In psoriasis, polymorphonuclear leukocytes are consistently present in the early psoriatic lesion and in actively spreading plaques. CD11b, which is part of the β2-integrin receptor Mac-1, plays an important role in various biological functions of the polymorphonuclear leukocyte such as leukocyte adhesion to endothelium, extravasation, tissue migration and degranulation. In the present study we investigated the possibility of systemic differences in leukocyte CD11b-expression between patients with extensive plaque psoriasis and healthy volunteers. Venous blood samples were obtained from 15 patients with extensive plaque psoriasis (Psoriasis Area and Severity Index greater than 10.0), and from 15, matched, healthy controls. Both unstimulated and in vitro leukotriene B4-stimulated leukocytes were stained for CD11b, which was quantified using flow cytometry methods. A tendency towards decreased basal CD11b-expression was observed on leukocytes from psoriatic patients compared to healthy subjects. After in vitro stimulation with leukotriene B4 (LTB4), the difference between psoriasis patients and controls increased further and was statistically significant. Patients with unstable psoriasis (increasing size of individual lesions and/or pinpoint papules around chronic plaques) proved to have even lower unstimulated and LTB4-stimulated CD11b expression. No correlation was found between CD11b expression and severity of psoriasis using the PASI-score. Interestingly, the relative CD11b up-regulation (ratio CD11bLTB4-stimulated/CD11bunstimulated) was virtually the same in both groups. Therefore, the signalling pathway from leukotriene B4-receptor binding up to CD11b expression on the leukocyte surface, was essentially normal in psoriasis. It is hypothesised that the decreased CD11b expression in psoriasis patients is caused by leukocyte compartmentalisation. (Key words: blood, CD11b, flow cytometry, leukotriene B4, polymorphonuclear leukocyte, psoriasis.)

Integrin adhesion molecules are of great importance in intercellular and cell-matrix interactions [9]. They consist of non-covalently linked α- and β-chains and are categorised by their β-chain [10]. The Mac-1-integrin (CD11b/CD18) which is part of the β2-subpopulation, is known to be of particular importance to various PMN functions [11-13]. It is pivotal to PMN adhesion to vascular endothelium, extravasation, tissue migration, the oxidative burst, and degranulation. The Mac-1-receptor can be up-regulated by various biochemical compounds such as formyl-Met-Leu-Phe, platelet activating factor, interleukin 8, and leukotriene B4 [14-17]. To the best of our knowledge, there are no known active down-modulators of Mac-1. In particular, the up-regulation of CD11b by leukotriene B4 (LTB4) is of interest since LTB4 is produced in large quantities in psoriatic lesional skin [18-21]. LTB4 which is formed in the arachidonic acid cascade, is a potent upregulator of CD11b. It causes a rapid increase in cell-surface presence through qualitative and quantitative up-regulation [11, 22-24]. We chose to evaluate CD11b as it is far more specific for PMN than CD18, which is also present on other leukocyte subsets.
In psoriatic skin, up to the most peripheral zone of the psoriatic lesion, the number of CD11b-positive cells is increased compared to normal skin [25], which suggests that the expression of CD11b by PMN is an important factor in the pathogenesis of psoriasis. So far, no information is available on CD11b expression by peripheral blood PMN of psoriatic patients.

A flow cytometrical study was performed in order to investigate whether there are any systemic changes detectable in the basal levels of PMN CD11b expression in patients with extensive plaque psoriasis compared to healthy volunteers. Secondly, we assessed whether PMN from psoriatics respond abnormally to ex vivo LTB₄-stimulation, with respect to CD11b-up-regulation.

Materials and methods

Subjects

Venous blood samples were obtained from 15 patients with extensive plaque psoriasis. All systemic antipsoriatic treatments were stopped at least three months prior to the investigation, and local antipsoriatic therapy was stopped for at least two weeks. Fifteen healthy volunteers, without any history or signs of skin disease, served as the control group. Subjects were at least 20 years of age. Both groups were matched for gender and age. No systemic, anti-inflammatory or immunomodulating drugs were allowed. The severity of plaque psoriasis was assessed by the psoriasis area and severity index (PASI). Patients were judged to have extensive plaque psoriasis when PASI was greater than 10.0.

Unstable plaque psoriasis was defined as an increasing size of the individual lesions during the two weeks preceding the study and/or the occurrence of pinpoint papules around chronic plaques. In stable psoriasis, these signs were not present.

CD11b integrin up-regulation assay [26]

Blood specimens for assessment of ex vivo neutrophil CD11b surface-expression were obtained. Peripheral blood (4 ml) was collected by venepuncture, kept in ethylene-di-amine-tetra-acetic acid (EDTA) at 4°C and processed within 3 h of collection to prevent non-specific up-regulation of CD11b-expression as a result of neutrophil activation.

Blood samples were processed in triplicate using 90 µl aliquots which were incubated with LTB₄ (10 µl 1 x 10⁻⁷ M) in Hanks’ balanced salt solution (Sigma Chemical Corp., St. Louis, USA) containing 0.1% bovine serum albumin (HBSS-BSA), or with HBSS (10 µl) alone for 30 min at 37°C. Samples were then cooled and incubated in the dark for 30 min at 4°C with 10 µl (0.045 g/l) anti-human CD11b-fluorescein conjugate (Mo-1-FITC, Coulter Corp., Hialeah, USA). Erythrocytes were lysed and the remaining cells were washed with HBSS-BSA, fixed in 1% paraformaldehyde solution and stored at 4°C until analysis.

The analysis was always performed within one week of preparation of the leukocyte suspensions, because previous experiments showed that assessment within 7 days minimises storage artefacts.

Flow cytometry analysis

All specimens were analysed on an Epics Elite Flow Cytometer (Coulter, Luton, UK). Cells were excited with an air-cooled 488 nm argon laser set at 15 mW. Green fluorescence (FITC) was measured through a 525 nm (band width 30 nm) band pass filter. Calibration and sensitivity were checked by using FITC-labelled beads (Standard-Brite, Coulter Source, Hialeah, USA). Forward and side scatter were used for gating granulocytes only. For each sample 5,000 gated cells were analysed (Fig. 1).

Statistical analysis

For comparison between different groups, the Mann-Whitney test was used. Correlation between disease activity and CD11b-expression, and between CD11b-expression before and after in vitro LTB₄-stimulation was calculated using the Pearson test.

Results

Psoriatic patients (N = 15) and healthy controls (N = 15) were matched for gender and age. Table I summarizes the age of patients and healthy controls as well as the severity of psoriasis indicated by the PASI-score. Among the 15 patients with psoriasis, 8 patients had unstable psoriasis, characterised by aggravation of the lesions during the two weeks preceding the study and pinpoint papules around the chronic plaques. The ages of patients and healthy controls were comparable. All patients were suffering from severe plaque psoriasis as indicated by the PASI-score. The extent of skin involvement did not differ between patients with unstable and stable psoriasis.

Figure 1. Right angle scatter (RAS) versus forward scatter (FS) of a leukocyte suspension. Several distinct cell populations can be recognised: (a) unlysed erythrocytes and debris; (b) lymphocytes; (c) monocytes; (d) PMN. This population of PMN is gated out to assess the FITC-fluorescence of every cell which correlates with the number of CD11b molecules present on the cell surface.
Figure 2. CD11b surface expression (mean fluorescence units per cell) of PMN in psoriatic patients and healthy volunteers.

Legend:
- Healthy Volunteers
- Untreated psoriasis
- Untreated psoriasis (overall)
- Stable psoriasis
- LTB4-stimulated unstimulated
- Buffer

The figure shows a comparison of CD11b expression levels in psoriatic patients and healthy volunteers. The expression was measured in terms of mean fluorescence units per cell. The bars represent the expression levels for different conditions, with the y-axis indicating the relative expression.
The presence of CD11b on the cell surface of PMN was 1.07 ± 0.34 in the patient group. The LTP-1 showed a highly significant difference in vitro stimulation with LTP-1 was compared to controls.

The mean PMN CD11b expression was not significant (p = 0.06). However, there was a trend towards a decreased CD11b expression in both groups (p = 0.02 and p = 0.001 respectively). The LTP-1 stimulated PMN CD11b expression was lower in patients with unstable positions (p = 0.002 and p = 0.001 respectively). Both unstimulated and in vitro LTP-1 stimulated PMN CD11b expression was lower in patients and healthy volunteers. Both unstimulated and in vitro LTP-1 stimulated PMN CD11b expression was lower in patients and healthy volunteers. The differences between the overall group of patients and healthy volunteers were more pronounced than they were between the two groups. The differences between the overall group of patients and healthy volunteers were more pronounced than they were between the two groups.

No differences were observed between patients with stable and unstable positions with respect to basal levels of PMN CD11b expression. No differences were observed between patients with stable and unstable positions with respect to basal levels of PMN CD11b expression.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Mean ± SEM</th>
<th>N/A/P</th>
<th>Years of age</th>
<th>PAI</th>
<th>PASS</th>
<th>N/A/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls</td>
<td>0.08 ± 0.02</td>
<td>15</td>
<td>13</td>
<td>4.2</td>
<td>3.8</td>
<td>no applicable</td>
</tr>
<tr>
<td>Patients with unstable positions</td>
<td>0.76 ± 0.18</td>
<td>15</td>
<td>12.3</td>
<td>4.6</td>
<td>2.3</td>
<td>no applicable</td>
</tr>
<tr>
<td>Patients with unstable positions (overall group)</td>
<td>0.77 ± 0.16</td>
<td>25</td>
<td>12.3</td>
<td>4.6</td>
<td>2.3</td>
<td>no applicable</td>
</tr>
<tr>
<td>Patients with unstable positions (mean ± SEM)</td>
<td>0.76 ± 0.18</td>
<td>15</td>
<td>12.3</td>
<td>4.6</td>
<td>2.3</td>
<td>no applicable</td>
</tr>
</tbody>
</table>

Table 1. Mean and standard error of the mean (SEM) of age.
In active psoriatic lesions, PMN-influx has been reported in 78% of patients and in chronic plaque lesions, skin, whereas PMN with a more modest density of CD11b are present on these PMN after in vitro stimulation with LTB₄.

An alternative explanation is habituation of peripheral blood PMN to increased LTB₄-levels in psoriatic skin. This hypothesis is supported by the observation that in psoriatic skin a decreased accumulation of PMN occurs following a standardized stimulus with LTB₄ [28, 29]. Repeated LTB₄ applications resulted in a decreased PMN accumulation as compared to the response following a single application [29]. However, in view of the fact that the relative CD11b up-regulation by LTB₄ in psoriatics proved to be essentially normal, this hypothesis is not supported by the observations in the present study.

Active down-modulation of CD11b is another possible mechanism that could explain the decreased PMN CD11b expression in psoriatic patients. It may be possible that such a defence mechanism exists in order to prevent massive cutaneous damage due to the abundant skin presence of PMN. To the best of our knowledge there are no known active down-modulators of CD11b. However, integrin α-units, like CD11b need divalent cations (calcium or magnesium) for their adhesive functions, and receptor function can be rapidly modulated through phosphorylation reactions [9]. It might well be possible that CD11b can be down-modulated on a functional level by changes in cation-concentrations and phosphorylase activity.

Since LTB₄-induced signalling in psoriatic PMN is essentially normal, the question arises as to what extent LTB₄ is relevant to the CD11b up-regulation of psoriatic PMN in vivo. The role of LTB₄ in psoriasis has been challenged further by the modest effects of 5-lipoxygenase inhibitors in the treatment of psoriasis [30-33].

The decreased CD11b expression on peripheral blood PMN in psoriasis remains an intriguing finding, and further studies should be aimed at mediators and factors involved in PMN compartmentalisation in psoriasis, and at mechanisms involved in the in vivo regulation of CD11b in psoriatic patients.


