Monocyte-derived dendritic cells over-express CD86 in patients with systemic lupus erythematosus

Patrice Decker¹, Ina Kötter², Reinhold Klein², Beate Berner²
Hans-Georg Rammensee¹

Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease of unknown aetiology with skin, joint, renal, cardiovascular and nervous manifestations. It is characterized by a large amount of circulating autoantibodies (autoAb) with diverse specificities as a result of abnormal activation of autoreactive B and T-helper lymphocytes. Dendritic cells (DCs) play a key role in regulating immune responses, either by priming naïve T-cells [1] or tolerizing autoreactive T-cells [2]. T-cell priming requires mature DCs whereas immature DCs can induce T-cell tolerance. Thus, dysregulation of DC maturation towards chronic DC activation may skew self-antigen presentation from tolerance to autoimmunity.

Recently, DCs have been suggested to be involved in SLE development [3] and myeloid/plasmacytoid DCs (mDCs/pDCs) have been found at lower concentrations in patient blood as compared with normal individuals [4]. Since DCs are known to migrate to lymph nodes upon activation (as reviewed in [5]), it has been hypothesized that blood DCs are activated in lupus patients, resulting in low blood cell numbers. As a consequence, it is rather difficult to analyse activation states of blood DCs since activated DCs are supposed to leave the bloodstream. As an alternative, it is possible to use DCs derived in vitro from monocytes. Importantly, monocytes have been shown to differentiate to DCs in vivo [6] in mice. Thus, it might be possible to overcome the problem of migration of activated blood DCs by analysing monocyte-derived DCs (MDDCs) activation in vitro and avoid any resulting bias in data interpretation.

Interestingly, autoAb production in mouse lupus has been shown to be preferentially dependent on CD86 [7]. Although peripheral blood B lymphocytes show an increased CD86 expression in lupus patients [8], no or little difference was observed for CD86 or CD80/CD86 expression, respectively on non-stimulated monocytes between lupus patients and normal donors [9, 10]. Moreover, CD86 expression on lupus MDDCs has not been investigated, except recently [11] where no difference between lupus and normal MDDCs was observed using a different approach and where cytokine secretion was not investigated. Before the latter study was published, we have thus decided to compare the activation state of MDDCs derived from lupus patients and normal donors and we show for the first time that lupus MDDCs mature spontaneously as observed by CD86 over-expression. This result might partly explain how the peripheral tolerance is broken in SLE patients and why autoreactive T-cells are activated.

Materials and methods

Donors

Twenty-two SLE patients fulfilling the American College of Rheumatology criteria and 16 normal individuals (3 men/13 women, mean age = 31 yrs), all randomly selected, were involved in this study. Patient characteristics are described in Tables 1 and 2. All samples were collected between 8:00 AM and 1:00 PM and were treated in the same conditions. Experiments with human cells have been approved by the local ethic committee (reference 146/2001V) and samples were obtained after informed consent of the donors. In all patients, the disease activity score (SLEDAI) was determined.

1Institute for Cell Biology, Department of Immunology, University of Tübingen and 2Internal Medicine II, University Hospital, Tübingen, Germany.

Submitted 28 July 2005; revised version accepted 31 January 2006.

Correspondence to: Dr Patrice Decker, Institute for Cell Biology, Department of Immunology, Auf der Morgenstelle 15, D-72076, Tübingen, Germany. E-mail: patrice.decker@uni-tuebingen.de
<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>SLEDAI (yrs)</th>
<th>PRED (mg/day)</th>
<th>CQ (mg/day)</th>
<th>HCQ (mg/day)</th>
<th>MMF (mg/day)</th>
<th>CSA (mg/day)</th>
<th>MTX (mg/day)</th>
<th>AZA (cumul.)</th>
<th>ANA anti-DNA (cumul.)</th>
<th>anti-ENA (cumul.)</th>
<th>ANA anti-DNA (d.o.b.)</th>
<th>anti-ENA (d.o.b.)</th>
<th>Organ involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>29</td>
<td>0</td>
<td>4</td>
<td>5</td>
<td>250</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>62</td>
<td>0</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>41</td>
<td>0</td>
<td>18</td>
<td>4</td>
<td>0</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>SSA/B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>50</td>
<td>6</td>
<td>2</td>
<td>10</td>
<td>0</td>
<td>400</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>47</td>
<td>4</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>–</td>
<td>– &gt;1:1280</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>39</td>
<td>0</td>
<td>5</td>
<td>7.5</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>15</td>
<td>0</td>
<td>+</td>
<td>SSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>32</td>
<td>2</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25</td>
<td>+</td>
<td>+ &gt;1:1280</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>64</td>
<td>8</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>–</td>
<td>– &gt;1:1280</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>28</td>
<td>0</td>
<td>11</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>250</td>
<td>0</td>
<td>+</td>
<td>+ SSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>50</td>
<td>8</td>
<td>1</td>
<td>6.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>40</td>
<td>10</td>
<td>2</td>
<td>7.5</td>
<td>0</td>
<td>400</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>95</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>41</td>
<td>6</td>
<td>10</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>+</td>
<td>+</td>
<td>&gt;1:1280</td>
<td>90</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>54</td>
<td>2</td>
<td>7</td>
<td>2.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>75</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>26</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>75</td>
<td>+</td>
<td>+ SSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>27</td>
<td>4</td>
<td>8</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>&gt;1:1280</td>
<td>–</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>56</td>
<td>4</td>
<td>2</td>
<td>10</td>
<td>0</td>
<td>400</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>&gt;1:640</td>
<td>–</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>28</td>
<td>0</td>
<td>8</td>
<td>10</td>
<td>0</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>18</td>
<td>F</td>
<td>55</td>
<td>4</td>
<td>18</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>41</td>
<td>6</td>
<td>1</td>
<td>50</td>
<td>0</td>
<td>400</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>Sm &gt;1:1280</td>
<td>95</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>40</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>21</td>
<td>F</td>
<td>39</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>150</td>
<td>0</td>
<td>0</td>
<td>150</td>
<td>0</td>
<td>+</td>
<td>U1RNP, SSA</td>
<td>–</td>
</tr>
<tr>
<td>22</td>
<td>F</td>
<td>43</td>
<td>8</td>
<td>7</td>
<td>7.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>150</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Yrs, years; SLEDAI, disease activity score; PRED, prednisolone; CQ, chloroquine; HCQ, hydroxychloroquine; MMF, mycophenolate mofetil; CSA, cyclosporine A; MTX, methotrexate; AZA, azathioprine; ANA, anti-nuclear antibody (normal, <1:80 by immunofluorescence); anti-DNA, anti-double-stranded DNA (normal, <60 IU/ml by ELISA); anti-ENA, anti-extractable nuclear antigen (antibody specificities are indicated); cumul., cumulative data (i.e. at any time point); d.o.b., at the date of blood collection; CNS, central nervous system; NT, not tested.
CD86 over-expression by lupus dendritic cells

Sample preparation
A total of 30–50 ml of heparinized blood was collected for each donor and was immediately used for peripheral blood mononuclear cells (PBMCs) isolation by standard gradient centrifugation with Ficoll-Hypaque (PAA). The PBMCs were washed several times before use. In most experiments, one normal donor was compared with one SLE patient. In a few experiments, two or three patients were compared with one normal donor. In each individual experiment, normal and lupus bloods were treated exactly the same.

MDDC preparation and maturation
The MDDCs were prepared from plastic-adherent PBMCs (1 h adherence step) cultured in the presence of 100 ng/ml granulocyte-macrophage colony-stimulating factor (GM-CSF, Leucomax 400, Sandoz) and 40 ng/ml interleukin (IL)-4 (R&D) for 6–8 days as described previously [12] in order to induce monocyte differentiation, except that the medium used was X-VIVO 15 (BioWhittaker) in the absence of serum. We checked medium. At day 3, cells were harvested and analysed by flow cytometry.

Flow cytometry
Immature MDDCs (non-adherent cells obtained at day 6–8) were analysed by flow cytometry, with a gate according to FSC/SSC parameters, for CD1a, CD11c, CD14, CD86 and human leucocyte antigen (HLA)-DR cell surface expression using specific monoclonal antibodies (mAb) and corresponding isotype controls (Pharmingen) as well as with propidium iodide using a FACScalibur apparatus with CellQuest software (Becton Dickinson). At day 3 of maturation, cells were harvested and DC maturation was checked by flow cytometry by measuring CD86 cell surface expression in order to estimate CD86 up-regulation.

Cytokine production
Cytokine secretion in culture supernatants was measured by sandwich ELISA using mAb pairs or ELISA kit (Pharmingen) specific for TNF-α, IL-6, IL-8 and IL-10, according to manufacturer’s recommendations. Cytokine concentrations were calculated according to standard curves and normalized according to the percentage of MDDCs in the preparation. Cytokine secretion was determined for MDDCs with or without activation signal. The resulting activation-induced secretion was determined using the ratio: concentration upon activation/concentration without activation. In each experiment, the secretion ratio between lupus and normal DCs was calculated for each cytokine.

Statistical analysis
In order to minimize the variation between experiments (differentiation duration, number of PBMCs available), the study was designed as follows. For flow cytometry analysis, cells were always stained with a specific Ab and with the corresponding isotype control. In all cases, when analysing mean fluorescence intensity (MFI) for a given cell surface molecule, the specific signal was calculated by dividing the signal measured with the Ab by the signal measured with the isotype control (MFI Ab/MFI isotype)
in order to take into account eventual background differences. We thus focused on expression levels. Then, MFI of lupus and normal MDDCs were compared by pair in each experiment by calculating the ratio: MFI lupus/MFI normal, meaning that we analysed over-expression of the given cell surface molecule in patients as compared with normal individuals. The population of MFI ratios was transformed using decadic logarithm function (to get a normally distributed population) and then analysed by a two-tailed one-sample Student’s t-test against the expected value of 0, i.e. log(1), which should be observed if both groups behave similarly. We thus tested the probability to get two groups with similar characteristics. When analysing percentages of cells positive for a given cell surface molecule, the marker was set according to the corresponding isotype control and fixed at 1% of positive cells as a background in all experiments. The difference in the percentages of positive cells between lupus and normal cultures was analysed as described above, using the ratio of percentages (lupus/normal) calculated for each SLE patient–normal donor pair. Difference in the percentages or concentrations of PBMCs and MDDCs between samples from normal donors and lupus patients or difference in the mean CD86 ratio between patient subgroups were analysed as described above. Values were transformed using decadic logarithm function but then analysed by two-tailed two-sample Student’s t-tests.

Cytokine concentrations were determined by ELISA and the ratios between the concentration produced by lupus MDDCs and the concentration produced by normal MDDCs was calculated for all cytokines in each individual experiment. The population of cytokine ratios was then transformed using decadic logarithm function and analysed by a two-tailed one-sample Student’s t-test against the expected value of 0, i.e. log(1).

Correlations were determined using Spearman correlation test. The Spearman correlation coefficient (R) and the probability (P) are reported.

**Results**

**Comparison of cell content between normal and lupus samples**

First of all, the PBMC concentrations (million cells per millilitre of blood) calculated for fresh blood after Ficoll centrifugation were compared between normal and lupus blood. As shown in Table 3, lupus blood contained significantly fewer PBMCs as compared with normal blood (P < 0.0001). According to this result, we decided to start with exactly the same PBMC number (instead of the same blood volume) for normal and lupus MDDC cultures in each individual experiment. Importantly, Scheinecker et al. [4] found no difference in the percentage of monocytes among PBMCs between lupus patients and normal individuals, suggesting that starting with the same PBMC number for lupus and normal cultures will not introduce a bias in our experiments. After in vitro differentiation for 6–8 days, immature MDDCs were harvested and analysed by flow cytometry. In all experiments, the DC gate used only contained CD14-negative, but CD11c-positive cells. Interestingly, the numbers of immature MDDCs obtained from both normal and lupus cultures did not differ (Table 3), indicating that the differentiation rate from monocytes is similar in both groups (calculated from the starting PBMC number). It also suggests that if an intrinsic defect is responsible for low myeloid DC numbers in patients, this defect does not depend on differentiation from monocytes. Interestingly, the percentage of immature MDDCs in lupus cultures was negatively correlated with the SLEDAI (P = 0.031). When we compared normal and lupus immature MDDC numbers obtained from the same volume of blood, we observed decreased DC numbers for SLE patients (Table 3) although the difference was not statistically significant (P = 0.065). On the contrary, a clear positive correlation was observed between the PBMC concentration and the immature MDDC concentration (calculated from the starting blood volume), either for the lupus group (Spearman correlation coefficient R = 0.53; P = 0.018; Fig. 1) or for all donors together (R = 0.52; P = 0.002; Fig. 1). Thus, the higher the PBMC concentration, the higher the number of harvested immature MDDCs.

**Lupus immature MDDCs over-express CD86**

In each individual experiment, lupus and normal MDDCs were compared. Importantly, it has already been shown that there is no difference for CD86 expression between lupus and normal monocytes [10]. After in vitro differentiation of normal and lupus monocytes for 6–8 days, immature normal and lupus MDDCs were harvested, analysed by flow cytometry and compared for the expression of cell surface molecules. The ratio of the MFI of a given cell surface molecule between lupus and normal MDDCs was calculated in order to get the level of over-expression of this cell surface molecule in lupus patients. Both normal and lupus immature MDDCs were CD14⁺CD11c⁺HLA-DRᵇwb with no obvious difference between the two groups. On the contrary, although both normal and lupus immature MDDCs were CD86⁺positive, a clear difference was observed in the level of CD86 expression. Thus, we observed that CD86 was over-expressed on lupus immature MDDCs in the absence of DC activation signal (as summarized in Fig. 2). An example of the CD86 over-expression by immature lupus MDDCs as compared with immature normal MDDCs is shown in Fig. 3. Whereas both lupus and normal immature MDDCs show a similar staining with the isotype control (as expected), a clear CD86 over-expression is observed with lupus MDDCs as compared with normal MDDCs. Interestingly, the whole MDDC population over-expresses CD86 and not only a sub-population. Thus, most patients (15 out of 20) over-express CD86 (ratio > 1, Fig. 2) and seven patients (35%) have a CD86 expression 50% higher than normal individuals (ratio ≥ 1.5, mean ratio = 2.54, Fig. 2). As a mean, the level of CD86 expression was 55% higher on lupus MDDCs as compared with normal MDDCs and this difference was statistically significant (P = 0.025). In order to prove that analysis was not biased by using

---

**Table 3. Cell counts**

<table>
<thead>
<tr>
<th></th>
<th>PBMCs (M/ml blood)</th>
<th>MDDCs (% PBMCs)</th>
<th>MDDCs (M/ml blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SLE</td>
<td>Normal</td>
<td>SLE</td>
</tr>
<tr>
<td>n</td>
<td>22</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>Geomean</td>
<td>0.9</td>
<td>1.64</td>
<td>0.94</td>
</tr>
<tr>
<td>CI</td>
<td>0.72–1.11</td>
<td>1.36–1.97</td>
<td>2.24–4.13</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.0001</td>
<td></td>
<td>0.64</td>
</tr>
</tbody>
</table>

*a* Concentrations of PBMCs and immature MDDCs were determined for both normal individuals and SLE patients and were compared. M, million; Geomean, geometric mean; CI, confidence interval (95%); P, probability as determined by Student’s t-test. Concentrations are expressed in terms of million cells per millilitre of starting blood or in terms of percentage of starting PBMCs.
ratios, we tested the correlation between CD86 expression and CD86 over-expression on lupus MDDCs. Thus, we could show that the level of CD86 expression on immature lupus MDDCs positively correlates with CD86 over-expression by these cells ($R = 0.48; P = 0.033$). When we analysed the percentage of CD86-positive MDDCs (percentage ratio between lupus and normal cells), we observed that lupus DC cultures contained more CD86-positive DCs. As a mean, lupus cultures contained 26% more CD86-positive DCs than normal cultures (ratio $= 1.26$). However, this difference did not reach statistical significance ($P = 0.064$). Nevertheless, a strong and statistically significant positive correlation was observed between the ratio of CD86 MFI and the ratio of the percentages of CD86-positive DCs ($R = 0.73; P = 0.0003$). Thus, the higher the CD86 over-expression by lupus MDDCs, the higher the concentration of CD86-positive DCs in lupus cultures as compared with normal cultures. No difference was observed for HLA-DR expression between lupus and normal immature MDDCs, either for the level of expression or for the percentage of positive cells. Moreover, the percentage of propidium iodide-positive MDDCs was similar in lupus and normal cultures. This result, together with the observation that the percentages of immature MDDCs among starting PBMCs are similar in lupus and normal cultures (Table 3), suggests that the survival of lupus DCs is not affected, especially not by the therapy.

Upon incubation with either TNF-α and PGE2 or LPS and IFN-γ, both lupus and normal MDDCs matured and up-regulated CD86, as observed by increases in MFI and percentages of positive cells. At day 3 of maturation, CD86 expression reached a similar level in both normal and lupus MDDCs (data not shown). Thus, the higher the CD86 over-expression by lupus MDDCs, the higher the concentration of CD86-positive DCs in lupus cultures as compared with normal cultures. No difference was observed for HLA-DR expression between lupus and normal immature MDDCs, either for the level of expression or for the percentage of positive cells. Moreover, the percentage of propidium iodide-positive MDDCs was similar in lupus and normal cultures. This result, together with the observation that the percentages of immature MDDCs among starting PBMCs are similar in lupus and normal cultures (Table 3), suggests that the survival of lupus DCs is not affected, especially not by the therapy.

Upon incubation with either TNF-α and PGE2 or LPS and IFN-γ, both lupus and normal MDDCs matured and up-regulated CD86, as observed by increases in MFI and percentages of positive cells. At day 3 of maturation, CD86 expression reached a similar level in both normal and lupus MDDCs (data not shown). Thus, the higher the CD86 over-expression by lupus MDDCs, the higher the concentration of CD86-positive DCs in lupus cultures as compared with normal cultures. No difference was observed for HLA-DR expression between lupus and normal immature MDDCs, either for the level of expression or for the percentage of positive cells. Moreover, the percentage of propidium iodide-positive MDDCs was similar in lupus and normal cultures. This result, together with the observation that the percentages of immature MDDCs among starting PBMCs are similar in lupus and normal cultures (Table 3), suggests that the survival of lupus DCs is not affected, especially not by the therapy.

Cytokine secretion

Supernatants of DC cultures where harvested 24 h after culture in medium alone or after activation with TNF-α and PGE2 or LPS and IFN-γ and were tested for the presence of TNF-α, IL-6, -10 and -8. In each experiment, the secretion ratio between lupus and normal DCs was calculated for each cytokine. This ratio was thus
calculated for the secretion with or without activation and for the resulting activation-induced secretion (as defined in the Materials and methods section).

TNF-α and IL-10 were not detected in the absence of DC activation signal, either with normal or lupus MDDCs (data not shown). Regarding IL-6, a slight secretion was detected in the absence of activation signal with both normal and lupus MDDCs, but at a similar extent (mean concentration = 122.9 pg/ml). On the contrary, a strong IL-8 secretion was observed with both normal and lupus MDDCs without any activation signal (mean concentration = 5.8 ng/ml). Nevertheless, no statistically significant difference was observed between normal and lupus MDDCs. Upon activation, all cytokines were secreted at higher levels (for example, mean IL-6 concentration = 390.0 pg/ml after TNF-α/PGE2 activation; mean IL-8 concentration = 19.6 ng/ml after TNF-α/PGE2 activation), but no significant difference was observed between normal and lupus MDDCs regarding the final cytokine concentrations or the activation-induced cytokine concentrations (data not shown). Finally, as shown in Fig. 4 a clear positive correlation was observed between CD86 over-expression and activation-induced IL-6 secretion ratios (lupus vs normal MDDCs; $R = 0.70$; $P = 0.010$), indicating that the more lupus DCs over-express CD86, the more they over-induce IL-6 secretion in the presence of TNF-α/PGE2 as compared with normal DCs.

**Correlations with clinical parameters**

Interestingly, a negative correlation was observed between MDDC concentrations (expressed in million cells per millilitre of starting blood) and C3 levels in SLE patients ($P = 0.022$). Moreover, we found a negative correlation between C3 levels and anti-nuclear antibody (ANA) levels ($P = 0.021$). Regarding CD86 over-expression, no correlation was observed with SLEDAI, patient age, disease duration or differential blood cell counts. Importantly, there was no correlation between CD86 over-expression and CRP or ESR levels. Likewise, no correlation was observed between CD86 over-expression and neopterin, C3/C4, ANA or anti-DNA autoAb levels.

Interestingly, we have observed a negative correlation between the mycophenolate mofetil (MMF) doses used in patients and ANA levels ($P = 0.039$) as well as between MMF doses and activation-induced IL-6 secretion ratios ($P = 0.020$), supporting the use of MMF in lupus therapy.

In order to exclude a role of medication in CD86 levels, we have analysed correlations between CD86 expression or over-expression and the doses of each drug used in SLE patients. Importantly, no correlation was found between CD86 expression and the dose of any drugs. Regarding CD86 over-expression, a negative correlation was observed with MMF: the higher the MMF dose the lower, the CD86 over-expression ($P = 0.035$). A similar tendency was observed with azathioprine (AZA), although it did not reach.
Discussion

We show for the first time that lupus immature MDDCs over-express CD86 as compared with normal MDDCs in the absence of any DC stimulus. Interestingly, lupus MDDCs with the highest spontaneous CD86 over-expressions also over-induce IL-6 secretion upon activation. Importantly, CD86 over-expression cannot be attributed to the therapy. These results support the hypothesis that DCs are over-activated in vivo in SLE patients, resulting in DC migration to lymph nodes and low blood DC concentrations.

We have decided to analyse MDDCs and not blood DCs. Indeed, DCs have been suggested to be involved in lupus development, but have also been shown to circulate at low concentrations in patient blood as compared with normal individuals [3, 4]. It was thus suggested that lupus DCs might be activated in patients, leading to DC migration from blood to lymph nodes and resulting in low blood concentrations. As a consequence, studies on the activation state of blood DCs in vivo might be biased, since over-activated DCs should migrate and leave blood circulation.

Only recently, MDDCs from lupus and normal donors have been compared [11] where CD86 over-expression by lupus MDDCs was not observed. Nevertheless, several reasons can explain the differences observed. First of all, Köller et al. [11] have used monocytes obtained by positive selection using CD14 magnetic beads whereas we used untouched monocytes. It has been observed that monocytes obtained by CD14-positive selection differ from those obtained from untouched monocytes [13, 14]. Thus, soluble factors secreted during DC cultures by CD14-activated monocytes might influence in vitro MDDC differentiation as well as the behaviour of the immature MDDCs obtained after 6–8 days of differentiation. In the latter study, the authors showed that immature MDDCs derived from adherent monocytes or CD14-positive monocytes obtained by positive selection expressed comparable levels of CD86 molecules. This was clear when comparing the percentages of CD86-positive DCs. Nevertheless, when comparing the level of expression, the authors also observed that the CD86 MFI was clearly increased in immature MDDCs obtained from untouched monocytes as compared with MDDCs obtained from positively selected monocytes (MFI = 799 vs 207, respectively). Thus, monocytes obtained by positive selection might be activated in culture and already exhausted after a few days, before differentiating in MDDCs.

The presence of serum in medium during MDDC preparation, as well as the nature of the serum used, have also been shown to influence DC differentiation [15]. Thus, immature MDDCs prepared in the presence of fetal calf serum (FCS) expressed higher levels of CD86 than MDDCs cultured in serum-free medium. This could again explain the differences observed with the study by Köller et al. [11] where medium containing 10% FCS was used. In the latter study, it is possible that the authors could not detect any difference in CD86 expression between MDDCs from lupus patients and MDDCs from normal donors because FCS was used, meaning that CD86 was higher in both DC groups and thus, decreasing the CD86 expression ratio between both groups and thus lowering the sensitivity.

Interestingly, we have found that the differentiation rate from monocytes to DCs is similar for both lupus patients and normal individuals (when starting with the same PBMC numbers) indicating that a deficient differentiation process of monocytes is not involved in low myeloid DC numbers in lupus blood. On the contrary, if instead of starting with the same PBMC numbers for normal and lupus DC cultures we start with the same blood volume, the number of MDDCs obtained from lupus blood is lower than the number obtained from normal blood and this observation fits with a previous study reporting low blood DC numbers in SLE patients [4]. In agreement, we have shown that the percentage of MDDCs in lupus cultures is negatively correlated with the SLEDAI. Moreover, although immature MDDCs from lupus patients spontaneously over-express CD86, they need activation to secrete high amounts of cytokines, as do normal MDDCs. These results mean that normal and lupus MDDCs behave similarly with regard to cytokine secretion and thus, the high concentrations of DC-derived cytokines observed in patient blood cannot be explained by intrinsic high cytokine secretion levels by lupus MDDCs. Nevertheless, lupus MDDCs with the highest CD86 over-expressions also show the highest activation-induced IL-6 secretion ratios. Interestingly, IL-6 has been reported to be present at higher concentrations in the sera of patients with active disease [16]. Contrarily to the present study, monocytes from lupus patients have been shown to have a limited differentiation flexibility towards MDDCs [17]. Nevertheless, in the later study authors analysed...
MDDCs early at day 3 of differentiation in the presence of very low concentrations of GM-CSF and IL-4 and did not analyse CD86 expression.

The CD86 over-expression on lupus MDDCs could be explained by several mechanisms. First, the regulation of CD86 expression at the cell surface might be impaired and might represent an intrinsic defect in SLE. Second, a serum soluble factor might be present in patients and could be involved in DC maturation. Likewise, it could be a soluble factor secreted in lupus DC cultures. In agreement, we have recently shown that nucleosomes, the main auto-antigen in lupus and found as a circulating complex in patients, induce DC maturation via a MyD88-independent pathway upon direct interaction with these cells [18]. This manifests by CD86 up-regulation on DCs and secretion of cytokines known to be associated with lupus. Third, IFN-α has been detected in the serum of lupus patients [19] and has been shown to induce normal monocytes to differentiate into mature DCs [3] as estimated by CD86 up-regulation. Nevertheless, DC cultures were performed without IFN-α or lupus serum in the present study but sensitization of monocytes before the cell culture might favour differentiation. We did not measure serum levels of IFN-α in blood donors and did not try to correlate them with CD86 over-expression.

Due to low DC numbers (as expected), it was not possible to perform functional analysis such as mixed lymphocyte reactions (MLRs). Nevertheless, we have analysed cytokine secretion. Moreover, it is well established that CD86 is an important molecule for T-cell activation and as mentioned above, autoAb production in mouse lupus has been shown to be preferentially dependent on CD86 [7]. MLRs would give information on the capacity of DCs to stimulate T-cells but not on the exact molecular mechanism. It could not be excluded that other proteins influencing T-cell activation are up- or down-regulated at the surface of lupus DCs. Thus, strong T-cell activation in the presence of lupus DCs could not be attributed for sure to CD86. On the contrary, by focusing on CD86 over-expression we highlighted a molecule which might be involved in a mechanism associated with lupus pathogenesis. Importantly, we also found that CD86 over-expression by lupus MDDCs correlates with high activation-induced IL-6 secretion as compared with normal MDDCs. It should be noted that no study has analysed MLRs. DCs phenotype and cytokine secretion together so far with lupus MDDCs. A few have analysed MLRs and MDDC phenotype but not cytokines. We did not analyse MLRs, but we describe DC phenotype and cytokine secretion.

In conclusion, lupus immature MDDCs over-express CD86 furnishing strong signal 2 for efficient T-cell activation. Moreover, high CD86 over-expression by lupus DCs is associated with high activation-induced IL-6 secretion as compared with normal DCs. Thus, lupus MDDCs are probably more efficient antigen-presenting cells that might efficiently co-stimulate T-cells even in the absence of DC activation signal. This mechanism may reflect a state facilitating T-cell activation and thus, might favour the break of peripheral tolerance in patients and might sustain continuous T-cell activation.

### Acknowledgements

We thank Dr. Martin Eichner (Department for Medical Biometry, University of Tübingen, Germany) for help in statistical analysis. This work was supported by a fortune grant (Forschungsprogramm der Tübingen Medizinischen Fakultät, Nr. 1199-0-0) and a Fritz Thyssen Stiftung grant (Az. 10.03.2.123) to P.D.

The authors have no conflicts of interest.

### References

17. Steinbach F, Henke F, Krause B, Thiele B, Burmester GR, Hiepe F. Monocytes from systemic lupus erythematosus patients are severely impaired in their activation capacity.

### Key messages

- Lupus MDDCs spontaneously over-express CD86, suggesting that they are pre-activated.
- Activation-induced IL-6 secretion correlates with CD86 over-expression.
- The abnormal high CD86 expression might be important for the immunopathogenesis of SLE.
Clinical Vignette

An unusual presentation of polyarticular tophaceous gout

A 60-yr-old man presented with a 15-yr history of episodic, inflammatory polyarthritis with subcutaneous tophi over the left elbow. He was diagnosed with polyarticular gout after gouty crystals were demonstrated from joint fluid. He was treated with colchicine and allopurinol. He was seen after 1 month when he complained of the spontaneous development of multiple intradermal bullae over the lateral and medial aspects of the feet, heels and in the skin overlying the tendoachillis (Fig. 1; see also supplementary Figs 2 and 3 available at Rheumatology Online). The deposits were mildly painful and yellowish white without signs of inflammation. Aspiration from the deposits revealed chalky fluid containing needle-shaped crystals. The patient was diagnosed as tophaceous gout with bullous tophi.

Tophi are usually firm nodular subcutaneous deposits, but may rarely be intradermal pustules or plaques. Tophi presenting as superficial bullae have only once been reported in the English literature [1]. That case was precipitated by a burn causing local tissue injury in one patient. In our patient, the tophi presented as intradermal bullae only on the margins of the sole and heel. Since acute or repetitive trauma is a known precipitating factor of gouty attacks, it could be postulated that imperceptible trauma to the feet precipitated the tophaceous bullae. The skin deposits did not invite the inflammatory response, possibly because the patient was on colchicine.

The authors have declared no conflicts of interest.

Supplementary data

Supplementary figures are available at Rheumatology Online.

D. KHANNA, A. SHRIVASTAVA

Department of Clinical Immunology and Rheumatology, KLES Hospital and Medical Research Centre, Belgaum, Karnataka, India

Correspondence to: A. Shrivastava, Department of Clinical Immunology and Rheumatology, KLES Hospital and Medical Research Centre, Belgaum, Karnataka, India. E-mail: arun453@yahoo.com

References


FIG. 1. Feet showing bullous tophi.