48.28 | 40.4-192 | 1.44 | 0.05 * |
| CD23 | 6.26 | 3.78 | 3.03-15.4 | 5.55 | 11.37 | 0.94-41.5 | -1.13 | 0.02 * |
| FcεRI | 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⁺ 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⁺ 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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292

293

294

295 **REFERENCE LIST**

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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.

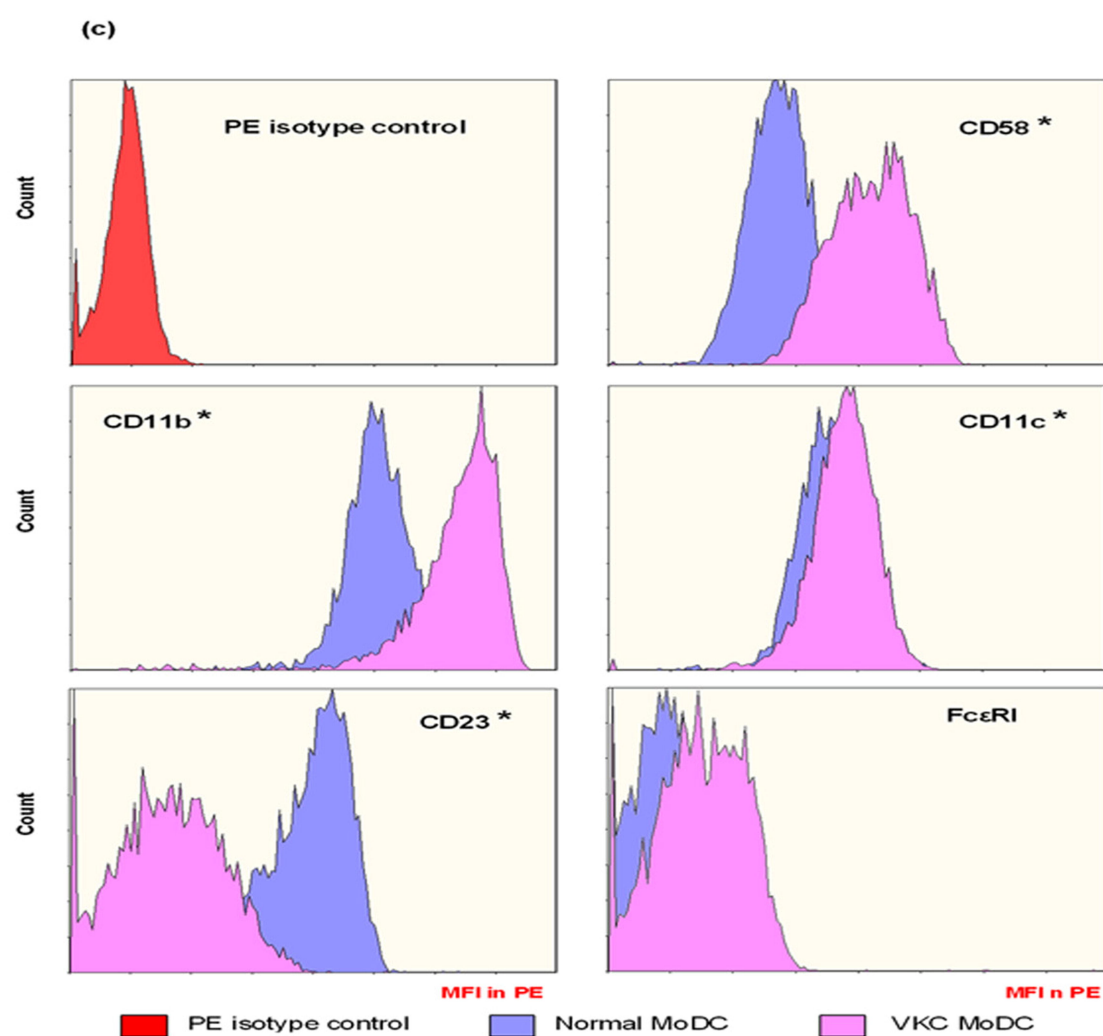
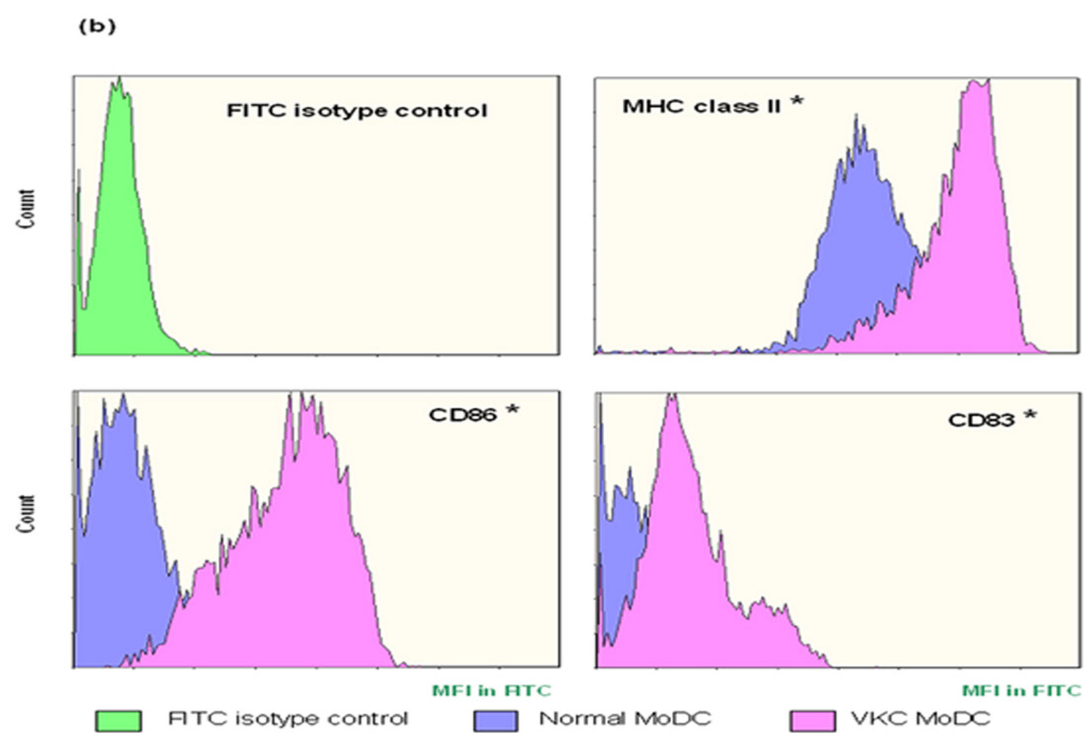
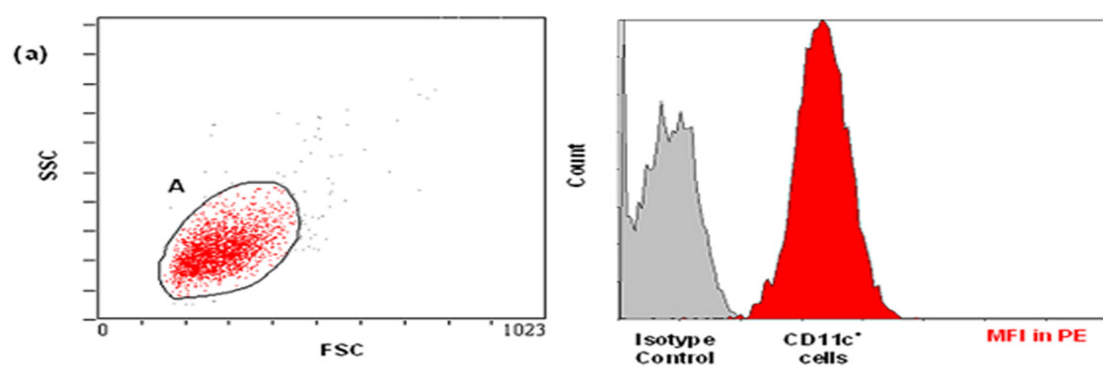
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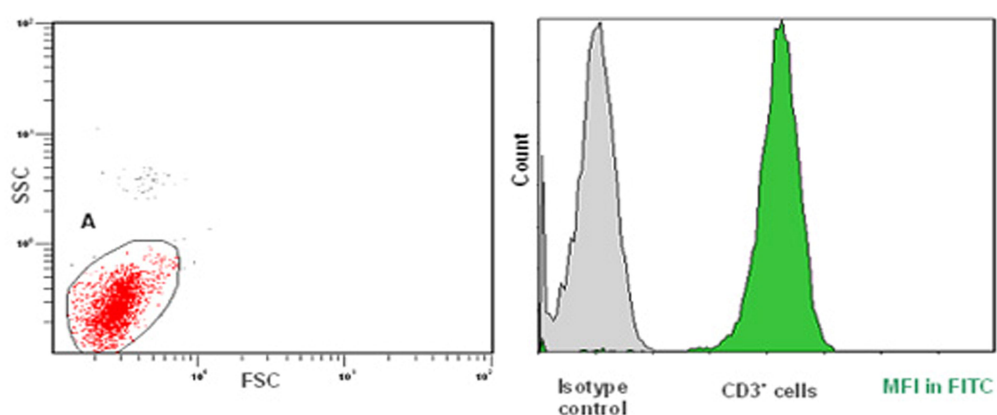
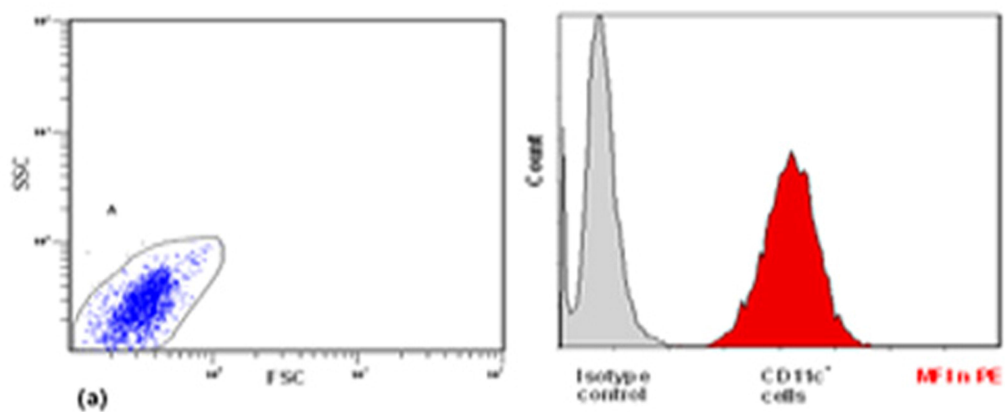
385 **Table 1:** Background patient information.

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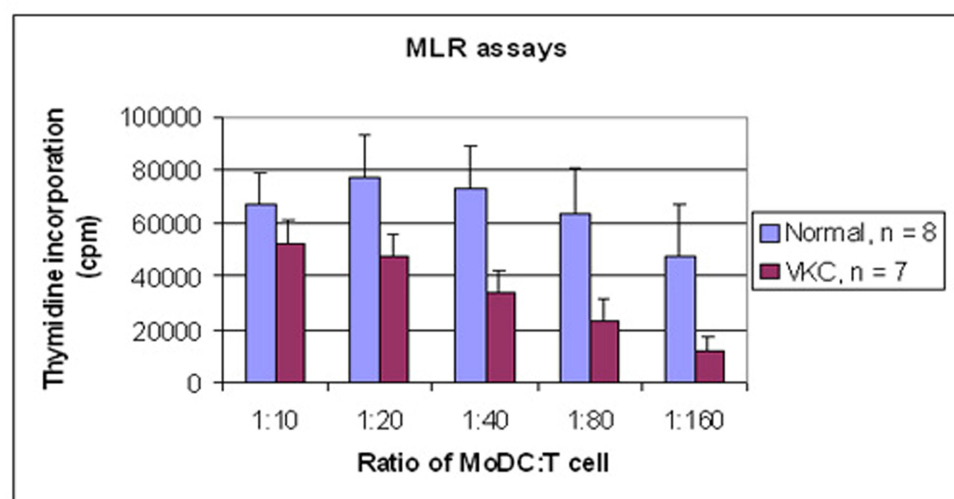
387 **Table 2:** The mean fluorescent intensity (MFI) recorded for each marker measured during flow
388 cytometric analysis. * Denotes a statistically significant result.

389





(b)



(c)