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Interleukin-8 receptor-mediated chemotaxis of normal human epidermal cells

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Normal human keratinocytes show chemotactic behavior towards interleukin-8 (IL-8). Under physiological conditions this cytokine seems to be present in an equilibrium between monomeric and dimeric forms, as indicated by Western blotting data. Radioligand binding studies suggest that keratinocyte chemotaxis is mediated by receptors specific for IL-8 dimers. IL-8 receptor-specific mRNA can be detected in a keratinocyte cell line by polymerase chain reaction.

Interleukin-8 receptor; IL-8 dimer; Human keratinocyte

1. INTRODUCTION

The possible involvement of interleukin-8 (IL-8) in various inflammatory skin diseases, particularly psoriasis, has been subject of a number of recent studies [1–3]. IL-8 has been described as a potent stimulator for neutrophils [4,5] and T-lymphocytes [6], but it may also directly influence the function of epidermal cells. Since chemotactic effects of IL-8 on epidermal cell lines (SCL-II, HaCat) had been described [7], we were interested whether this proinflammatory cytokine could influence the chemotaxis of normal human epidermal cells as well. The directed migration of human keratinocytes is dependent upon the presence of specific cell surface receptors. Therefore, to study the putative IL-8 receptors, radioligand binding assays with [^{125}I]IL-8, and mRNA-PCR specific for the IL-8 receptor from neutrophils [15] were performed on cultured normal human keratinocytes. Specificity of binding was assessed by competition studies with the chemotactic peptides fMLP and NAP-2, as well as with the endothelial 79 amino acid form of IL-8. To compare the binding characteristics of IL-8 on various cell types, human neutrophils and NIH 3T3 cells were included in the study.

2. MATERIALS AND METHODS

2.1. *Labelling of IL-8*

Human recombinant interleukin-8 containing 72 amino acids with a molecular weight of 8 kDa was iodinated by the chloramine-T method [8] to obtain a specific activity of 350–500 Ci/mmol.

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2.2. *Electrophoresis and Western blotting*

Electrophoresis of unlabeled IL-8 and 125J-IL-8 was performed on SDS-PAGE as described [9]. Bands were electro-blotted to Fluorotrans membranes (Pall, Portsmouth, UK). Filters were incubated with goat anti-human IL-8 neutralizing antiserum (R&D Technology, Minneapolis, USA). Bound primary antibody was visualized by first incubating with donkey anti-goat IgG antiserum (IgG) coupled to phosphodiesterase (Boehringer Mannheim, Germany; dilution 1:2,500 in PBS) and subsequently with 3,3',4,4',-tetraaminobiphenyl (DAB; Sigma, Deisenhofen) as enzyme substrate.

2.3. *Epidermal cell preparation and culture*

Normal keratinocytes were isolated from newborn foreskin and cultured as described earlier [10]. Human squamous cell carcinoma cells (SCL-II) and 3T3 fibroblasts were grown in DMEM containing 10% FCS.

2.4. *Isolation of granulocytes*

Polymorphonuclear leukocytes (PMNL) were prepared from normal human volunteers by Ficoll/Hypaque gradient centrifugation as described [11].

2.5. *Binding assays*

Cultured human keratinocytes, SCL-II cells, NIH 3T3 cells and PMNL (2×10^5 cells each) were incubated with 2 nM [^{125}I]IL-8 at 4°C for 4 h in the presence of increasing concentrations (0.1 nM–1 μM) of unlabeled IL-8 (72aa form), endothelial IL-8 (79aa form), NAP-2 or fMLP (Sigma, Deisenhofen, Germany). Cells were washed, lysed and cell bound radioactivity was measured in gamma-counter (Pharmacia-LKB, Uppsala, Sweden).

2.6. *Chemotaxis assay*

Chemotaxis of freshly separated human epidermal cells was analysed in modified Boyden chambers with membranes of 10 μm pore size as described [7].

mRNA-PCR: Total RNA from SCLII cells was separated from DNA using LiCl. From the cDNA sequence published for the high affinity receptor in human neutrophils [15] primers were synthesized flanking a 300 bp fragment (L-primer: 5'CAG-ATCCACAGATGT-GGGAT3': R-primer: 5'TCCAGCCATTCACCTTGGAG3'). Reverse transcription was specifically primed using the R-primer. After

30 amplification cycles the product was visualized by EtBr-stained agarose gel electrophoresis.

3. RESULTS AND DISCUSSION

Freshly separated human epidermal cells showed significant chemotactic response towards IL-8. The magnitude of the response was comparable to that of fibroblast conditioned medium (FCM) (Fig. 1). These findings in normal epidermal cells correlate very well with earlier studies in the epidermal cell line SCL-II [7]. Since it is well established that chemotaxis is mediated through specific receptors we tried to identify IL-8 receptors on human keratinocytes by radioligand binding experiments. The results of our competition binding studies are shown in Fig. 2. In accordance to published data [12] binding of ^{125}I-labeled IL-8 to PMNL could be displaced by excess unlabeled IL-8 with a K_i of 4 nM. In contrast, no displacement could be obtained in NIH 3T3 mouse fibroblasts. Surprisingly, in normal human keratinocytes and to a somewhat lesser degree in SCL-II cells increasing concentrations of unlabeled IL-8 led to an increase in cell bound radioactivity. This seemingly paradoxical phenomenon was not observed when NAP-2, the endothelial form of IL-8 (79aa) or fMLP were used as competitors (Fig. 3). In search for a reasonable explanation for these results we assumed concentration-dependent dimerization of IL-8. This assumption is based on the demonstration of IL-8 dimers in solution by NMR [14]. In fact, we could show the presence of a 16 kDa band in PAGE which showed IL-8 immunoreactivity in Western blots (Fig. 4). The data prove the presence of IL-8 dimers under physiological conditions of neutral pH. The phenomenon of concentration-dependent dimerization has a precedent in the dimer formation by human growth hormone [13]. Thus the observed increase of [^{125}I]IL-8 binding in the presence of cold ligand can best be explained by concentration-dependent formation of IL-8 dimers and dimer-specific receptor binding on keratinocytes.

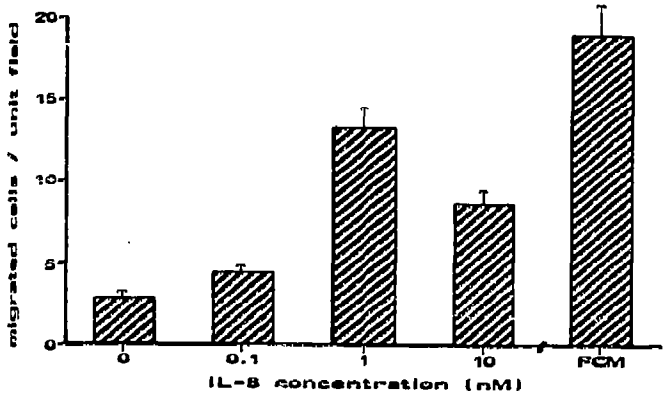

Fig. 1. Chemotaxis of normal human epidermal cells. Freshly isolated cells were analysed in modified Boyden chambers. Membranes had a pore size of 10 μm.

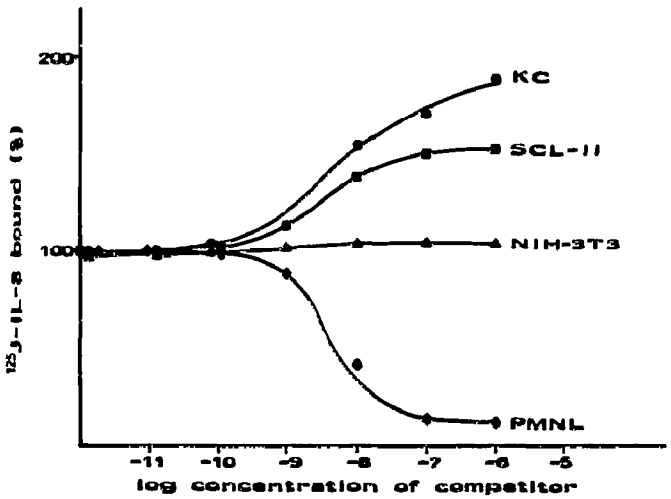

Fig. 2. Comparison of different cell types in competition experiments using [^{125}I]IL-8 as radioligand. In duplicate assays cultured normal and carcinoma-derived (SCLII) human keratinocytes, NIH 3T3 mouse fibroblasts and PMNL (2×10^5 cells each) were incubated with 2 nM [^{125}I]IL-8 for 4 h at 4°C in the presence of increasing concentrations (0.1–1 μM) of unlabelled IL-8. Cells were washed, lysed in 0.5 N NaOH and cell bound radioactivity was measured in a gamma-counter.

Computerized analysis of a saturation binding curve (not shown) suggests that the binding of [^{125}I]IL-8 originates from a specific binding (K_d=1 nM, B_{max}=14,400 receptors/cell) superimposed by a nonspecific binding.

Further support for the presence of specific IL-8 receptors in epidermal cells comes from the demonstration of mRNA transcripts by mRNA-PCR. Using primers specific for the high affinity IL-8 receptor described for PMNL [15] an amplification product can be achieved showing the exact size predictable from the published cDNA sequence (Fig. 5).

In summary, we could show for the first time that normal human keratinocytes possess IL-8 receptors,

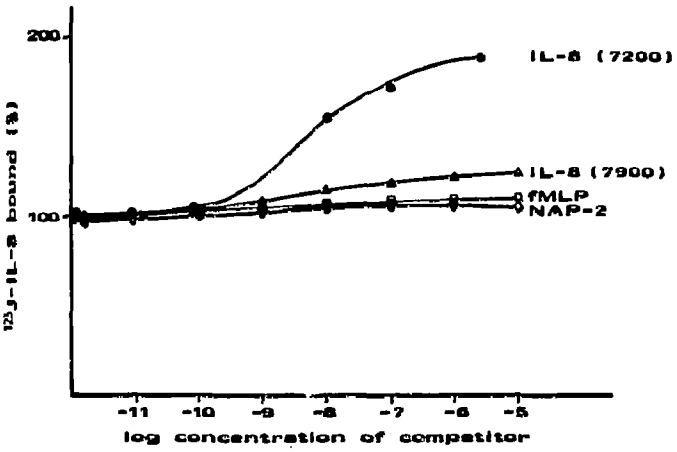

Fig. 3. Comparison of different competitors for binding of [^{125}I]IL-8 to cultured normal keratinocytes. Incubation conditions were as described under Fig. 2.

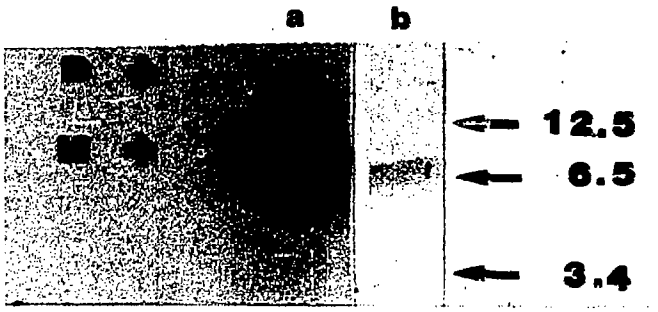

Fig. 4. IL-8 dimers shown by electrophoresis through 17.5% PAA/SDS. M=8 kDa monomer, D=16 kDa dimer. (Lane a) Autoradiography of 5 μg [^{125}I]L-8; (lane b) Western blot of 5 μg IL-8 and subsequent immunological detection with anti-IL-8 antiserum as described in Materials and Methods.

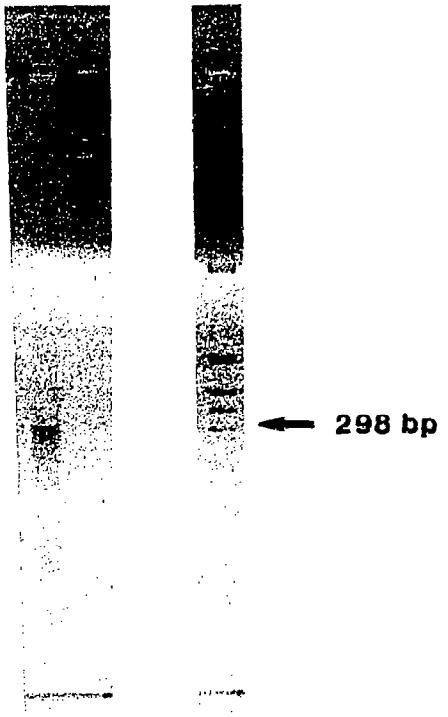

Fig. 5. Electrophoresis of the PCR-fragment amplified from high affinity IL-8 receptor mRNA. For sequence of primers used for cDNA synthesis and amplification, see Materials and Methods. (Lane 1) 300 bp PCR-product; (lane 2) negative control; (lane 3) 1 kb ladder (Gibco BRL, Eggenstein, Germany) as size marker.

which are likely to be involved in IL-8 induced chemotaxis of epidermal cells. These data suggest that IL-8 could play a role in the activation of epidermal cells in skin conditions with increased IL-8 levels, such as psoriasis.

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