

Short communication

Different expression of adhesion molecules and tetraspanins of monocytes of patients with atopic eczema

Background: Atopic eczema (AE) and psoriasis vulgaris (Pso) represent the most frequent chronic inflammatory skin diseases, which have a high number of characteristics in common but differ in their clinical picture and immunological background. A shared feature of both AE and Pso is a high recruitment of distinct proinflammatory cells from the blood into the skin at the initiation of the disease. A multistep adhesion cascade via different adhesion receptors consisting of 'tethering' and 'rolling' mediated by selectins, α -integrins and β -integrins and the 'arrest' of the cells is initiated during this process.

Aims of the study: To evaluate the expression of adhesion molecules and tetraspanins of monocytes of patients with AE and Pso in comparison with healthy controls.

Methods: We analysed the expression of adhesion molecules and tetraspanins on monocytes freshly isolated from the peripheral blood of patients with AE ($n = 40$) and Pso ($n = 65$) during exacerbation of their disease in comparison with healthy, non-atopic controls ($n = 50$).

Results: A high number of similarities between monocytes of patients with AE and patients with Pso, and disease-related differences in the expression of CD62L, CD62P, CD11a, CD11b, CD11c, CD49b, CD49d, CD49e and CD18 and the tetraspanins CD9, CD53, CD63 and CD151, which were elevated on monocytes of patients with AE could be observed.

Conclusion: A distinct expression pattern of adhesion molecules and tetraspanins on monocytes of patients with AE and Pso might influence the recruitment process of inflammatory precursor cells and facilitate new approaches for therapeutic strategies aimed at interrupting the very earliest steps of the fateful recruitment process.

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Atopic eczema (AE) and psoriasis vulgaris (Pso) are the most common chronic inflammatory skin diseases (1, 2). Both of them are genetically inherited and dominated by a complex immunological network. From the clinical point of view, pruritic erythematous skin lesions of the flexural folds characterize AE, while psoriasis is characterized by erythematous plaques, which are typically found at the elbows and knees of the patients. In AE, CD4-activated T-helper 2 (Th2) T-cells, which produce interleukin (IL)-4, IL-5 and IL-13 prevail especially in the acute phase, while Pso is predominated by Th1 immune responses which are driven by interferon (IFN)- γ -secreting T cells.

Abbreviations: AE, atopic eczema; NA, non-atopic, non-psoriatic healthy donors; Pso, psoriasis; RFI, relative fluorescence intensities; MFI, mean fluorescence intensities.

In the initiation of both diseases, homing of distinct proinflammatory cells such as monocytes and T cells into the skin is regarded as a crucial mechanism which forms the basis for the outcome of the inflammatory immune response in the skin (3–5).

The extravasation of monocytes from the blood into the peripheral tissue consists of tethering, rolling, adhesion and diapedesis and involves a high number of adhesion molecules such as selectins, α -integrins, β -integrins or tetraspanins. Adhesion receptors stabilize the interaction of monocytes with corresponding receptors on the vascular walls of the blood vessels. Further on, it is known that monocyte binding to endothelial cells of the blood vessels is deeply dependent on the state of inflammation, and monocytes have to activate several adhesion pathways in order to leave the blood circulation. In this process, adhesion molecules serve not only as a kind of 'mechanical bond', but also as selective

recognition molecules, which direct the tissue-specific trafficking of these cells.

Materials and methods

Patients and diagnostic criteria

A total of 40 patients (21 females, 19 males) with moderate to severe AE (age 33 ± 13 years; range 14–68) (serum IgE level 1000 ± 800 kU/l), 65 patients (34 females, 31 males) with Pso (age 50 ± 17 years; range 23–81) (serum IgE level 298 ± 489 kU/l) and 50 healthy volunteers (NA; 28 females, 22 males) (serum IgE level 59 ± 104 kU/l), without AE or a positive family history for atopy, atopic eczema or psoriasis were recruited (age 30 ± 7 years; range 22–56). The protocol was approved by the local ethics committee and a written informed consent was obtained from all patients and controls included in the study. AE was diagnosed according to the criteria of Hanifin and Rajka.

Reagents

Fluorescein isothiocyanate (FITC)-labeled F(ab')₂ fragments of goat-anti-mouse were purchased from Dianova (Hamburg, Germany), phycoerythrin (PE)-labeled monoclonal antibody (mAb) against CD14 (M5E2) and unlabeled mouse-IgG2a (G155-178) from Becton Dickinson (Mountain View, CA, USA). The mAb against CD11a (HI11.1), CD11b (ICRF44), CD11c (B-ly6), CD41 (HIP8), CD49a (SR84), CD49b (12F1-H6), CD49c (C3II.1), CD49d (9F10), CD49e (IIA1), CD62E (68-5H11), CD62L (Dreg-56), CD62P (AC1.2), CD18 (CR3/CR4 6.7), CD29 (MAR4), CD61 (RUU-PL7F12), CD104 (450-9D), CD9 (M-L13), CD37 (M-B371), CD53 (HI29), CD63 (H5C6), CD81 (JS-81) and CD151 (14A2.H1) were purchased from Becton Dickinson. The mAb mouse-anti-CD82 (B-L2) was purchased from Acris (Hiddenhausen, Germany). All other chemicals were from Sigma Chemicals Co (St. Louis, MO, USA) unless otherwise indicated.

Analysis of total-serum IgE and allergen-specific IgE

Serum levels of total and allergen-specific IgE against birch pollen in the sera of the patients were analyzed with the Immulite system (DPC Biemann, Bad Nauheim, Germany).

Monocyte isolation

To evaluate the quantitative differences of monocytes, peripheral blood from the donors was drawn into tubes containing ethylenediaminetetraacetic acid (EDTA) and monocytes were isolated with a modified density-gradient protocol after using Nycoprep® (Nycomed, Oslo, Norway) as described previously (6).

Flow cytometric analysis

Labeling of monocytes was performed as reported previously in detail (7). The viable CD14⁺ monocytic cell population was analyzed on a FACSCalibur (Becton Dickinson). For quantitative evaluation, two-dimensional dot-plot analysis was performed and the CD14 population was gated out manually by a combination of forward and side scatter (FSC/SCC) and FL2/FL3 (CD14⁺/7AAD) gates. The mean fluorescence intensities (MFI) were determined by Cell-Quest software (Becton Dickinson). The values of the relative

fluorescence intensity (RFI) were calculated by the following equation:

$$RFI = \frac{MFI(\text{specific staining}) - MFI(\text{control staining})}{MFI(\text{control staining})}$$

Statistical analysis

Statistical analysis using Mann–Whitney *U*-test was performed with SPSS 12.0 for Windows (SPSS, Chicago, IL, USA). Calculated values shown were the percentage of positive cells or RFI of positive cells $\pm$ standard error of mean (SEM).

Results and discussion

The expression pattern of adhesion molecules and tetraspanins of monocytes directs the adhesion, diapedesis and migration of these cells from the blood vessels into the peripheral tissue.

Selectins interact with their ligands on endothelial cells and are capable of slowing down monocytes within the blood vessels. Therefore, in the first set of experiments, we studied the expression of selectins on the cell surface of monocytes isolated from the peripheral blood of patients with AE, Pso and NA. We observed an increased expression of L-selectin (CD62L) on monocytes of patients with AE compared with monocytes of patients with Pso and NA (Fig. 1). In contrast, the surface expression of P-selectin (CD62P) was higher on monocytes of patients with AE but low on monocytes from patients with Pso (Fig. 1). E-selectin (CD62E) was not expressed on monocytes at all (data not shown). The

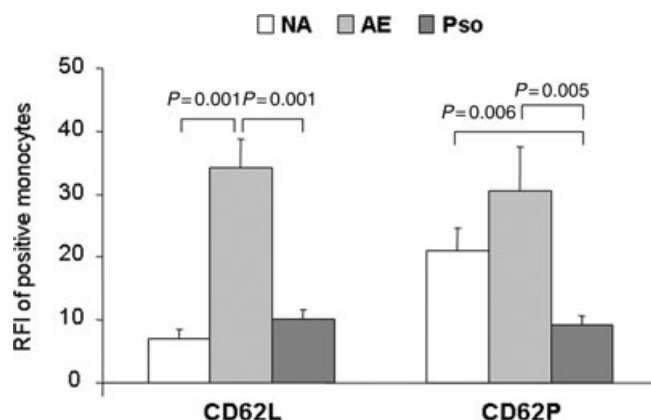


Figure 1. Surface expression of selectins of monocytes of patients with AE and Pso in comparison with healthy controls. Surface expression of CD62L and CD62P on freshly isolated monocytes of healthy volunteers (NA), patients with atopic eczema (AE) and patients with psoriasis (Pso). The RFI $\pm$ SEM of cells expressing the selectins is depicted on the y-axis. Open columns show the data from healthy volunteers, light grey columns show the data of patients with atopic eczema and dark grey columns show the data of patients with psoriasis.

enhanced expression of CD62L on monocytes of patients with AE might support cluster formation of CD62L, which is necessary to reduce the velocity of monocytes in the laminar blood flow (8). Because of the reduction of velocity, in the next step CD62P might be capable of interacting with its ligands on endothelial cells, thereby mediating the rolling and tethering of monocytes (8). Integrins have a pivotal role in the adhesion of monocytes to endothelial cells of the blood vessels and the extravasation of monocytes through the blood vessel wall into the tissue. To evaluate the expression pattern of integrins, we studied in the next set of experiments the expression of the α - and β -chains of integrins on monocytes of the different patient subgroups. We found that the expression

of CD11a, CD11b, CD11c, CD49b, CD49d, CD49e, CD18 and CD29 was increased on monocytes of patients with AE compared with patients with Pso, whereas monocytes of patients with AE showed a decreased expression of CD49c (Fig. 2A, B and C). Monocytes from patients with Pso displayed only an enhanced expression of CD49a in comparison with monocytes of healthy controls. No difference in the expression of CD41 on monocytes has been detected between the groups (data not shown) and CD104 was not expressed on monocytes at all.

The enhanced expression of CD18 and CD11a on monocytes of patients with AE implicates an enhanced expression of the complete CD11a/CD18 (LFA-1) heterodimer on these cells (Fig. 2A and C). This might cause an enhanced adhesion of monocytes of patients with AE to endothelial cells. It has been described that β_2 -integrins are important for the diapedesis of monocytes from the blood into the tissue in some diseases (9). Therefore, an enhanced expression of CD29 and CD49d on monocytes of patients with AE might lead to a higher capacity of these cells to migrate into the skin.

Another group of surface molecules involved in cell activation and migration are tetraspanins. Tetraspanins

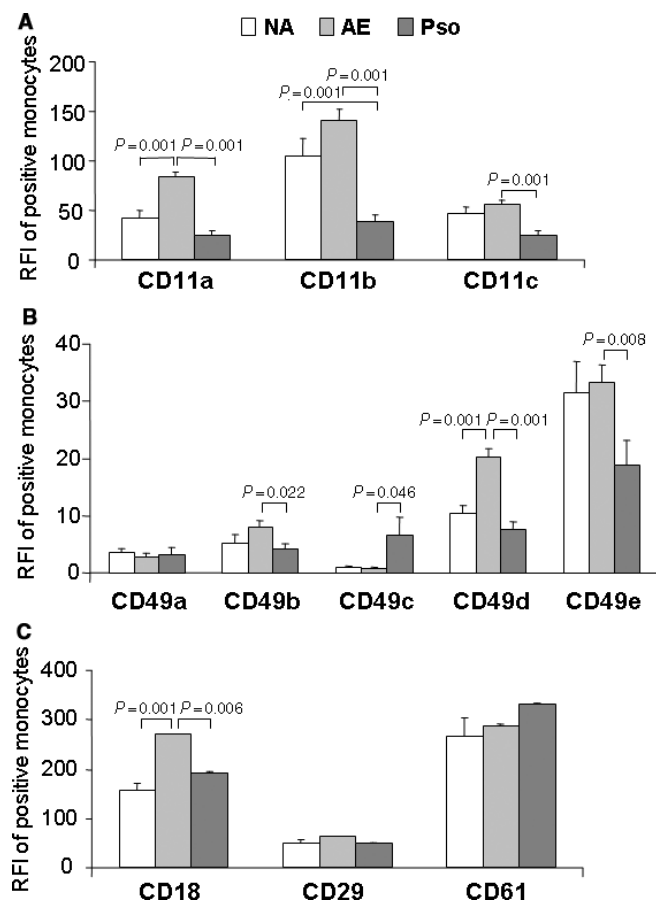


Figure 2. Surface expression of α -chains and β -chains of integrins of monocytes of patients with AE, Pso and healthy controls. Surface expression of (A) α -chains of integrins CD11a, CD11b, CD11c; (B) α -chains of integrins CD49a, CD49b, CD49c, CD49d, CD49e; (C) β -chains of integrins CD18, CD29 and CD61 on freshly isolated monocytes of healthy volunteers (NA), patients with atopic eczema (AE) and patients with psoriasis (Pso) are shown. The RFI $\pm$ SEM of cells expressing the α -chain of integrins is depicted on the y-axis. Open columns show the data from healthy volunteers, light grey columns show the data of patients with atopic eczema and dark grey columns show the data of patients with psoriasis.

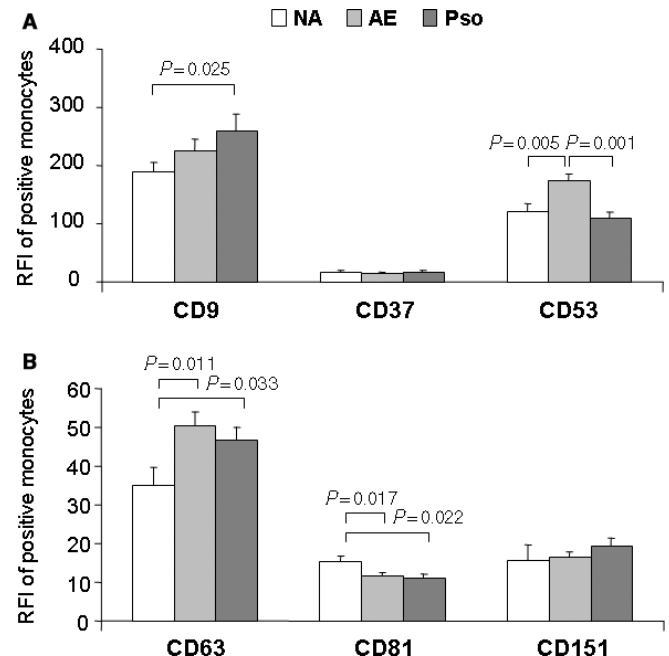


Figure 3. Surface expression of tetraspanins of monocytes of patients with AE and Pso in comparison with healthy controls. Surface expression of the tetraspanins: (A) CD9, CD37, CD53, and B: CD63, CD81 and CD151 on freshly isolated monocytes of healthy volunteers (NA), patients with atopic eczema (AE) and patients with psoriasis (Pso) is shown. The RFI $\pm$ SEM of cells expressing the tetraspanins is depicted on the y-axis. Open columns show the data from healthy volunteers, light grey columns show the data of patients with atopic eczema and dark grey columns show the data of patients with psoriasis.

interact with each other, thereby creating the 'tetraspanin-web' and are in close contact with several other proteins on the cell surface and in the cytoplasm, e.g. integrins, multiple Ig-superfamily members, proteoglycans, complement-regulatory proteins, growth factor receptors and ligands (10). Thereby they are capable of modulating the adhesion, migration, activation and proliferation of cells (10).

We found an enhanced surface expression of the tetraspanins CD9 and CD53 on monocytes from patients with Pso compared with NA (Fig. 3A). Further on, surface expression of CD63 was enhanced on both monocytes of patients with Pso and AE (Fig. 3A), whereas the surface expression of CD81 was downregulated on monocytes from patients with AE and Pso compared to monocytes from NA (Fig. 3B). Taken together, monocytes of patients with AE expressed elevated levels of the tetraspanins CD9, CD53, CD63 and CD151 in comparison with monocytes from patients with Pso and NA. In contrast, no difference has been found in the expression pattern of CD82 between monocytes of patients with AE or Pso and healthy donors (data not shown).

It is known that most of the tetraspanins interact with β_1 - or β_2 -integrins, which are also upregulated on the surface of monocytes of AE patients (11). As a consequence of the high tetraspanin expression, the affinity and avidity of monocytes from patients with AE for intracel-

lular adhesion molecule (ICAM)-1, ICAM-2, ICAM-3 and fibrinogen might be enhanced (12, 13) resulting in an enhanced adhesion of monocytes from patients with AE to endothelial cells and extracellular ligands such as laminin, fibronectin and fibrinogen (14, 15).

Taken together, mechanisms involved in the recruitment of inflammatory cells into the skin are crucial for the initiation of acute flare-ups of chronic eczematous skin diseases such as AE or Pso. Therefore, a detailed knowledge about the disease specific repertoire of adhesion molecules and tetraspanins, as well as the delineation of disease-specific and general characteristics in the expression profile of these molecules, which might represent a shared feature of different inflammatory skin diseases, are important for the development of effective therapeutic strategies. These treatment strategies might be aimed at targeting adhesion molecules or tetraspanins to selectively prevent the very earliest steps of the recruitment cascade.

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