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Both interleukin-8 receptors independently mediate chemotaxis

Jurkat cells transfected with IL-8R1 or IL-8R2 migrate in response to IL-8, GROα and NAP-2

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Abstract

Neutrophil leukocytes, the target cells for interleukin-8 and related CXC chemokines, bear high numbers of two types of IL-8 receptors (IL-8R1 and IL-8R2). By cDNA transfection Jurkat cell lines were generated that stably express either IL-8R1 or IL-8R2 (J-IL8R1 and J-IL8R2). J-IL8R1 expressed 4,000 ± 1,000 copies of IL-8R1, and bound IL-8 with high affinity (K_d 1–4 nM) and GROα and NAP-2 with low affinity (K_d 200–500 nM). J-IL8R2 expressed 17,000 ± 3,000 copies of IL-8R2, and bound all three chemokines with high affinity. Both transfectants showed a similar degree of chemotactic migration after stimulation with IL-8, GROα and NAP-2. All three chemokines were equally potent as attractants of J-IL8R2, whereas IL-8 was 300 to 1,000-fold more potent than GROα or NAP-2 as attractant of J-IL8R1. The potencies, therefore, agree with the affinities of the ligands to IL-8R1 and IL-8R2. Our results demonstrate that both IL-8 receptors function independently, and mediate chemotaxis in response to IL-8 and other CXC chemokines.

Key words: Inflammation; Chemotaxis; Interleukin-8; Receptor; Transfection; Jurkat cell

1. Introduction

Chemokines constitute a novel family of small cytokines which attract and activate leukocytes, and are of major importance as mediators of inflammation [1,2]. Two subfamilies are distinguished depending on the arrangement of the first two of four conserved cysteines, which are either separated by one amino acid (CXC chemokines) or adjacent (CC chemokines). IL-8 and several other CXC chemokines act mainly on neutrophils. Like other chemotactic agonists, they induce shape changes, chemotaxis, a transient rise of intracellular free Ca^{2+} ($[Ca^{2+}]_i$), granule exocytosis, integrin upregulation, formation of bioactive lipids, and the respiratory burst [2,3].

Biochemical studies have shown that neutrophils express two distinct IL-8 receptors [4,5] which are now termed IL-8R1 and IL-8R2 [2]. IL-8 binds to both receptors with high affinity, whereas GROα, GROβ, GROτ and NAP-2 bind with high affinity to IL-8R2 (K_d 0.2–2.5 nM) and low affinity to IL-8R1 (K_d 200–500 nM) [4–7]. The cDNAs for IL-8R1 and IL-8R2 were cloned and shown to code for homologous proteins of 350 and 360 amino acids with seven putative transmembrane domains, which are typical for G-protein coupled receptors [8,9]. IL-8R1 and IL-8R2 have high amino acid sequence identity except for the amino- and carboxyl-terminal regions (Fig. 1). The amino-terminal region, which may form part of the chemokine binding pocket through a disulfide link to the third extracellular loop [10], is likely to determine ligand recognition and affinity [11,12], while the carboxyl-terminal region is thought to be important for signalling and receptor desensitization [2]. Recombinant IL-8 receptors were previously expressed transiently in mammalian cells and shown to have similar binding properties as their natural counterparts and to mediate $[Ca^{2+}]_i$ changes [13–15].

The role of IL-8R1 and IL-8R2 in the induction of chemotaxis and other neutrophil functions has not been studied. In particular, it is not clear whether the two receptors mediate the same cellular responses and can

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Abbreviations: IL-8R1/2, human interleukin-8 receptor type 1/2; J-IL8R1/2, Jurkat transfectants expressing IL-8R1/2; GROα and NAP-2 (neutrophil-activating peptide 2) are two chemotactic cytokines related to IL-8; αCD3, OKT3, monoclonal antibody recognizing CD3 on T lymphocytes.

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function independently of each other. In the present study, cDNAs coding for IL-8R1 or IL-8R2 were stably transfected into Jurkat cells, and the functional responses elicited by stimulation with IL-8, GROα and NAP-2 were studied. Since lymphocytes are implicated in inflammation, we reasoned that Jurkat cells, which are derived from a human T cell leukemia, would be able to transduce chemokine receptor signals. The present results show that both IL-8 receptors function independently and mediate chemotaxis in response to IL-8 and other CXC chemokines.

2. Materials and methods

2.1. Cells and transfectants

The human T cell leukemia line, Jurkat, (a kind gift of Dr. A. Tobler, Central Hematology Laboratory, University Hospital, Bern) and Jurkat transfectants were maintained in RPMI 1640 medium with glutamin, penicillin, streptomycin and 10% FCS. For cell culture of the transfectants the medium was supplemented in addition with G-418 (Gibco/BRL) at 0.8 mg/ml.

DNA for IL-8R1 was synthetized by PCR using the primers SER1/H3, 5′-CCAAGCTTACCATTGCTGAAACTGAAGAGG, and ASR1/Xba, 5′-CCTCTAGATCAGAGGTTGGAAGAGACATTG, and reverse transcription products from neutrophil RNA as template, resulting in a 1,112 bp fragment that contained the entire coding region for IL-8R1. Following subcloning into pcDNA-1 (Invitrogen Corp.), nucleotide sequencing [16] revealed identity with the published sequence [8]. cDNAs for human IL-8R2 were isolated as described previously [15]. Jurkat cell lines that stably express one or the other IL-8 receptor, J-IL8R1 and J-IL8R2, were obtained as described by Moser et al. [15]. Briefly, the cells were washed twice with PBS and incubated on ice for 5 min ($5–8 \times 10^6$ cells/800 μl PBS) with 20 μg linearized pcDNA-1/IL-8R1 or pcDNA-1/IL-8R2 (clone 202 [15]) and 10 μg linearized $pSV2_{neo}$ vector. After electroporation (250 V, 960 μF, pulse time 30–40 ms) with a gene pulser (Bio-Rad), the cells were placed on ice for 10 min, diluted 100-fold with prewarmed RPMI 1640 medium containing 10% FCS and cultured for 48 h. The medium was supplemented with 0.8 mg/ml G-418 (Gibco/BRL), and the culture was continued until the control cells (no $pSV2_{neo}$ added) started to die (10–14 days). G-418 resistant Jurkat cell clones were obtained by limiting dilution and cell lines expressing IL-8R1 or IL-8R2 were identified by ^{125}I-IL-8 binding. One stable clone expressing IL-8R1, was isolated and termed J-IL8R1. Of eight clones expressing IL-8R2, the one with the highest ^{125}I-IL-8 binding was selected and termed J-IL8R2.

2.2. Binding and functional assays

Chemokines were chemically synthesized [17], and IL-8 was labelled with radioactive iodine [4]. Binding studies were performed as described [4] using samples of 4×10^6 J-IL8R1 or J-IL8R2 cells, 2 nM ^{125}I-IL-8 and increasing concentrations of unlabeled IL-8, GROα or NAP-2.

Changes of cytosolic free calcium concentration ($[Ca^{2+}]_i$ were determined with cells loaded with fura-2 by incubation for 30 min at 37°C with 0.1 nmol fura-2/acetoxymethylester per 10^6 cells [18]. Loaded cells (5×10^6 cells/ml in loading buffer) were stimulated at 37°C with a chemokine or αCD3 (OKT3, Ortho Diagnostic Systems), and the fluorescence-related $[Ca^{2+}]_i$ changes were recorded [18].

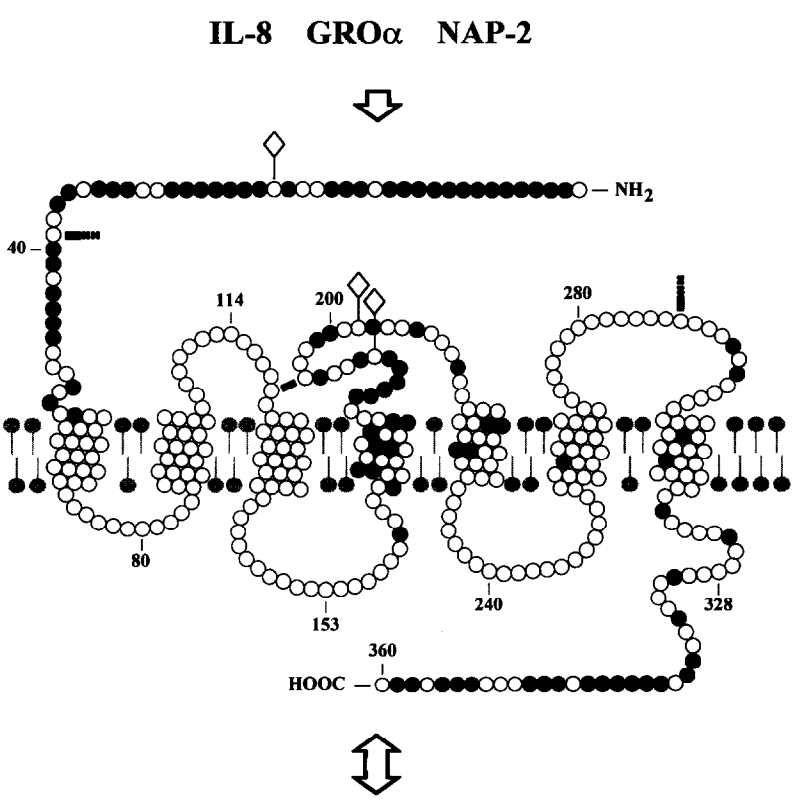

Fig. 1. Schematic representation of the human IL-8 receptors. The 360 amino acid sequence of IL-8R2 is shown. The sequence was aligned with that of IL-8R1 (350 amino acids) allowing for gaps [2], and residues that differ after alignment are shown as filled circles. Diamonds designate putative N-linked glycosylation sites, and pinhead symbols represent lipids in the plasma membrane. Two putative disulfide bridges, formed by Cys^{119}/Cys^{196} and Cys^{39}/Cys^{287}, respectively are indicated by thick lines [10]. Note the extensive sequence similarity between IL-8R1 and IL-8R2 in most transmembrane domains. Highest sequence divergence is in the amino- and carboxy-terminal regions.

Cell migration was measured in 48-well microchemotaxis chambers (Neuro Probe, Inc., Cabin John, MD) [19]. Chemattractants diluted in HEPES-buffered RPMI 1640 supplemented with 1% pasteurized plasma protein (PPL SRK, Swiss Red Cross Laboratory, Bern, Switzerland) were added to the bottom wells and 100,000 cells suspended in the same medium to the upper wells. A polyvinylpyrrolidone-free polycarbonate membrane with 5 nm pores (Nucleopore) separating upper and lower wells was coated with type IV collagen [20]. After incubation at 37°C for 3 h in humidified air with 5% CO_2, the membrane was removed, washed on the upper side with PBS, fixed, and stained. Migrated cells were counted microscopically at 1,000 × magnification in five randomly selected fields per well. All assays were performed in triplicate.

3. Results and discussion

3.1. Chemokine binding to Jurkat transfectants

Binding studies were performed with the two stably transfected cell lines, J-IL8R1 and J-IL8R2, which were found to express 4,000 ± 1,000 and 17,000 ± 3,000 receptors per cell, respectively. The competition for binding of ^{125}I-IL-8 by unlabelled IL-8 and GROα is shown in Fig. 2. Both transfectants bound IL-8 with equally high affinity (K_d 1–4 nM), whereas GROα exhibited high-affinity binding to J-IL8R2 (K_d 3 nM) and low-affinity binding to J-IL8R1 (K_d 300 nM). Similar results were obtained with NAP-2 yielding K_d values of 6 and 450 nM for J-IL8R2 and J-IL8R1, respectively. These results indicate that IL-8R1 and IL-8R2 can be distinguished by their binding of GROα and NAP-2 but not of IL-8. Jurkat cells that were not transfected with IL-8R1 or IL-8R2 did not bind ^{125}I-IL-8.

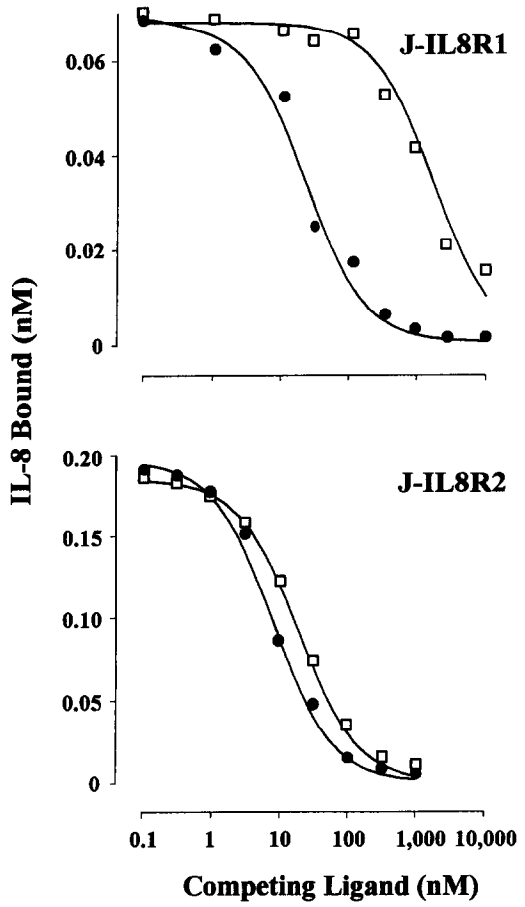

Fig. 2. Cold ligand competition of ^{125}I-IL-8 binding to J-IL8R1 and J-IL8R2. 4×10^6 transfected cells were incubated at 4°C with 2 nM ^{125}I-IL-8 in the presence of increasing concentrations of IL-8 (●) or GROα (□) and further processed as described in section 2. 60–80% and 40–50% of IL-8R1 and IL-8R2 receptors, respectively, were occupied in the absence of unlabelled ligands.

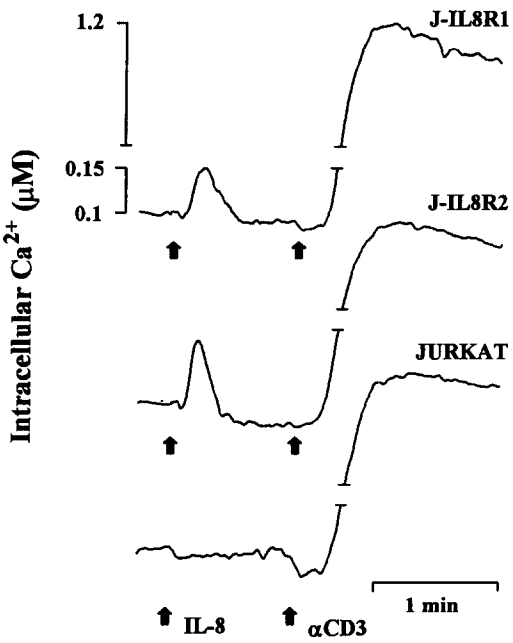

Fig. 3. $[Ca^{2+}]_i$ changes induced in J-IL8R1, J-IL8R2 and non-transfected Jurkat cells. Fluorescence changes in Fura-2 loaded cells (5×10^6/ml) were monitored during sequential stimulation (arrows) with 100 nM IL-8 and 2 μg/ml αCD3, and $[Ca^{2+}]_i$ was calculated [18].

The binding properties of the transfected receptors are closely comparable to those of the natural IL-8 receptors in human neutrophils [4–6]. The present findings are in agreement with previous observations in transiently transfected E293 cells [13]. Two distinct recombinant IL-8 receptors were also expressed in COS cells [14]. In this case, however, GROα or NAP-2 did not compete for ^{125}I-IL-8 binding to IL-8R1 and bound to IL-8R2 with markedly lower affinity than IL-8, suggesting that COS cells may be less convenient for the study of recombinant IL-8 receptors.

3.2. Cytosolic free Ca^{2+} changes

A rapid and transient rise in $[Ca^{2+}]_i$ is an early event after stimulation of leukocytes with chemotactic agonists. As shown in Fig. 3, stimulation with IL-8 induced a small, but significant $[Ca^{2+}]_i$ rise in J-IL8R1 and J-IL8R2, but not in non-transfected Jurkat cells. This indicates that both transfected IL-8 receptors are functional, and mediate similar signal transduction events as in neutrophils [3]. The maximal $[Ca^{2+}]_i$ levels after stimulation with 100 nM IL-8 did not exceed 250 nM. A higher and

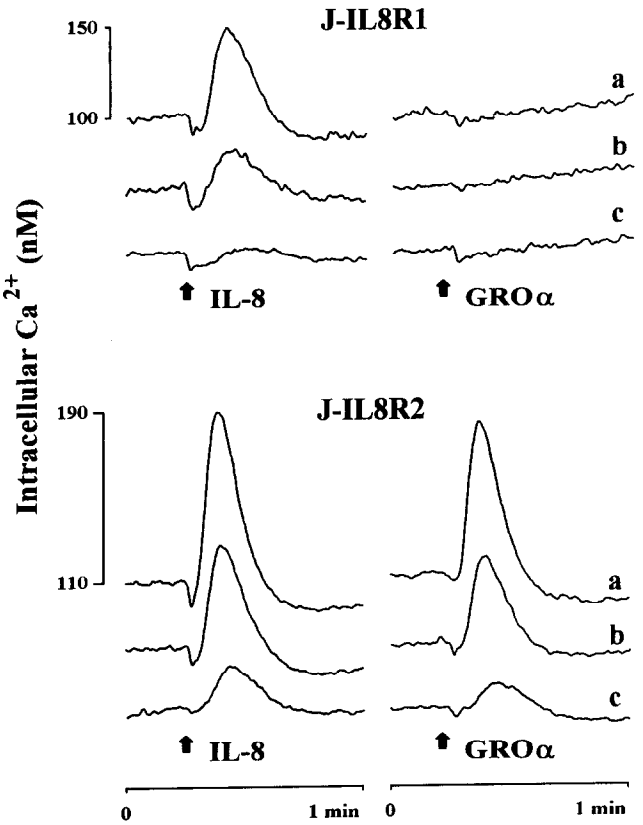

Fig. 4. $[Ca^{2+}]_i$ changes induced in J-IL8R1 and J-IL8R2 cells by IL-8 and GROα. Fura-2 loaded cells (5×10^6/ml) were stimulated with 100, 10, and 1 nM IL-8 or GROα (a–c) and the $[Ca^{2+}]_i$ changes determined [18]. The recordings are representative for three independent experiments.

more protracted rise was obtained after addition of αCD3. The $[Ca^{2+}]_i$ rise induced by IL-8 was concentration dependent in both J-IL8R1 and J-IL8R2 (Fig. 4). Stimulation with up to 100 nM GROα (Fig. 4) or NAP-2 (not shown) induced virtually identical, concentration-dependent $[Ca^{2+}]_i$ changes in J-IL8R2, but no $[Ca^{2+}]_i$ rise in J-IL8R1. The $[Ca^{2+}]_i$ response in J-IL8R1 was consistently weaker than in J-IL8R2. Since both tranfectants bound IL-8 with high affinity, these differences may have resulted from the approximately 4-fold difference in the density of the expressed receptors.

As expected on the basis of the receptor binding data, mutual cross-desensitization of the Ca^{2+} response was obtained with IL-8 and GROα in J-IL8R2 but not in J-IL8R1 (not shown). These observations are in agreement with our former data in neutrophils (which bear both types of IL-8 receptors) where stimulation with IL-8 abrogated the response to GROα whereas GROα only partially prevented the response to IL-8 [4]. Addition of EGTA to the assay buffer to chelate extracellular Ca^{2+} did not prevent the rise in $[Ca^{2+}]_i$, but reduced the rate of increase by about 30% indicating that Ca^{2+} release from intracellular stores and Ca^{2+} influx through the plasma membrane occurred upon stimulation.

3.3. Chemotaxis

Chemotaxis is considered the most important functional response to CXC chemokines. Using the transfected cells, we, therefore, compared the capacity of each of the two IL-8 receptors to mediate this response. As shown in Fig. 5, IL-8-dependent migration was observed with both J-IL8R1 and J-IL8R2. The effect was concentration dependent and bimodal, which is characteristic for leukocyte chemotaxis in vitro. In some experiments, as shown in Fig. 5, no bimodal curve was obtained for J-IL8R1 chemotaxis in response to GROα or NAP-2 up to 1 μM. With both transfected cells, maximum migration in 3 h amounted to 175 ± 46 (± S.D.) cells per five high-power fields (mean of four separate experiments), and was obtained at IL-8 concentrations of 1 nM, which corresponds closely to the optimum chemotactic concentration for human neutrophils. GROα and NAP-2 were chemotactic for either type of transfected cells, but the concentrations yielding maximum effects differed by up to a thousand-fold, namely 1–10 nM for J-IL8R2 and

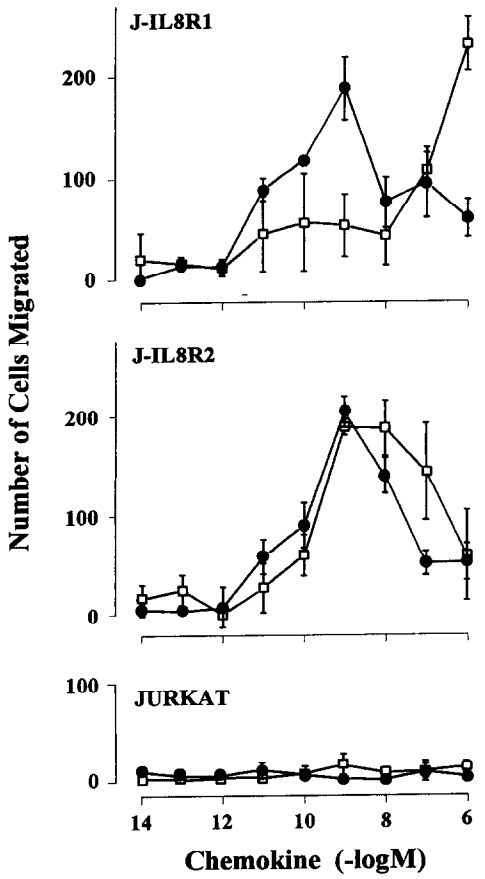

Fig. 5. In vitro chemotaxis of J-IL8R1, J-IL8R2 and non-transfected Jurkat cells. Concentration-dependent effects of IL-8 (●) and GROα (□) on cell migration were determined as described in section 2. Results are expressed as the mean number (± S.D.) of migrating cells per five high-power fields of triplicate assays. Number of cells migrated in the absence of added chemokines (random migration) were 22 ± 4 and 8 ± 5 for transfected and normal Jurkat cells, respectively. Data shown are representative for four independent experiments.

0.3–1 μM for J-IL8R1 (Fig. 5). Non-transfected Jurkat cells did not respond to IL-8, GROα or NAP-2.

Since GROα and NAP-2 at concentrations up to 100 nM did not induce $[Ca^{2+}]_i$ changes in J-IL8R1, it was somewhat surprising to observe a full chemotactic response. It is known, however, that shape changes in neutrophils are not affected by depletion of mobilizable storage pool Ca^{2+} [21,22], and that neutrophil migration on certain surfaces is not related to $[Ca^{2+}]_i$ changes [23].

While considerable information is available on granulocyte chemotaxis and migration, little is known about the mechanism of migration of lymphocytes. Several chemoattractant receptors have been characterized in myeloid cells, and all shown to belong to the superfamily of seven-transmembrane-domain receptors [2]. Seven-transmembrane-domain receptors have also been identified in lymphocytes through cDNA cloning. EBI1 [24], BRL1 [25] and MDR15 (Barella et al., in preparation) are strongly expressed in normal and leukemic human B cells, and share 30–40% sequence identity with the IL-8 receptors. Another receptor, 6H1, was shown to be expressed primarily in activated chicken T lymphocytes [26]. High expression in both myeloid and lymphoid cells, including Jurkat cells, was reported for a human receptor termed pBE1.3 [27] or LESTR52 [28], which was isolated from human monocyte cDNA libraries. The ligands for these receptors are still unknown. Because of their relatedness to the IL-8 receptors, they may mediate lymphocyte migration, although, at least for MDR15 and LESTR52, none of the known chemoattractants were shown to bind ([28] and Barella et al., in preparation).

Several papers have reported that IL-8 is chemotactic for human T lymphocytes [1]. The effects, however, are much less pronounced than those observed for neutrophils [2]. We were unable to detect $[Ca^{2+}]_i$ changes [29] or significant migration responses by human blood lymphocytes challenged with IL-8 (T. Geiser, unpublished results). Our present data show that leukemic T cells are able to migrate, but only respond to CXC chemokines when transfected with cDNAs for one of the IL-8 receptors. In an earlier study, we showed that lymphocytes and particularly Jurkat cells express low levels of IL-8R1 mRNA (that could be demonstrated only by reverse transcriptase-PCR) while mRNA for IL-8R2 was not detected [15]. The lack of demonstrable specific binding of IL-8 is in agreement with these observations indicating that the level of IL-8 receptors on lymphocytes is extremely low. Lymphocytes have been shown to respond to CC chemokines, including RANTES, MIP-1α, MIP-1β [30–33] and the CXC chemokine IP10 [33], which is inactive on neutrophils [34].

Two main conclusions can be drawn from our study. (i) Both IL-8 receptors, IL-8R1 and IL-8R2, which are expressed on human neutrophils mediate chemotaxis in response to IL-8 and related CXC chemokines. They are equally responsive to IL-8, and can function independently of each other. (ii) T lymphocytic cells are able to migrate in response to CXC chemokines when they express the appropriate receptors. Cells that express IL-8R2 can be stimulated equally well with IL-8, GROα and NAP-2, and are thus likely to be responsive to all CXC chemokines known to be active on neutrophils. Despite their low binding affinity, GROα and NAP-2 are able to attract cells that exclusively bear IL-8R1 receptors when supplied at concentrations close to their K_d values.

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