Increased expression of adhesion receptors in both lesional and non-lesional psoriatic skin

O. J. de Boer 1,2, I. M. M. J. Wakelkamp 1, S. T. Pals 2, N. Claessen 2, J. D. Bos 1, P. K. Das 1,2

1 Department of Dermatology, University of Amsterdam, Academisch Medisch Centrum, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands
2 Department of Pathology, University of Amsterdam, Academisch Medisch Centrum, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

Received: 16 June 1993

Abstract. Adhesion receptors and their ligands play a vital role in the immune system. We studied the expression of different adhesion receptors, using single- and double-staining immunohistochemical techniques, in both lesional and non-lesional skin specimens from seven psoriasis patients and in skin biopsy specimens from eight normal healthy controls.

Our results showed an overall increased expression of several adhesion receptors in both lesional and non-lesional psoriatic skin. We consistently found an increased expression in particular of ICAM-1 and E-selectin on endothelial cells, and ICAM-1 on T cells and Langerhans cells. In contrast, a weak expression of VCAM-1 was found on endothelial cells and mononuclear cells in lesional psoriatic skin specimens alone. Interestingly, LFA-1 was also expressed on Langerhans cells, with a greater frequency in skin from lesional than from non-lesional sites, but was never expressed in skin from normal healthy individuals. Furthermore, significantly increased numbers of Langerhans cells and T cells with a positive reactivity for MAb HECA-452 were found in both lesional and non-lesional psoriatic skin.

We hypothesize that the enhanced expression of adhesion receptors on migrating immunocompetent cells and endothelial cells of psoriatic skin in general facilitates the increased influx of activated T lymphocytes and other immunocomponent cells into the skin, and thus underscores the generalized character of the disease.

Key words: Psoriasis - Adhesion receptors - CLA - Immunohistochemistry

Psoriasis is an inflammatory skin disease of unknown aetiology which spontaneously flares and subsides. Histologically, psoriatic skin is characterized by hyperproliferation of keratinocytes, increased vasculature and cellular infiltrates predominantly comprising neutrophils, memory CD4+ T cells and CD1a+ dendritic cells [5, 7, 36]. In lesional psoriatic skin, clusters of activated T cells are often seen in close contact with macrophages and Langerhans cells (LC) in both the papillary and reticular dermis [6]. The role of these immunocomponent cells in psoriasis is unknown, but the beneficial effect of immunosuppressive drugs (e.g. cyclosporin A) indicates that T cells play an important role in the pathogenesis of the disease [25, 49].

The extravasation of lymphocytes from the vascular compartment into sites of chronic inflammation, for example psoriatic skin, is a receptor-mediated process [24]. Several receptors expressed by endothelial cells and lymphocytes involved in adhesion and homing into various tissues have been identified [11, 32]. Recently it has been reported that 80–90% of the lymphocytes in inflammatory skin diseases, including psoriasis, express the cutaneous lymphocyte-associated antigen (CLA) recognized by MAb HECA-452 [40]. This molecule has been suggested to be the homing receptor for lymphocyte migration into skin [40]. One of its ligands, E-selectin [3] (formerly known as ELAM-1), has been found to be upregulated in lesional psoriatic skin [21]. As well as lymphocytes and endothelial cells, keratinocytes also show an increased expression of several adhesion receptors, for example VLA-3, -5, -6 [26] and ICAM-1 [20, 22].

The increased expression of various adhesion receptors in lesional skin reflects an ongoing local immune response, accompanied by a frequent influx of inflammatory cells into the lesions. The cellular influx presumably represents an important feature in the chain of events leading to the perpetuation of the disease. It is known that minor trauma to clinically non-lesional skin can lead to the development of lesions. Indeed, several studies have shown that abnormalities in non-lesional skin are related to cellular activation [14, 42]. In addition, Pellegrini et al. [38] have found that keratinocytes in both lesional and non-lesional psoriatic skin show an altered integrin distribution on their surface. In most other studies concerning adhesion receptor expression, the immunohistochemistry of lesional psoriatic skin has been compared with either non-lesional or normal healthy skin.
as the only control [34], and the data have not always been evaluated simultaneously.

In this study we applied single- and double-staining immunohistochemical techniques to examine objectively the interacting cell types expressing various adhesion receptors in both lesional and non-lesional skin. The results were then compared with those obtained from normal human skin. Our study concentrated on those adhesion receptors that are presumed to be responsible for cellular influx into the skin [39]. We found that there is a generalized upregulation in the expression of adhesion receptors in psoriasis, supporting the view that clinically non-lesional skin is in an activated state [15, 16, 29].

### Patients and methods

#### Patients

Seven patients (three males, four females; mean age 39.3 ± 14.6 years) who had not received local or systemic treatment during the previous 2 months were selected for the study. The status of the disease was scored by means of psoriasis area severity index (PASI) [17] and was found to be in the range 2.5–16. The disease activity of all the patients as compared with previous visits was assessed as stable. All patients gave their informed consent to donate skin specimens for investigation. Biopsy specimens from eight age-matched healthy volunteers with no history of skin abnormalities and skin specimens obtained during plastic surgery were used as normal controls.

#### Specimens

Punch biopsy specimens (4 mm), were removed from the trunk under local lidocaine/adrenalin anaesthesia, immediately frozen in liquid nitrogen and stored at −80 °C. Specimens of lesional psoriatic skin were always taken from the inside border of the lesional plaque. Additional biopsy specimens were taken from a non-lesional area of the same donor at a location away from the lesional plaque. Additional biopsy specimens were taken from the trunk under local lidocaine/adrenalin anaesthesia, immediately frozen in liquid nitrogen and stored at −80 °C. Specimens of lesional psoriatic skin were always taken from the inside border of the lesional plaque. Additional biopsy specimens were taken from a non-lesional area of the same donor at a location away from the lesional site where no newly developing lesions had been observed by the patients for the previous 2 months. Cryostat sections (5 μm) were cut, fixed in cold acetone (4 °C), and stored at −20 °C until use.

#### Monoclonal antibodies and conjugates

Leu4 (CD3), Leu3a (CD4), Leu2a (CD8) were obtained from Becton & Dickinson (Mountain View, Calif, USA). OKT6 (CD1a) was purchased from Ortho Diagnostics (Raritan, N.J., USA). Anti-F-VIII-related antigen (specific for endothelial cells), CD68 (clone EBM-11) and CK-1 (cytokeratin) were from Dakopatts (Glostrup, Denmark). Anti-LFA-1 (CD11a, clone CLB-LFA-1/2) was obtained from the central laboratory of the Red Cross Blood Transfusion Service, Amsterdam, The Netherlands. Anti-ICAM-1 (CD54) was from British Biotechnology, Abingdon, UK. Anti-E-selectin (clone I.2B6) was a gift from Dr. D. Haskard, Rheumatology Unit, Postgraduate Medical School, London, UK [30]. Anti-VCAM-1 (clone 4B9) was a gift from Dr. J. Harlan, Harborview Medical Center, Seattle, Wash. [10]. Horseradish peroxidase (HRP)-conjugated rabbit anti-mouse immunoglobulins (RAM), HRP-conjugated rabbit anti-fluorescein isothiocyanate (FITC) Ig, biotin-conjugated rabbit anti-mouse Ig (RAM-HRP), and streptavidin–biotin complex with HRP (SABC-HRP) were obtained from Dakopatts. Isotype-specific AP-conjugated goat anti-mouse IgG1 and HRP-conjugated goat anti-mouse IgG2a, IgG2b and IgM were obtained from Southern Biotechnology (Birmingham, AL, USA). AP-conjugated RAM was obtained from TAGO (Burlingame, Calif.).

### Immunohistochemistry

Single immunoenzymatic stainings were performed using a three-step indirect peroxidase technique [31] and for stainings with VCAM-1 the streptavidin–biotin immunoperoxidase method was used [23]. Briefly, in the former technique MAb were applied to the sections, which were then incubated with RAM-HRP followed by SAR-HRP. In all cases peroxidase activity was visualized by 3-amino-9-ethyl carbazole [19]. The sections were counterstained with haematoxylin. In the streptavidin–biotin method the sections, after incubation with the primary antibody, were further incubated with RAM-BIO followed by SABC-HRP. Control sections were incubated following the same method, but with substitution of the MAb by PBS.

Depending on the MAb combination double immunoenzymatic stainings were performed using three different techniques that have been optimized and are routinely used in our laboratory [30, 31]. For most of the double-staining experiments combinations of an FITC-labelled and an unlabelled MAb were used as the primary steps [32]. Using this technique, double stainings with CD3, CD4, CD8, and CD1a on the one hand, and HECA-452, LFA-1, VCAM-1 and ICAM-1 on the other were performed. The second double-staining method consisted of the combination of a polyclonal and a monoclonal antibody [33]. This double-staining protocol was used with the combinations F-VIII/ICAM-1 and F-VIII/E-selectin. In the third double-staining protocol, highly specific second-step antibodies were used only when the primary antibodies had different isotypes [47]. This protocol was used for the double stainings with CD68 against LFA-1 and HECA-452.

### Evaluation of results

Sections were examined by light microscopy. For the quantification of the percentages of the different cell types expressing relevant adhesion receptors, double- and single-stained cells were counted in each of the following layers: epidermis, free in the papillary dermis, perivascular in the papillary dermis, free in the reticular dermis and perivascular in the reticular dermis [7]. The cell numbers were first expressed as a percentage of the total number of a particular subgroup of immunocompetent cells within each skin compartment. Finally, the mean percentages of cell types expressing different adhesion receptors were determined by totalling the number of each particular subgroup of single- and double-stained cells over the whole tissue section.

Statistical evaluation of the results was carried out using one-way analysis of variance (ANOVA) followed by a two-sample t-test. P-values < 0.05 were considered significant.

### Results

#### Expression of adhesion receptors on the various cell types

The expression of adhesion receptors on the various cell types was evaluated first by single immunohistochemical staining of serial sections, and the exact localization of