

## Interleukin-8 Receptors in Normal and Psoriatic Polymorphonuclear Leukocytes

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**Polymorphonuclear leukocyte (PMNL) infiltration is an important characteristic in psoriatic lesions. The proinflammatory 8-kD peptide interleukin-8 (IL-8) is present in psoriatic scales and possesses a high chemotactic activity on human neutrophils, which may relate to its role in psoriasis. Its chemotactic activity is mediated via specific receptors on PMNL. The goal of our work was to ascertain whether PMNL infiltration in psoriasis can be accounted for by functional abnormalities of the circulating PMNL due to alterations in the IL-8 receptor density or affinity (or both). Results of radioligand binding studies performed in 10 psoriatic patients, 10 patients with atopic eczema and 11 normal controls showed no difference in receptor affinity ( $K_d$ ) between the groups. However, a slight but significant elevation in IL-8 receptor density was seen on PMNL from psoriatic individuals ( $31,230 \pm 3,237$  binding sites per cell) compared to those from normal volunteers ( $24,152 \pm 2,643$ ) and atopic eczema patients ( $24,092 \pm 2,743$ ). Increased number of IL-8 receptors may, besides elevated cutaneous IL-8 concentrations, contribute to the intraepidermal accumulation of PMNL in psoriasis. Key words: Interleukin-8; Receptors; Psoriasis; Leukocytes.**

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The possible involvement of IL-8 in various skin diseases, particularly psoriasis, has been the subject of a number of recent studies (1–3). The cytokine has been isolated from psoriatic plaques (2) and has been shown to be a selective chemoattractant for neutrophils (4, 5) acting via specific receptors. Recent reports described approx 20,000 IL-8 receptors per cell on human neutrophils, exhibiting a single type of high affinity binding with a  $K_d$  of  $8 \times 10^{-10}$  mol/l (6) or 75,000 receptors per cell with a  $K_d$  of  $4 \times 10^{-9}$  mol/l (7). The goal of the present investigation was to ascertain whether the intraepidermal accumulation of polymorphonuclear leukocytes (PMNL) in psoriasis can be accounted for, in addition to increased interleukin-8 (IL-8) levels, also by functional abnormalities of the circulating PMNL due to alterations in the IL-8 receptor density and/or affinity. For this purpose, we measured the

binding of IL-8 to PMNL in patients with psoriasis and compared these findings with those in another chronic inflammatory dermatosis (atopic eczema) and healthy controls.

### PATIENTS AND METHODS

#### Patients

Ten patients with psoriasis (5 males and 5 females, mean age 48.7 years, range 19 to 86 years), 10 patients with atopic eczema (5 males and 5 females, mean age 34.7 years, range 20 to 61 years) and 11 healthy volunteers (4 males and 7 females, mean age 46.9 years, range 17 to 81 years) participated in the study.

The psoriasis group consisted of 5 patients with the chronic plaque type, 2 patients with the guttate type, 2 patients with the localized, and 1 patient with the generalized pustular type. The extent of cutaneous involvement was up to 10% of the body surface in 2 patients, 10–25% in 2 patients, and more than 25% in 6 patients.

Atopic eczema has been diagnosed according to clinical criteria as previously described (8). The extent of disease was 10–25% of the body surface in 2 patients and more than 25% in 8 patients.

Excluded from the study were patients who had received treatment for their skin disease within the previous 4 weeks.

#### Preparation of PMNL

PMNL were isolated as previously described (9). Briefly, blood was centrifuged at 150 g for 15 min. The plasma was discarded, cell pellet was suspended in saline and layered over a Ficoll-Metrizoate solution at a density of 1.077 g/ml. After centrifugation at 1,450 g for 20 min, the PMNL layer was taken off and washed with saline. Erythrocytes were removed by hypotonic lysis with distilled water, platelets by repeated centrifugation in bovine serum albumin solution (Sigma, Deisenhofen, Germany). The final PMNL pellet was resuspended in Hanks' balanced salt solution with phenol red (HBSS; Flow, Meckenheim, Germany) containing 10 mM HEPES buffer (pH 7.3; Flow). The resulting cell suspension contained more than 90% neutrophils with a viability of more than 95% as assessed by trypan blue exclusion.

#### Measurement of the binding of [ $^{125}$ J]-IL-8 to PMNL

Human recombinant interleukin-8 containing 72 aminoacids with a molecular weight of 8 kD was a kind gift from Dr. I. Lindley (Sandoz, Vienna, Austria) and was iodinated by the chloramine-T method (7) to obtain a specific activity of 350–500 Ci/mmol. Aliquots of PMNL suspension ( $10^6$  cells) were pipetted into Eppendorf tubes, centrifuged and the supernatant was discarded. Then increasing concentrations (0.1–10 nmol/l) of [ $^{125}$ J]-IL-8 in 200  $\mu$ l buffer were added to the cell pellet to start the incubation, which makes it possible to determine the dissociation constant  $K_d$  and the receptor density  $B_{max}$  of the cells. Incubation was performed for 90 min at 4°C to avoid ligand internalization (7). After incubation, cells were washed three times with icecold PBS. Radioactivity was determined in a Minigamma 1275 gamma counter (LKB, Freiburg, Germany). Nonspecific binding was determined in the presence of 0.1–10  $\mu$ mol/l unlabelled IL-8 which was pipetted together with [ $^{125}$ J]-IL-8. Specific binding was defined as total binding (measured in the absence of unlabelled IL-8) minus nonspecific binding.

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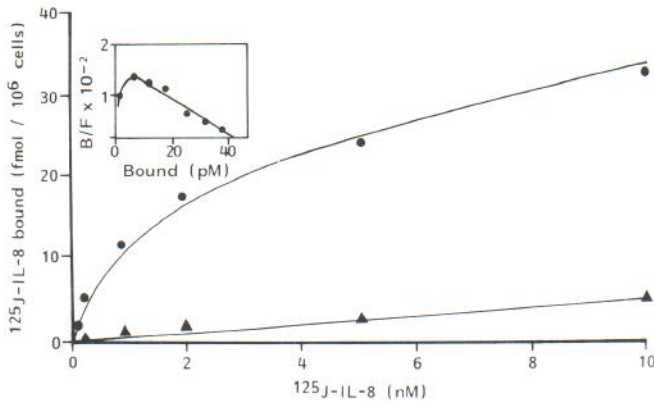


Fig. 1. Binding of IL-8 to normal PMNL as a function of increasing concentrations of [ $^{125}\text{J}$ ]-IL-8. Total binding (●), nonspecific binding (▲) The insert shows the Scatchard plot derived from the specific binding data.

#### Analysis of binding data

All binding experiments were done in duplicate. Computer analysis of binding data was performed by nonlinear curve fitting program MxN-FIT (10). Student's *t*-test was used for statistical analysis.

## RESULTS

Fig. 1 illustrates the binding of [ $^{125}\text{J}$ ]-IL-8 to PMNL as a function of increasing concentrations of the radioligand. Curve fitting assuming the presence of a single class of binding sites gave a  $K_d$  of  $3.92 \pm 0.55$  nmol/l and a receptor density of  $24,152 \pm 2,643$  sites/cell of healthy controls. Curve fitting according to a two-site model did not improve the fit, suggesting that [ $^{125}\text{J}$ ]-IL-8 interacts with a single class of binding sites under the experimental conditions used. The Scatchard analysis of specific IL-8 binding showed a curvilinear pattern, similar to that obtained by Samanta et al. (6).

Fig. 2 summarizes the IL-8 receptor density on PMNL in patients with psoriasis, atopic eczema and healthy controls, as obtained by batch analysis of the saturation curves. Psoriatic PMNL showed a significantly elevated number of IL-8 receptors ( $31,230 \pm 3,237$  receptors per cell) compared with normal ( $24,152 \pm 2,643$  sites/cell,  $p < 0.05$ ) or atopic PMNL ( $24,092 \pm 2,743$  receptors/cell,  $p < 0.05$ ).

No difference was observed between  $K_d$  values from normal healthy and atopic eczema or psoriatic PMNL ( $K_d$  of  $3.92 \pm 0.55$  nmol/l,  $3.86 \pm 0.24$  nmol/l,  $4.09 \pm 0.47$  nmol/l;  $p > 0.05$ ) (Fig. 3). Within the psoriasis and atopic eczema group, binding parameters did not seem to differ between patients with different types and degrees of disease.

## DISCUSSION

Psoriatic lesions are characterized by, among other things, a pronounced inflammatory infiltrate which may be, at least partly, caused by the chemotactic activity of IL-8, since Northern blot analysis of psoriatic epidermis clearly demonstrated IL-8 mRNA which was not detected in normal skin. Moreover, psoriatic epidermis showed IL-8 immunoreactivity in the basal and upper levels which was absent in normal skin (1). The goal of the present investigation was to ascertain whether functional abnormalities of the circulating PMNL due to alter-

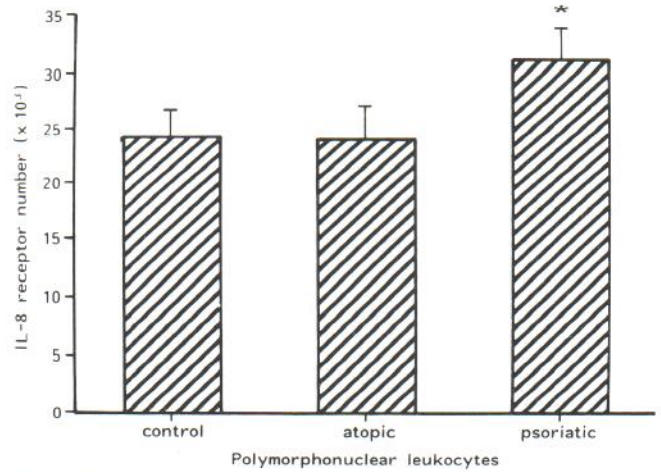


Fig. 2. IL-8 receptor density expressed as receptor number per PMNL obtained from healthy controls ( $n = 11$ ), patients with atopic eczema ( $n = 10$ ) and psoriasis ( $n = 10$ ). Values represent mean  $\pm$ SD.

ations in the IL-8 receptor density and/or affinity may contribute to the inflammatory infiltration in psoriatic skin. For this purpose, we performed radioligand binding studies in PMNL of patients with different types of psoriasis. For comparison, we studied a group of patients with another inflammatory dermatosis (atopic eczema) and healthy controls.

Using classical radioligand binding assays for the detection of cell surface receptors, we were able to demonstrate the presence of high-affinity IL-8 receptors on PMNL which was consistent with the findings of others (6, 7).

Psoriatic PMNL exhibited a significant increase in specific IL-8 binding compared to cells from healthy controls and atopic eczema patients. Scatchard plot analysis of binding data revealed that the increase in IL-8 binding in psoriasis was due to increased  $B_{\text{max}}$  (receptor number per cell) rather than an altered binding affinity.

No significant difference was found between healthy controls and patients with atopic eczema with regard to their IL-8 binding parameters. The mean receptor densities were vir-

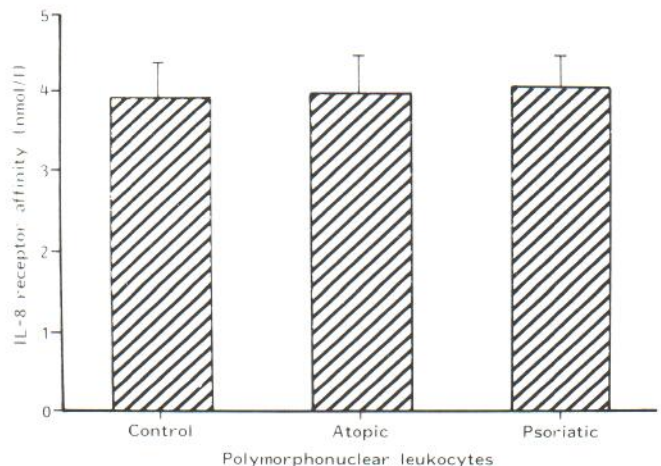


Fig. 3. Receptor affinity for IL-8 expressed as dissociation constant ( $K_d$ ) in PMNL obtained from healthy controls ( $n = 11$ ), patients with atopic eczema ( $n = 10$ ) and psoriasis ( $n = 10$ ). Values represent mean  $\pm$ SD.

tually identical in the two groups of patients and mean receptor affinity, moreover, in all three groups.

Elevated cutaneous levels of the chemoattractant protein IL-8 were identified in psoriasis (3). Thus, it is likely that elevated cutaneous levels of IL-8 may play a role in the initiation of the inflammatory infiltrate in psoriasis.

Our findings of a slightly elevated receptor density for IL-8 in psoriatic PMNL suggest, however, that PMNL accumulation in psoriatic skin may result not only from an excess of cutaneous IL-8, but also from increased receptor-mediated migration of psoriatic cells towards the chemotactic IL-8 stimulus. Both factors acting together may thus contribute to the inflammatory infiltrate in psoriasis. This is not the case in atopic eczema, which is characterized by predominantly mononuclear inflammatory infiltrate and normal numbers of PMNL IL-8 receptors. The use of specific IL-8 receptor antagonists should, therefore, prove to be of particular interest for the treatment of this disease.

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