

## Expression and Function of $\beta_2$ Integrin CD11B/CD18 on Leukocytes from Patients with Psoriasis

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**The expression of  $\beta_2$  integrin CD11b on granulocytes and monocytes from patients with psoriasis vulgaris and pustular psoriasis was examined by flow cytometry. The amount of CD11b expressed on both granulocytes and monocytes was greater in 4 patients with pustular psoriasis than in 16 patients with psoriasis vulgaris. Its expression correlated with the development of pustules on the skin. No difference was seen between healthy blood donors and patients with active psoriasis vulgaris. Three patients with pustular psoriasis were followed during retinoid treatment. Granulocytes and monocytes showed a decrease in CD11b expression after administration of retinoids, in parallel with clearing of the skin. The adherence of granulocytes isolated from psoriasis patients was tested on cultured human umbilical vein endothelium. No significant difference in adherence was observed between control cells and cells from patients with active psoriasis vulgaris. These data indicate that the development of micro-abscesses in the dermis in psoriasis vulgaris is not related to enhanced  $\beta_2$  integrin function. The increased CD11b expression found in patients with pustular psoriasis may, however, serve as a triggering factor for pustule formation in pustular psoriasis. Key words: L-selectin; flow cytometry; adhesion.**

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Psoriasis is a local inflammatory disease of the skin. The early histological changes seen in a psoriatic lesion include invasion of neutrophils into the epidermis and formation of Munro abscesses in the dermis (1, 2). Other  $\beta_2$  bearing cells are also found in the lesions (3). Mediators found in psoriatic scales, which may cause an influx of inflammatory cells into the skin, are chemotactic substances such as leukotrienes (4, 5) cytokines (6, 7) and the complement component C5a/C5a desArg (8). Some of these mediators are generated by activated keratinocytes (9), which in turn can activate endothelial cells to increase their expression of adhesion molecules.

Accumulation of neutrophils at the site of inflammation is dependent on the expression of adhesion molecules. Three families of adhesion molecules are involved:  $\beta_2$  integrins; the immunoglobulin supergene family; and selectins (10). In order for freely moving leukocytes in the bloodstream to egress into the surrounding tissue, an interaction between the cells and the endothelial cells of the vessel wall must occur. An early event in this process is the rolling action of leukocytes, initiated by the expression of P- and E-selectins on activated endothelial cells and L-selectin on leukocytes (11, 12). The subsequent enhanced adhesion, and migration into the tissue involves upregulation

and activation of CD11b/CD18 on neutrophils and ICAM-1 on endothelial cells (13, 14).

Several reports have focused on the role of neutrophils in patients with active forms of psoriasis (4, 15–17). The oxidative metabolism in these cells is enhanced as a sign of neutrophil activation in patients with pustular psoriasis during the initial acute phase with pustule formation, and is close to normal at the later non-pustular stage (18). Neutrophils can be activated by chemotactic stimuli, such as complement factor C5a and leukotriene B<sub>4</sub> (LTB<sub>4</sub>) found in psoriatic scales (8, 17). Upon activation by inflammatory mediators or selectin–ligand interactions, CD11b/CD18 is mobilized from intracellular pools (19) to the neutrophil surface. Enhanced expression of CD11b/CD18 could therefore reflect an early sign of inflammatory triggering. Several cell populations with CD11b or CD18 are found in psoriasis tissue (3). Since the neutrophil is one of the predominant CD11b/CD18 cells in a psoriatic infiltrate, we wanted to investigate whether there is enhanced expression of adhesion molecules on the surface of circulating neutrophils during formation of micro-abscesses and pustules.

In the present investigation, we compared the leukocyte expression of CD11b and L-selectin in different forms of psoriasis and examined how these cells adhere to human vascular endothelial cells (HUVEC). By relating pustule formation and activity of the disease with the expression and function of CD11b on neutrophils and monocytes, we attempt to understand whether disease activity correlates with an upregulation of CD11b and adhesion.

### MATERIALS AND METHODS

#### Patients

Forty-three patients with extensive spreading psoriasis were treated at the Department of Dermatology, University Hospital, Linköping. The diagnosis was made upon established clinical and histological criteria. None of the patients was on oral medication when entered in the study. A total of 27 patients were examined for CD11b and/or L-selectin, and the last 16 for adhesion.

Included within the patient group were 4 patients, 2 women and 2 men, age 49, 61, 76 and 79 years, respectively, with bouts of generalized pustular psoriasis. They were investigated for CD11b expression when untreated. Three of them were examined before treatment, during etretinate treatment and when their skin had cleared.

Two of the 43 patients with a previous history of pustular psoriasis of von Zumbusch type and not on any medication were sampled at different time intervals over a period of 1 year. Of these 2 patients, 1 was sampled during bouts of pustules (first sampling time) and the other patient during different stages of erythroderma. The small number of patients with active forms of untreated pustular psoriasis is due to the limited number of patients with pustular psoriasis not treated with retinoids in Sweden.

#### Control group

Blood samples from 1 or more healthy blood donors were always analysed in parallel when leukocytes from psoriasis patients were

examined for CD11b and L-selectin expression. A group of 40 blood donors was accumulated during the study. All the donors were analysed for CD11b, 21 were analysed for L-selectin and 20 for leukocyte adhesion to HUVEC.

#### Monoclonal antibodies

Mouse anti-human CD11b phycoerythrin-conjugated antibodies and the isotypic control serum were purchased from Becton-Dickinson (Mountain View CA, USA). Mouse anti L-selectin FITC-conjugated antibodies were purchased from Immunotech (Kemila, Stockholm, Sweden).

#### Preparation and staining of cells

Leukocyte separation was performed as described previously (20). Briefly, EDTA blood was hemolysed at 15°C with a freshly prepared ammonium chloride solution. To avoid spontaneous upregulation of CD11b (20) the cells were kept at 4°C after haemolysis. To induce CD11b upregulation the cells were incubated at 37°C for 15 min with or without the chemotactic peptide *f-met-leu-phe* (f-MLP) at a final concentration of  $5 \times 10^{-7}$  M (Sigma, St. Louis, MO, USA). The cells were then incubated with 10 µl monoclonal antibodies for 30 min, washed twice in cold PBS-EDTA, resuspended in the same buffer and immediately examined in a flow cytometer. The L-selectin expression was analysed on leukocytes incubated at 4°C only.

#### Flow cytometry

The cells were analysed in a Coulter Epics flow cytometer (Coulter Electronics Inc., Hialeah, FLA, USA). The instrument had a 15 mW air-cooled argon laser, with an excitation wavelength of 488 nm. The optical alignment was checked with Immunocheck (Coulter Co. FLA, USA) and the PMT voltage standardized with Standard Brite (Coulter Co.) before each experiment.

The white blood cells appeared as 3 distinct populations on a scattergram according to their size and granularity; lymphocytes, monocytes and granulocytes. The mean fluorescent intensity (MFI) of each individual cell was analysed for monocyte and granulocyte populations. The cut-off for positive fluorescence was defined as the 99th percentile of the fluorescence of the isotypically stained cells (control cells).

#### Human endothelial cells

HUVEC were isolated from umbilical cord veins. The method used was mainly that described by Jaffe et al. (21). Briefly, the vein was flushed with PBS pH 7.4 and incubated with collagenase H (2 mg/ml) (Boehringer-Mannheim, Bromma, Sweden) in cell culture medium M199 (Life Technologies, Stockholm, Sweden) for 15 min at 37°C. The cells were added to 96-well tissue culture plates (Corning, NY, USA). After 1 week, the cells were confluent as judged from microscopic examination, and were used for adhesion experiments.

#### Adhesion experiments

Lipopolysaccharide (LPS) from *Salmonella typhimurium* (Sigma) at a final concentration of 1 µg/ml was incubated with the cultured endothelial cells for 4 h at 37°C and washed once. Granulocytes from the psoriasis patients and the blood donors were separated on Ficoll-Hypaque (Nygaard, Oslo, Norway) by density gradient centrifugation (22). Cells ( $3-5 \times 10^3$ ) were allowed to adhere to the HUVEC cells for 30 min at 37°C and then washed once. The peroxidase content of adherent granulocytes in each well was determined with a 3,3',5,5'-tetramethylbenzidine liquid substrate system (Sigma). The percentage of adherence was determined by dividing the absorbance values of the adherent cells with the absorbance values of the total amount of cells added to the wells (23).

#### Statistical evaluation

Comparison between groups was made using unpaired Student's *t*-test (two tailed). A *p*-value of less than 0.05 was considered significant.

## RESULTS

### Expression of integrin CD11b/CD18

CD11b expression was monitored in a control group of blood donors, patients with extensive and active psoriasis vulgaris and patients with generalized pustular psoriasis. Monocytes from the blood donors showed a mean fluorescence intensity (MFI) of 15.7 at 4°C. After 15 min incubation at 37°C, a rise to 41.0 MFI was seen for the monocytes. Further stimulation with fMLP caused a 50% rise in the CD11b expression to 60.9 MFI (Fig. 1a). A similar pattern was observed for granulocytes (Fig. 1b). No increase in CD11b expression for either the monocytes or the granulocytes was observed in the psoriasis vulgaris group despite active disease and leukocytosis. In contrast, both granulocytes and monocytes from the patients with active pustular psoriasis showed significantly higher MFI values at both 4°C and 37°C. Not only did these cells express more CD11b molecules on their surface when isolated

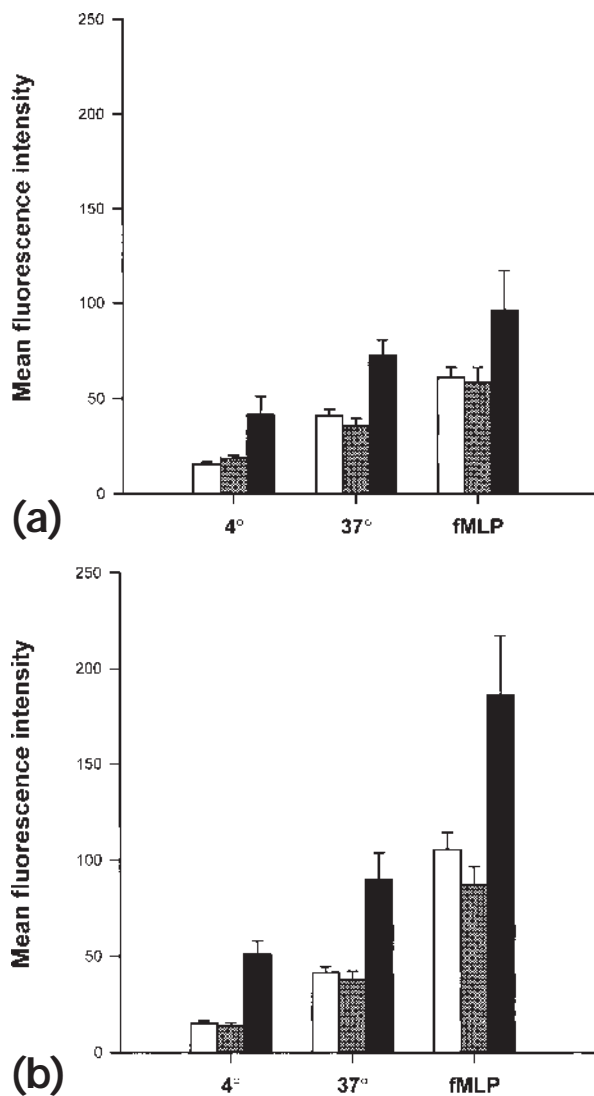


Fig. 1. CD11b expression in (a) monocytes and (b) granulocytes isolated from whole blood. The cells were kept on ice or incubated at 37°C with or without fMLP. The figure shows healthy blood donors (□) ( $n=40$ ), patients with psoriasis vulgaris (▨) ( $n=16$ ) and patients with pustular psoriasis (■) ( $n=4$ ).

from the blood, but they also had a larger intracellular pool, since activation at 37°C with or without fMLP caused an up-regulation of CD11b that significantly exceeded ( $p < 0.001$ ) that of the control cells.

Both the psoriasis vulgaris patients and the pustular psoriasis patients showed signs of leukocytosis, as reflected by the increased number of cells in the cell preparations for the flow cytometer analysis (Table I).

*Expression of L-selectin*

Expression of L-selectin was measured on leukocytes kept at 4°C since incubation at 37°C causes a decrease in MFI (values not shown). No difference between the blood donors and the psoriasis vulgaris patients was seen. The MFI values for L-selectin on granulocytes from 2 patients with pustular psoriasis were within the ranges of the normal blood donors (46.6 and 20.3 MFI, respectively).

*Adhesion to HUVEC*

Adhesion of granulocytes to HUVEC monolayers is shown in Fig. 2. The percentage of granulocytes adhering to non-activated HUVEC was low for both controls and psoriasis vulgaris patients (11 and 8%, respectively).

There was a significant increase in the number of adhering granulocytes when the endothelial cells were activated by LPS for 4 h. However, no difference in the percentage of adhering granulocytes was seen between control and psoriasis cells; both increased from 8–11% to 30%. The granulocytes stimulated with fMLP prior to incubation with unstimulated HUVEC showed no increase in adhesion despite increased CD11b expression. It was not possible to perform adhesion experiments with cells from pustular psoriasis patients since these cases were rare and acute. The HUVEC preparations were not available on these occasions.

*Effect of retinoid treatment on CD11b expression*

CD11b expression on leukocytes isolated from patients with active pustular psoriasis was evaluated before and during treatment with the retinoic acid derivative, etretinate. Fig. 3a shows the expression for CD11b on unstimulated monocytes for 2 of 3 patients and Fig. 3b the granulocytes from the same patients. A dramatic decrease in the amount of CD11b molecules expressed on the cell surface occurred during treatment. In parallel, their skin cleared of pustule formation and redness. A normal receptor expression was observed on the patients cells

Table I. The number of leukocytes in the cell preparation from controls and psoriasis blood from flow cytometry measurements

Group	n	Number of monocytes <sup>a</sup>	Number of granulocytes <sup>a</sup>
Psoriasis vulgaris	16	843 ± 139	12,864 ± 2,311
Pustular psoriasis	4	1,278 ± 273	15,952 ± 4,982
Blood donors	5	571 ± 174	4,336 ± 958

<sup>a</sup> Mean ± SEM for the number of cells analysed in a 25 µl cell suspension.

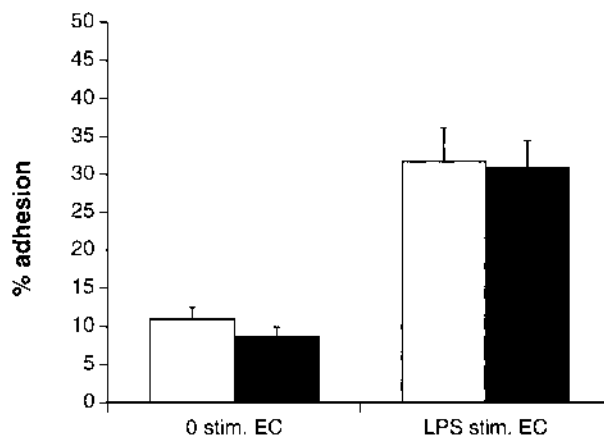


Fig. 2. Adhesion of granulocytes from blood donors (□) (n=20) and patients with psoriasis (■) (n=16). The endothelial cells were unstimulated (0 stim) or LPS stimulated.

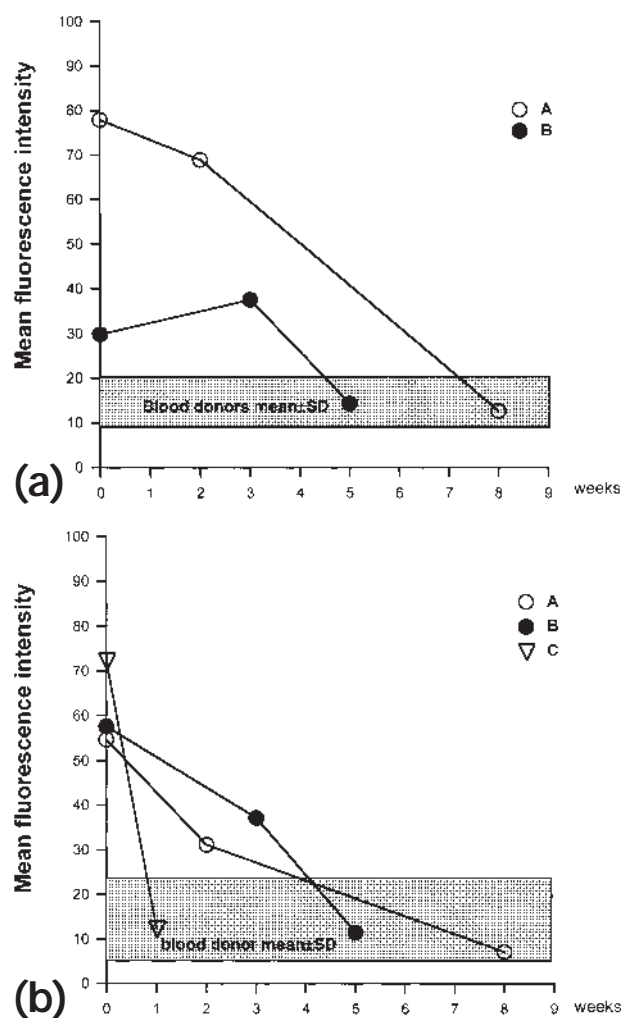


Fig. 3. CD11b expression in (a) monocytes and (b) granulocytes from patients A, B and C with pustular psoriasis before and during etretinate treatment. The shaded area depicts the blood donor group (n=40) (mean ± SD). Only the values for 4°C incubation are shown.

after 1, 5 and 8 weeks, respectively. One patient had an acral pustular psoriasis with a subsequent clearing of pustules and normal CD11b expression after 1 week of treatment.

#### CD11b expression in 2 patients tested over a period of time

Fig. 4 shows the CD11b expression on granulocytes of a patient who had had recurrent pustular psoriasis (von Zumbusch) but had no active disease at the time of the first blood sample. A month later she had some red scaling skin without any visible pustules. The expression of CD11b was somewhat decreased compared with that of controls and psoriasis vulgaris patients. On the third occasion the patient had active, spreading disease but no visible pustules and showed normal CD11b expression. The patient was not on any medication. The monocytes showed the same pattern of expression as the granulocytes (results not shown).

Another patient, shown in Fig. 5, with subacute annular generalized pustular psoriasis (24) was followed at intervals for 1 year. The patient had no fever during active disease and spontaneous healing occurred quickly. The patient was not on any medication when samples I–IV were collected.

The patient showed, at the time of the first blood sample (I), a widespread scaling rash with isolated annular formations of pustules. The monocytes and granulocytes showed a significantly increased CD11b expression. Two days later, at the time of the second sample (II), the pustules were drying up and no new pustules were seen. Although CD11b expression on the granulocytes had dropped to a normal level, the expression of CD11b on the monocytes remained high. A week later (III) the pustules had cleared and a slight redness with scaling remained in a few areas. At this time both the granulocytes and monocytes showed normal CD11b values. No significant changes were seen on the next occasion despite the patients suffering from red and scaling skin (IV). Etretinate was given after sample IV. Two months later the fifth blood sample (V) was taken at a time when the skin was almost clear. No changes in CD11b expression were observed.

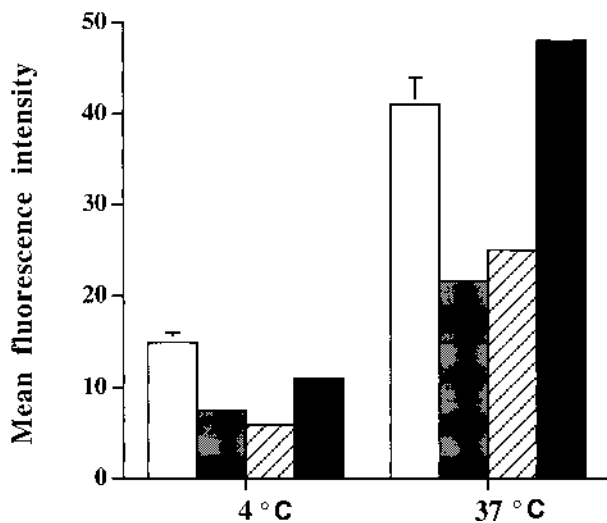


Fig. 4. CD11b expression in granulocytes held at 4°C and at 37°C for 15 min from 1 patient who had had recurrent pustular psoriasis on 3 separate occasions (▨, ▩ and ■) and the mean ± SE of 40 healthy blood donors (□).

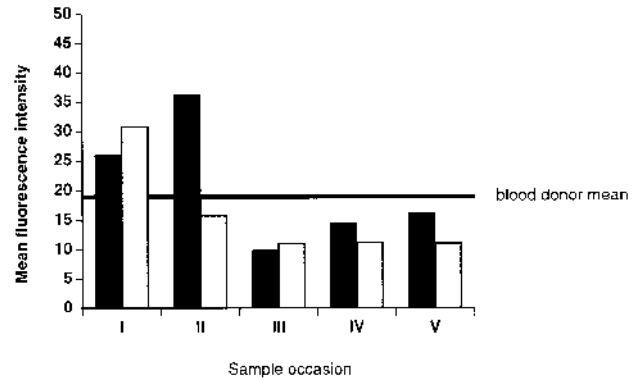


Fig. 5. The CD11b expression in monocytes ■ and granulocytes ▨ held at 4°C from a patient with subacute annular generalized pustular psoriasis at different stages of the disease. Initial pustule formation (I), maximal pustule formation (II), spontaneous healing had started (III), still active psoriasis but no pustules (IV) and treated and healed skin (V). Etretinate treatment was given after sample IV. The blood donor mean is based on  $n=40$ .

#### DISCUSSION

This study, although including only a limited number of patients suffering from active generalized pustular psoriasis, showed that peripheral blood granulocytes and monocytes from these patients with active disease expressed more CD11b on their cell surface than patients without pustules and patients with psoriasis vulgaris. The high CD11b expression correlated well with pustule formation and normalization of CD11b coincided with the clearing of the pustules. The delayed normalization of integrin expression in monocytes could be due to the slower turnover of monocytes compared with granulocytes. It is also suggested by van Pelt et al. (3) that CD11b bearing cells, other than neutrophils are found early in psoriatic lesions.

Cells from patients with generalized pustular psoriasis also showed a large intracellular and mobilizable pool of CD11b, since fMLP stimulation caused an enhanced expression that significantly differed from normal blood donors and the other psoriasis patients. It has previously been shown that fMLP treated cells expose 85% of the total amount of CD11b (25) which argues against a suboptimal stimulation of controls and other psoriasis patients.

The fact that no upregulation of CD11b or shedding of L-selectin was observed in neutrophils or monocytes from psoriasis vulgaris patients with inflamed skin and leukocytosis, indicate that inflammation *per se* is not necessarily associated with the upregulation of these molecules on circulating cells.

In fact van Pelt et al. (26) showed a decreased expression of CD11b on circulating neutrophils from patients with extensive plaque psoriasis. This could relate to our observation that 1 patient with pustular psoriasis at a time without visible pustules showed decreased CD11b expression.

There are several reports of increased adhesion molecules in both lesional and non-lesional skin from psoriasis patients (27–29) However, no difference in adhesion to HUVEC was discerned between granulocytes from controls and from psoriasis vulgaris patients despite activation of endothelial cells with LPS to enhance E-selectin and ICAM-1 expression (13, 30). Yokochi et al. (31) showed a slightly increased expression of the adhesion molecule on granulocytes, CD15s, which binds to E-selectin in patients with pustulosis palmaris et plantaris.

This was, however, not correlated with functional adhesion studies. They also showed a higher expression of CD11b on granulocytes in the patients being treated. This might explain why they observed a smaller difference in CD11b expression between the groups than we found in the present study.

The pronounced upregulation of CD11b in granulocytes and monocytes during pustule formation and normalization during retinoid treatment suggest that enhanced expression of  $\beta_2$  integrins may play a central role during pustule formation.

Although retinoids did not affect the expression of CD11b in unstimulated or stimulated blood donor granulocytes *in vitro*, they exhibit several anti-inflammatory effects. Degranulation and oxidative activation in neutrophils are suppressed (32, 33) and granulocytes from psoriasis patients treated with retinoids show reduced chemotactic activity (34). Topical application of retinoids blocks LTB<sub>4</sub> stimulated migration of leukocytes into the skin (35) and LTB<sub>4</sub> may play an important role in plaque and pustule formation (17). Thus, it is clear that retinoids, either applied topically or given orally, can regulate different functions of neutrophils.

The fact that a decrease in CD11b expression in patients with generalized pustular psoriasis parallels skin healing during retinoid treatment does not necessarily link the effect to cell adhesion and homing. It could also reflect an anti-inflammatory effect resulting in impaired generation of  $\beta_2$  integrin-activating inflammatory mediators.

The increased CD11b expression and enhanced mobilization of CD11b found in circulating cells from pustular psoriasis patients could therefore be a prerequisite for the massive migration of inflammatory cells into the inflamed skin and could be an early sign of a triggering factor for a pustular bout. This correlation is demonstrated in 1 patient with pustular psoriasis (Fig. 5). Initially, a high CD11b expression on granulocytes and monocytes was found together with pustules, but when no pustules were present, no increase in CD11b was detected even though the patient's skin still showed active disease.

The mechanism that controls cellular priming and upregulation of CD11b in pustular psoriasis is unclear. The fact that granulocytes from patients with generalized pustular psoriasis contain and express more CD11b during pustular stages of the disease offers a link between cell activation, pustule formation and inflammation in the skin. We hypothesize that the increased CD11b expression does not affect psoriasis vulgaris but can facilitate the acute form of generalized pustular psoriasis.

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