Modulation of IL-8 receptor expression on purified human T lymphocytes is associated with changed chemotactic responses to IL-8

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Abstract: Interleukin-8 is a member of the chemokine superfamily and is a major mediator of acute inflammation. Although IL-8 has been reported by some laboratories also to be a chemoattractant for T lymphocytes, this has been difficult to confirm and remains a controversial issue. By using freshly purified human T cells (90–95% $CD3^+$), we could demonstrate consistent directional migration of T cells to recombinant human IL-8. IL-8 was as potent as RANTES, MIP1α, and MIP1β in inducing T cell chemotaxis. Highly purified T cells, however, incubated at 37°C for more than 12 h or cultured overnight with anti-CD3 antibody cross-linked to plastic dishes, showed a markedly reduced capacity to migrate in response to IL-8. This was associated with a decrease in binding of radioiodinated IL-8 to T cells. Northern blot and polymerase chain reaction analyses showed that freshly purified T cells expressed mRNA for both IL-8 receptor type A and type B. Steady-state levels of mRNA for the IL-8RA and IL-8RB genes were also reduced by incubation of the cells with or without anti-CD3 for 12 h at 37°C. These results indicate that T cells are indeed one of the target cell populations for IL-8. The regulation of IL-8 receptor expression on T lymphocytes may contribute to the pathophysiological role of IL-8 in inducing the homing and infiltration of T cells. *J. Leukoc. Biol.* 57: 335–342; 1995.

Key Words: *T lymphocytes · interleukin-8 · IL-8 receptors · chemotaxis*

INTRODUCTION

A superfamily of "chemokines" has been identified, that selectively chemoattracts and activates specific leukocyte subpopulations (as reviewed in refs. 1–3). The members of the C-C chemokines are mainly chemotactic for monocytes, whereas the C-X-C chemokines, except for IP10 and PF4, chemoattract and activate neutrophils. A number of chemokines, including RANTES, MIP1α, and MIP1β of the C-C subfamily, as well as IP10 of the C-X-C subfamily, have been shown to induce T lymphocyte chemotaxis in vitro [4–7]. Although several reports indicated that interleukin-8 (IL-8) is capable of inducing T cell migration in vitro and T cell infiltration in vivo [8–13], this effect of IL-8 on T cells could not be reproduced by a number of investigators ([4, 9, 14] and reviewed in refs. 3 and 15). These observations, which were also made in our own laboratory [4, 8], motivated us to investigate further the in vitro effect of IL-8 on T cells. In addition, the presence of IL-8 receptors on T cells is still questionable ([16–18] and reviewed in ref. 3). In the present study, we observed that freshly purified human T lymphocytes were consistently chemoattracted by IL-8 and expressed both type A and B high-affinity receptors for IL-8. Incubation of purified T cells for more than 12 h with or without anti-CD3 cross-linked to plastic plates at 37°C resulted in decreased steady-state levels of receptor mRNA and ligand binding in association with reduced T cell chemotactic responses to IL-8.

MATERIALS AND METHODS

Reagents

Recombinant human (rh) IL-8 was a kind gift from Dainippon Pharmaceutical Company (Osaka, Japan). Other recombinant chemokines were purchased from Pepro Tech (Rocky Hill, NJ). ^{125}I-labeled IL-8 and ^{125}I-labeled GRO were purchased from Dupont NEN (Boston, MA), with specific activity of 2200 Ci/mmol.

Cells

Human peripheral blood enriched in mononuclear cells or lymphocytes was obtained from normal donors (National Institutes of Health Clinical Center, Transfusion Medicine Department, Bethesda, MD) by leukapheresis. The blood was centrifuged through Ficoll-Hypaque. The mononuclear cells (PBMCs) at the interface were washed twice with phosphate-buffered saline (PBS) and centrifuged through an isoosmotic Percoll (Pharmacia, Uppsala, Sweden) gradient as described [19] to remove monocytes. Human T cell enrichment columns (R&D Systems, Minneapolis, MN) were then used to prepare a purified human T cell population via high-affinity negative selection according to the manufacturer's instruction. This isolation procedure typically yielded

Abbreviations: CI, chemotaxis index; FACS, fluorescence-activated cell sorter; IL-8, interleukin-8; IL8RA, IL-8 receptor A; mAb, monoclonal antibody; PBMC, peripheral blood mononuclear cell; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; rhIL-8, recombinant human IL-8; RT, reverse transcription; SDS, sodium dodecyl sulfate.

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90-95% $CD3^+$ T cells. The cells were resuspended in chemotaxis medium (RPMI 1640, 1% bovine serum albumin, 25 mM HEPES) or cultured in RPMI 1640 medium containing 10% fetal calf serum (Hyclone, Logan, UT). For anti-CD3 antibody treatment, the T cells were adjusted to a concentration of 5×10^6 cells/ml and cultured on six-well tissue culture plates precoated with a 10 μg/ml solution of anti-CD3 monoclonal antibody (mAb) for 12 h at 37°C. Alternatively, purified T cells were incubated with anti-CD3 mAb in plastic dishes precoated with goat anti-mouse Fc antibody (Caltag, Los Angeles, CA). These treatments typically yielded "activated" T cells showing proliferation, increased cytokine production, and cytokine receptor expression as well as increased chemotaxis to selective chemokines [4, 5, 20].

Chemotaxis Assay

T lymphocyte migration was assessed by a 48-well microchemotaxis chamber technique [21]. A 25-μl aliquot of rhIL-8 or other reagents diluted in chemotaxis medium was placed in the lower compartment and 50 μl of cell suspension (5×10^6 cells/ml in medium) was placed in the upper compartment of the chamber. The two compartments were separated by a polycarbonate filter (5 μm pore size; Neuroprobe, Cabin John, MD) coated with 10 μg/ml collagen type IV (Gibco, Gaithersburg, MD) or 200 μg/ml gelatin (Sigma, St. Louis, MO) overnight at 4°C or for 1 h at 37°C. The chamber was incubated at 37°C for 3 h in humidified air with 5% CO_2. At the end of the incubation period, the filter was removed, fixed and stained with Diff-Quik (Harlew, Gibbstown, NJ). The number of migrated T cells in three high-power fields (400×) was counted by light microscopy after coding the samples. The results are expressed as the mean (± SD) value of the migration in triplicate samples and are representative of at least three experiments performed. In some experiments, the migration was expressed as a chemotaxis index (CI) calculated as follows:

$$CI = \frac{\text{migration to stimuli}}{\text{migration to medium}}$$

The coating of polycarbonate filters with matrix proteins enabled 75% of the migrated T cells to adhere to the undersurface of the filter. Of T cells that crossed the filter, 25% were recovered in the bottom of the assay wells. Because the number of cells falling into the bottom well was directly proportional to the number of cells adhering to the undersurface of the filter regardless of the stimuli used (medium or chemokines), only cells remaining on the filter were counted after staining.

The statistical significance of the number of cells migrating in response to stimuli versus baseline (migration toward control medium) was calculated using a Student's *t*-test.

Binding of ^{125}I-rhIL8 to human T lymphocytes

The binding of ^{125}I-IL8 to T cells was assessed as previously described [22]. Briefly, 2×10^6 cells were incubated in duplicate tubes with 0.1 ng of ^{125}I-IL-8 in a total volume of 200 μl of binding medium (RPMI 1640, 1% bovine serum albumin, 25 mM HEPES, 0.05% NaN_3). The nonspecific binding was determined by parallel incubation in the presence of 100-fold excess of unlabeled rhIL-8. After incubation at room temperature for 1 h, the cells were pelleted through a 10% sucrose/PBS cushion. The tips of the tubes containing cell pellets were cut and radioactivity was determined in a gamma counter (Clinigamma, Pharmacia, Gaithersburg, MD). The nonspecifically bound radioactivity in the presence of unlabeled rhIL-8 was subtracted from total bound radioactivity to yield specific binding. To determine the receptor number, the cells were incubated with 0.1 ng of ^{125}I-IL-8 plus a series of concentrations of unlabeled IL-8. After incubation, the cell pellets were collected as described above and the data were analyzed with a Macintosh program LIGAND [23]. The binding capacity of T cells for ^{125}I-GRO was also assessed.

RNA extraction and Northern blot analyses

Northern blots were carried out according to standard procedures [24]. RNA was isolated by lysing cells in guanidine hydrochloride and pelleting the RNA through a 5.4 M CsCl cushion. Then 15 μg of total RNA was fractionated on 1.2% agarose-formaldehyde gels, followed by Northern transfer to S&S Nytran membranes (Schleicher & Schuell, Keene, NH). Membranes were prehybridized with Hybrizol I (Oncor, Gaithersburg, MD) for 6 h at 42°C, then hybridized with ^{32}P (Amersham, Arlington Heights, IL) labeled cDNAs encoding regions of divergence between IL-8 receptor A (IL8RA) and IL8RB (A. Lloyd et al., unpublished data). A 245-bp *Acc*I fragment of IL8RA (nucleotides 1-245) and a 553-bp *Hha*I-*Hin*dIII fragment of IL8RB (nucleotides 698-1250) were chosen, which share only approximately 60% nucleotide homology in the analogous regions. After hybridization for 12 h at 42°C, the membranes were washed twice with 2× SSC (1× SSC: 0.15 M sodium chloride, 15 mM sodium citrate) and 0.1% sodium dodecyl sulfate (SDS) at 45°C for 10 min, twice with 2× SSC at 55°C for 10 min, and finally once with 0.1× SSC at 65°C for 10 min. The membranes were exposed to Kodak XAR films at −80°C with intensifying screens. RNA loading and transfer to membranes was checked by examination under ultraviolet light and hybridization of the blot with chicken β-actin probe (Boehringer Mannheim, Indianapolis, IN).

Southern analyses of polymerase chain reaction products

The following oligonucleotides were used for Southern analyses of polymerase chain reaction (PCR) products: IL8RA: 5'-ACA GAT CCA CAG ATG TGG GAT TTT GAT GAT-3' (nucleotides 63-92 of the coding region); IL8RB: 5'-AAG GTG AAG ATC TTA GTA ATT ACA GTT ACA-3' (nucleotides 72-101 of the coding region). PCR was performed by using a GeneAmp RNA PCR kit following the manufacturer's instructions (Perkin Elmer/Cetus, Norwalk, CT). Briefly, 1 μg of total RNA was reverse transcribed using oligo(dT)-16 as primer in a 20-μl reaction volume. The reverse transcript was used directly for amplification. PCR conditions were as follows: in a 100-μl reaction, 50 nmol of each primer, 1 mM dNTPs, 2 mM $MgCl_2$, plus 1× PCR reaction buffer II and 2.5 μl of Ampli Taq DNA polymerase. Primers for IL8RA or IL8RB were used: IL8RA sense primer 5'CCT GGC CGG TGC TTC AGT TAG ATC AAA CCA (nucleotides 1-30), IL8RA antisense primer 5' TCA CAG AGT CAA AGA TCG TAT GTC CCC GAC (nucleotides 160-131); IL8RB sense primer 5' GTC AGG ATT TAA GTT TAC CTC AAA AAT GAA (nucleotides 1-30), IL8RB antisense primer 5'-TAA AGG TCC CTA AGA CCA AGT GTA CCC CGC (nucleotides 162-133). Reactions were incubated in Perkin-Elmer/Cetus thermal cycler for 25 cycles (denaturation 1 min at 94°C, annealing 1 min at 55°C, and extension 1 min at 70°C). After extraction with chloroform,

TABLE 1. Dose Range of Chemotactic Activity of rhIL-8 for T Lymphocytes[a]

Donor	Number of migrated cells (Mean ± SD)				
	1	2	3	4	5
Medium	48 ± 11	84 ± 19	142 ± 25	28 ± 7	53 ± 19
RANTES (10 ng/ml)	89 ± 12[b]	337 ± 37[b]	329 ± 10[b]	77 ± 14[b]	148 ± 31[b]
IL-8 (ng/ml)					
1000		174 ± 17			
100	104 ± 15[b]	217 ± 27[b]	271 ± 37[b]	59 ± 20	134 ± 14[b]
50	133 ± 17[b]	348 ± 35[b]	329 ± 40[b]	140 ± 11[b]	135 ± 10[b]
10	121 ± 23[b]	231 ± 30[b]	206 ± 14[b]	94 ± 19[b]	143 ± 12[b]
1		114 ± 11	161 ± 40		108 ± 21

[a]T cells were purified from peripheral blood of healthy donors with a high-affinity T cell enrichment column. The chemotaxis assays were performed in a 48-well chemotaxis chamber. Results are expressed as the mean (± SD) number of migrated T cells in three high-power fields obtained in three replicates. The data are for five individuals out of 15 tested.
[b]$P < .05$ compared to medium control.

40 μl of PCR reaction sample was loaded on 1% agarose gel in TAE buffer. Products were visualized with ethidium bromide and the gel was subsequently denatured in 0.5 M NaOH, 1.5 M NaCl, neutralized in 10 M ammonium acetate, and transferred to Nytran membranes. Membranes were prehybridized in Hybrizol II (Oncor) for 6 h at 42°C. Oligonucleotide probes (50 ng) specific for a sequence internal to the primers used in the PCR were labeled with [^{32}P]dCTP (Amersham) using an oligonucleotide labeling kit (Boehringer). Probes were separated from free nucleotides by passing through a Chrom spin 10 column (Clontech, Palo Alto, CA) and added to the hybridization mix. After 16 h at 42°C, the membranes were washed in 2× SSC, 0.1% SDS at 55°C and exposed to Kodak XAR films for 12 h at −80°C.

RESULTS

T cells were purified by high-affinity negative selection columns immediately after obtaining the donated peripheral blood leukocytes. Purified T cell preparations were 90–95% CD3$^+$ with minor contamination by CD56$^+$ cells, which are believed to be natural killer cells that are not chemoattracted by IL-8 (D. Taub, unpublished observation). All cell preparations were devoid of granulocytes, B lymphocytes, and monocytes, as assessed by fluorescence-activated cell sorter (FACS) analysis using the relevant antibodies. Chemotaxis assays were performed as soon as the T cells were purified. The polycarbonate filters were coated with matrix proteins as previously reported to obtain T cell migration and adherence onto the lower surface of the filter with various chemotactic cytokines [4]. Gelatin and collagen type IV were equally effective (data not shown). Detailed dose-response experiments were performed with T cells from 15 healthy donors and all freshly prepared cells showed significant migration in response to IL-8. **Table 1** shows the results for five donors. Although there was donor-to-donor variation, all T cell preparations responded chemotactically to IL-8. The response curves were bell shaped (**Fig. 1**), typical of the response induced by chemoattractants for other leukocyte populations [22, 25, 26]. The optimal concentrations of IL-8 in inducing T cell migration ranged from 10 to 50 ng/ml. This was comparable in potency to the effect of C-C chemokines, RANTES, MIP1α, and MIP1β, which are chemotactic for T cells as well as monocytes [4, 7, 22, 25] and were used as positive controls (data not shown). In checkerboard analyses, T cells migrated toward a positive gradient of IL-8 (with higher IL-8 concentrations in the lower compartment of the chemotaxis chamber). There was no significant migration in the presence of negative IL-8 gradients with higher IL-8 concentration in the upper compartment containing the T cells (data not shown), confirming the observation by Ross et al. [10].

To further verify that T cell migration across polycarbonate filters was indeed induced by IL-8, a monoclonal anti-IL-8 antibody was utilized. At a concentration ratio of 10:1, this mAb inhibited 75% of the chemotactic effect of IL-8 on T cells (**Fig. 2**). The antibody did not significantly inhibit spontaneous background migration (to control medium) or migration to the positive control chemokines RANTES and MIP1β, indicating that the mAb specifically neutralized the chemotactic effect of IL-8 on T cells (Fig. 2).

High-affinity selection columns enabled us to obtain highly purified T cells within 2–3 h, which is significantly less than the 16 h necessary to prepare T cells in our previous

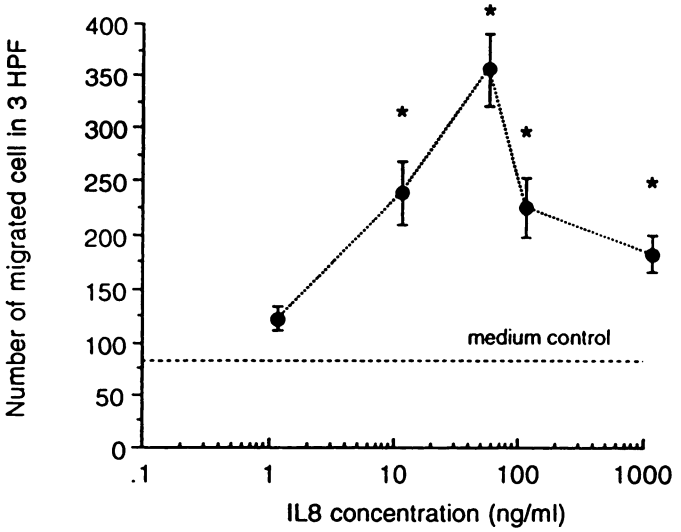

Fig. 1. Dose-response curve of T cell migration to IL-8. The migration assay was performed as described in Materials and Methods. The curve represents a single experiment out of 15 performed. The results are expressed as the mean (± SD) number of migrated cells in three high-power fields obtained in three replicates. *$P < .05$ compared to migration induced by medium alone.

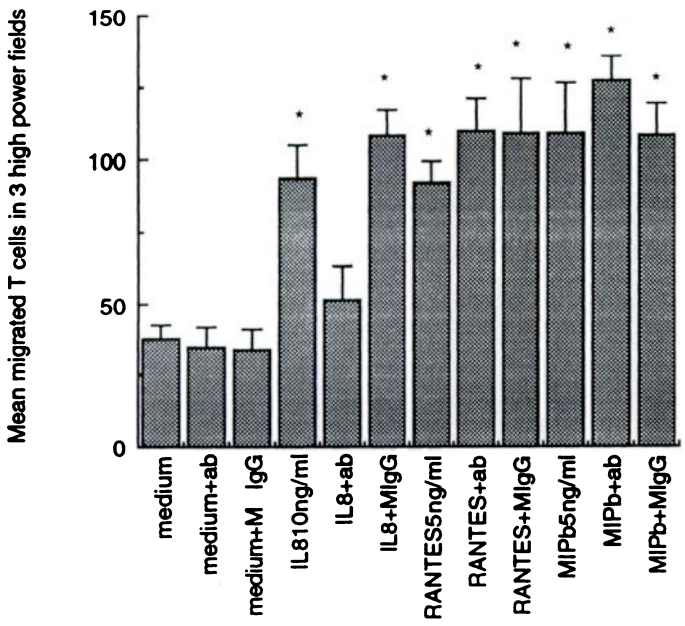

Fig. 2. Inhibition of T cell migration to IL-8 by anti-IL-8 monoclonal antibody. Chemokines at the indicated concentrations or medium alone were preincubated with an anti-rhIL-8 monoclonal antibody (clone 3C6) or irrelevant murine immunoglobulin G (IgG) for 30 min at 37°C. The mixture was then assayed for the chemotactic activity for T lymphocytes. $^*P < .05$ compared with corresponding medium control (medium alone, medium + ab, or medium + M IgG).

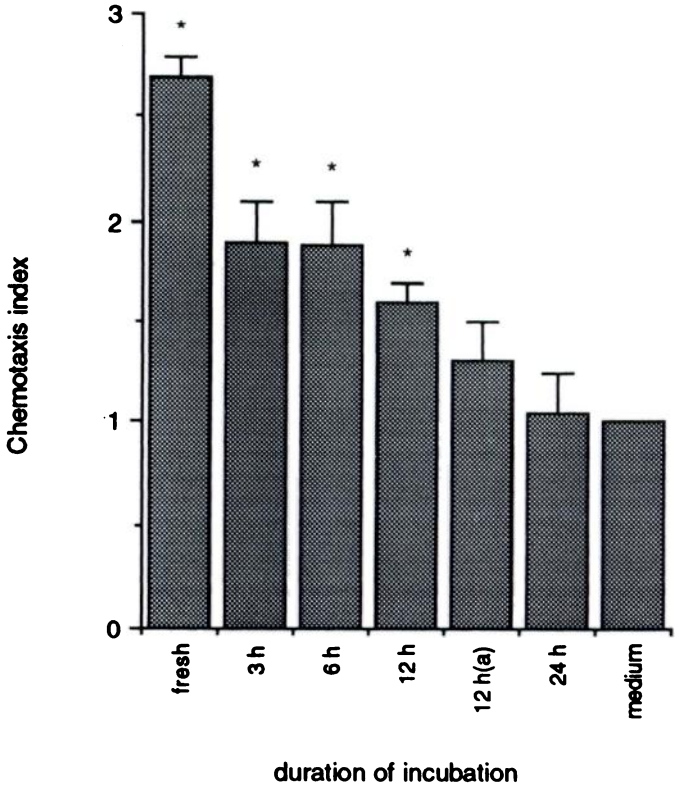

Fig. 3. Effect of duration of incubation on T cell migration induced by rhIL-8. The representative chemotactic response of T cells after different incubation periods to 10 ng/ml rhIL-8 is shown. A similar response pattern was seen in T cells from 15 donors. $^*P < .05$ compared to medium control.

report [4]. We therefore examined the effect of incubating purified T cells at 37°C for various lengths of time on the responsiveness to IL-8. Compared to freshly purified cells, incubated T cells showed a progressive reduction in the chemotaxis response to IL-8 with the increase in the duration of incubation at 37°C. After 3–6 h at 37°C, the migratory capacity of T cells from a particular donor to 10 ng/ml IL-8 decreased by 30%, and after 24 h the T cell migration to IL-8 was reduced to the baseline level (**Fig. 3**). T cells incubated with anti-CD3 monoclonal antibody fixed on plastic plates for 12 h at 37°C also exhibited marked diminution in migration to IL-8 (Fig. 3). In addition, PBMCs that were initially incubated with anti-CD3 antibody overnight and then processed to obtain highly purified T cells showed similar reduction in chemotaxis to IL-8 (**Table 2**). Thus T cells treated with anti-CD3 antibody even in the presence of accessory cells failed to be chemoattracted by IL-8. Further fractionation of freshly purified T cells into $CD4^+$ and $CD8^+$ subsets revealed no difference in the migratory capacity to IL-8 by these T cell subsets (data not shown). This contrasts with the results reported for MIP1α and MIP1β, which preferentially chemoattracted $CD8^+$ and $CD4^+$ T cell subpopulations, respectively [4].

We next examined whether the change in T cell chemotactic response to IL-8 was associated with modulation of receptor expression on the cells. Freshly purified T cells expressed a considerable number of high-affinity binding sites (K_d = 2 nM, R = 3600/cell; **Fig. 4A**). The binding data generated in this study fit best to the one-receptor species model. After 6 h of incubation at 37°C more than 50% of the binding sites were no longer detectable in displacement experiments. After 24 h of incubation at 37°C or treatment with anti-CD3 antibody at 37°C for 12 h the specific binding of ^{125}I-IL-8 on T cells was further reduced (Fig. 4B).

In Northern blot analyses, purified T cells expressed both IL8RA and IL8RB mRNA (**Fig. 5**). The steady-state levels of mRNA were reduced after incubation at 37°C for 12–24 h or by anti-CD3 activation at 37°C for 12 h. However, the mRNA levels remained constant when freshly purified T lymphocytes were kept at 4°C for 12 h (Fig. 5). Such refrigerated cells could also bind IL-8 and were chemoattracted by IL-8 (data not shown).

The mRNA synthesis of IL8RA and B in freshly isolated

TABLE 2. Effect of Anti-CD3 mAb Treatment on T Cell Binding for and Migration to IL-8

Cells	Specific binding for IL-8 (cpm)[a]	CI induced by IL-8 (50 ng/ml)[b]
Freshly purified cells	3784	2.7
Incubation at 37°C for 12 h		
Purified T cells[c]	2030	1.7
With PBMCs + anti-CD3[d]	89	1.1
Purified T cells + anti-CD3[e]	644	1.2

[a]T cells (2×10^6) cultured under different conditions were examined for ^{125}I-IL-8 binding as detailed in Materials and Methods. Specific binding was calculated by subtraction of nonspecifically bound cpm on the cells in the presence of 1000-fold excess of unlabeled IL-8 from total bound cpm in the absence of unlabeled ligand.

[b]Chemotaxis indices were calculated as the fold of migration in response to IL-8 versus migration to medium control.

[c]Purified T cells incubated in medium alone.

[d]T cells purified from PMBCs incubated with anti-CD3 mAb.

[e]Purified T cells incubated with anti-CD3 mAb cross-linked on plastic dish.

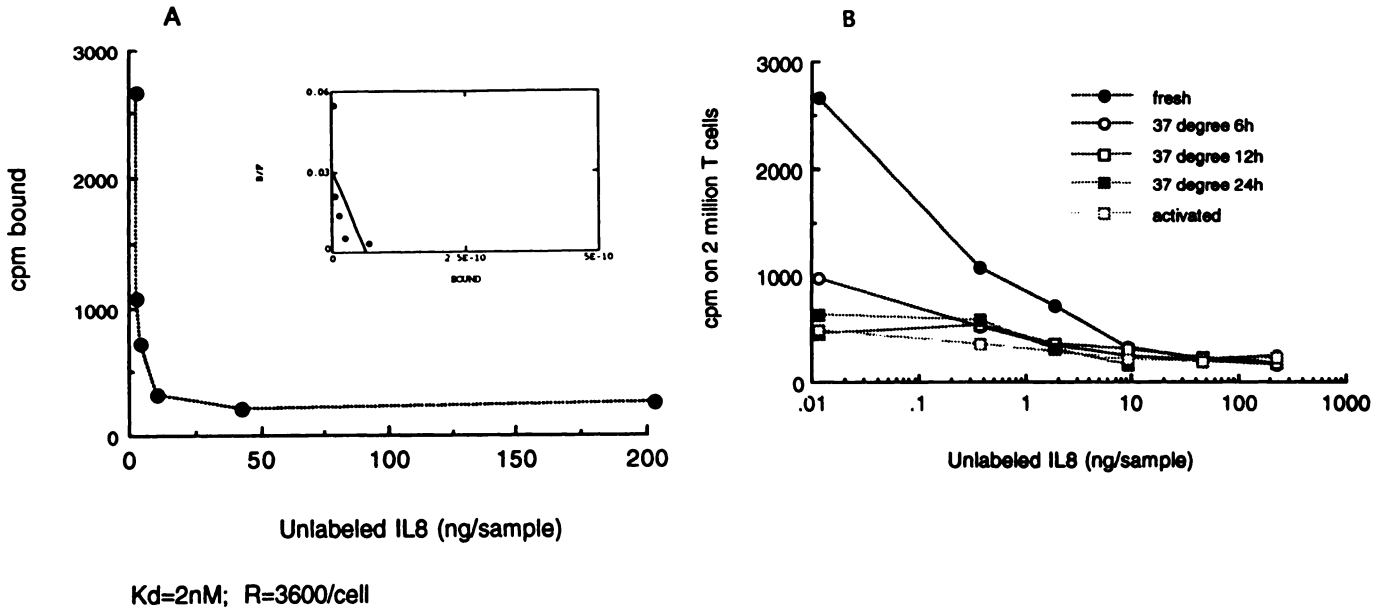

Fig. 4. Binding of T lymphocytes for radiolabeled IL-8. T cells (2×10^6) were incubated with 0.1 ng of ^{125}I-IL-8 in 200 μl of binding medium for 1 h at room temperature with different concentrations of unlabeled IL-8. After incubation, the cell suspension was centrifuged through a 10% sucrose/PBS cushion. The tips of tubes with cell pellets were then measured in a gamma counter for radioactivity. (A) Displacement curve and Scatchard plot (inset) obtained with freshly purified T lymphocytes. (B) Displacement curves generated with T cells after different incubation periods. At least five experiments were performed with essentially similar results.

T lymphocytes was further characterized by Southern analyses of the PCR products with internal probes. The primers for PCR covered the most divergent regions of IL8RA or IL8RB sequences. Thus the two internal probes did not cross-hybridize the products generated by IL8RA or IL8RB primers on Southern blotting (**Fig. 6**). Figure 6 shows that PCR products generated with IL8RA primers hybridized with the IL8RA internal oligonucleotide probe (lane 1) but not with IL8RB probe (lane 3). Conversely, the IL8RB primer-generated products hybridized only with the IL8RB

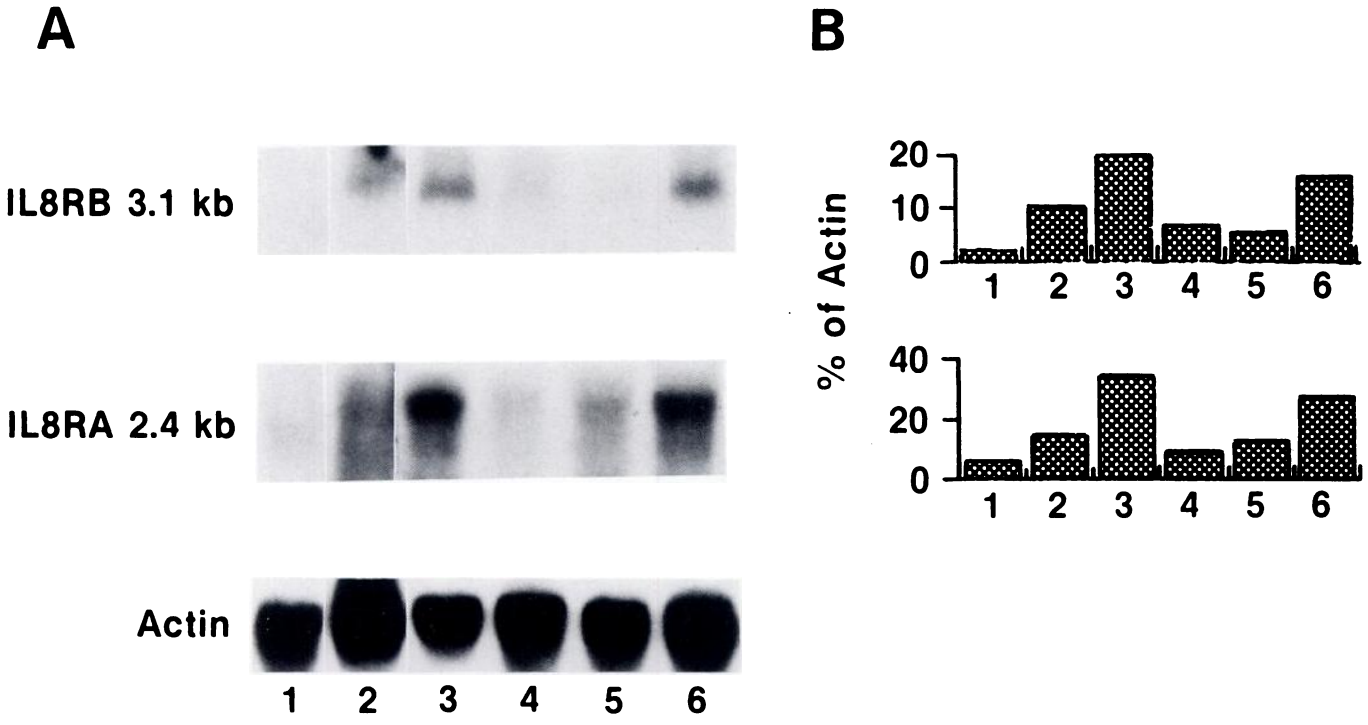

Fig. 5. Northern analyses of IL-8 R mRNA expression by T cells. 15 μg of total RNA from T cells under different culture conditions was electrophoresed on 1.2% formaldehyde-agarose gel. The blotting and hybridization were performed as described in Materials and Methods. (A) RNA samples were extracted from cells incubated at 37°C for 24 h (lane 1), at 37°C for 6 h (lane 2), at 4°C for 12 h (lane 3), and at 37°C for 12 h (lane 4); cells incubated with anti-CD3 antibody (lane 5); and freshly purified cells (lane 6). (B) Densitometric normalization of IL8R messages as determined by the proportion to actin message.

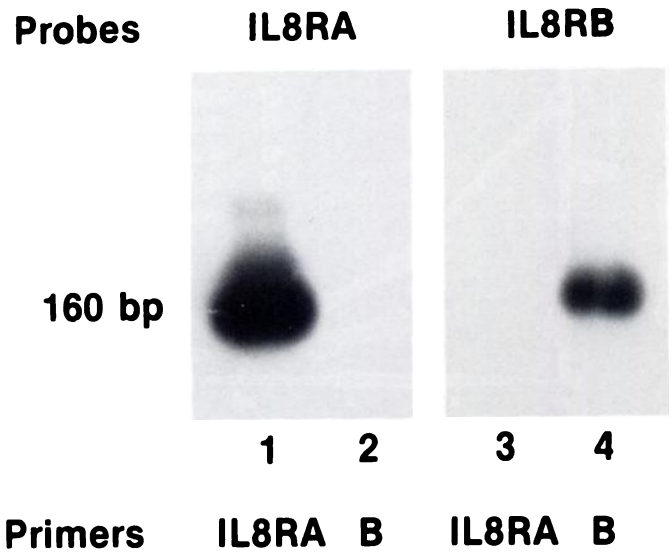

Fig. 6. Southern analysis of PCR products generated with IL8RA or IL8RB primers. 1 μg of total RNA extracted from fresh T cells was reverse-transcribed with oligo dT and amplified with specific primers. The products were then electrophoresed, transferred to a Nytran membrane, and hybridized with the internal probes. Lanes 1 and 2, IL8RA and IL8RB primer-generated products hybridized with IL8RA internal probe; lanes 3 and 4, IL8RA and IL8RB primer-generated products hybridized with IL8RB internal probe.

internal probe (lane 4), but not with IL8RA probe (lane 2), confirming that fresh T cells expressed transcripts for both IL8RA and IL8RB. In FACS analyses, about 20% of fresh T cells were stained by a specific anti-IL-8 RA antibody and 15% cells stained with anti-IL8RB antibody (a kind gift from Drs. J.K. Kim and C. Hebert, Genentech, South San Francisco CA, and data not shown).

Since fresh T lymphocytes expressed both IL8RA and IL8RB mRNA, the binding capacity for GRO/MGSA, a C-X-C chemokine that selectively binds with much higher affinity to IL8RB than to IL8RA (for review see ref. 27), was examined. Unlabeled IL-8 in excess competitively inhibited the binding of both ^{125}I-IL-8 and ^{125}I-GRO to T cells (**Fig. 7**). Although unlabeled GRO completely competed for ^{125}I-GRO binding to T cells, it only partially inhibited ^{125}I-IL-8 binding. This pattern of cross-competition was similar to that observed for neutrophils [1]. These results suggest that T cells expressed both IL8RA and IL8RB on the cell surface and that IL-8 bound to both receptors with high affinity. GRO binds to IL8RB with high affinity but can bind to IL8RA only with low affinity (reviewed in ref. 27). This correlates with our observation that GRO had considerably less chemoattractant activity than IL-8 for T lymphocytes (data not shown).

DISCUSSION

IL-8 was initially characterized as a neutrophil chemoattractant and activator. It was later shown also to induce T lymphocyte migration in vitro and infiltration in vivo [8–13]. It has been estimated that about 10% of the lymphocytes prepared from PBMCs were capable of responding to IL-8 [8, 9]. In addition to being chemotactic for T lymphocytes [8–13], IL-8 has been reported to induce a rapid and long-lasting increase in the level of inositol phosphates in lymphocytes [28]. However, the in vivo and in vitro chemoattractant effects of IL-8 on T cells have been difficult to reproduce in some laboratories ([4, 9, 14] and reviewed in refs. 3 and 15). For example, it has been reported that both unstimulated and stimulated T cells, if purified, failed to be chemoattracted by IL-8 ([4] and reviewed in refs. 3 and 15). In contrast, Wilkinson and Newman [29] reported that in the presence of monocytes, a proportion of polyclonally activated T cells responded to IL-8 in polarization and collagen gel invasion assays. The success with which we could consistently induce directional migration of freshly purified T cells with IL-8 in vitro prompted us to investigate the conditions that might influence the T cell response to IL-8. The major determinant was the length of time needed to prepare T cells and the duration of exposure of T cells to 37°C. In our study, highly purified and unstimulated preparations of human peripheral blood T lymphocytes consistently exhibited migratory activity toward IL-8 gradients when the cells were assayed immediately after purification or kept at 4°C for up to 12 h. However, T cells incubated at 37°C with or without anti-CD3 showed a progressive decrease in the number of cells undergoing chemotaxis with time in response to IL-8. This pattern of responsiveness contrasts with the observation that some of the C-C chemokines chemoattract and bind anti-CD3 antibody-treated T cells [4, 22]. Consequently, anti-CD3 treatment of T cells apparently differentially modulates receptor expression for different chemokines on T cells. IL-8 may be more involved in the homing of resting T cells than the other proinflammatory chemokines.

Two distinct IL-8 receptors, IL8RA and IL8RB, have been characterized and molecularly cloned [30, 31]. IL-8 binds to both receptors with high affinity, whereas GRO/MGSA recognizes IL8RB with high affinity and interacts with IL8RA with low affinity (reviewed in ref. 27). Although these receptors have been reported to be preferentially expressed by various cell types, the presence of both IL-8 receptors on T cells has not yet been very well documented ([16, 17] and reviewed in refs. 3 and 27). By reverse transcription (RT)-PCR analyses, Moser et al. [18]

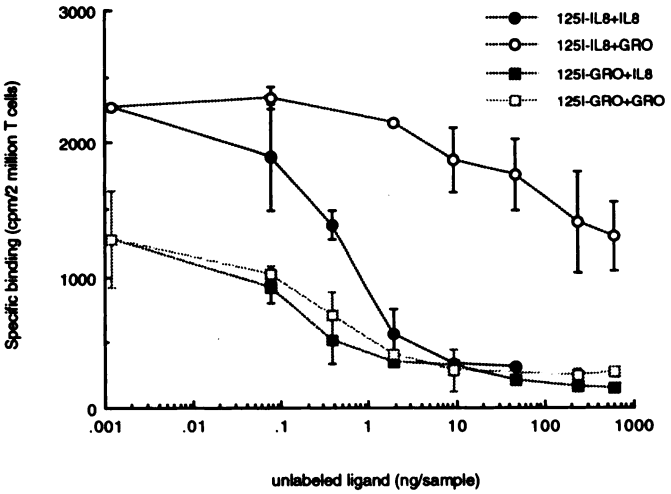

Fig. 7. Cross-competition of ^{125}I-IL-8 and ^{125}I-GRO binding on T lymphocytes by unlabeled ligands. T lymphocytes (2×10^6) were incubated with 0.1 ng of ^{125}I-IL-8 or ^{125}I-GRO in 200 μl of binding medium for 1 h at room temperature in the presence of series of dilutions of unlabeled IL-8 or GRO. The cells were then pelleted and measured for radioactivity as described in Materials and Methods. The data shown represent the mean (± SD) value of three experiments performed.

detected IL8R type 1 (IL8RA) transcripts in CD4+ T cells, phytohemagglutinin-treated blood lymphocytes, and a T cell line, Jurkat. A small proportion of peripheral T lymphocytes has been reported to be stained by fluoresceinated NAP-1/IL-8 [9]. In our study, Northern blotting revealed steady-state levels of mRNA for both IL8RA and IL8RB in fresh T cell preparations as well as T cells kept at 4°C for 12 h. This was confirmed by the results for RT-PCR products generated by IL8RA and IL8RB oligonucleotide primers. Binding studies with radiolabeled ligands provided further evidence for the presence of two types of IL-8 receptors on T cells. It must be noted that compared to neutrophils, almost 50% of which responded chemotactically to IL-8 and expressed higher levels of IL8RA and IL8RB, the proportion of T cells detectable by anti-IL8RA and anti-IL8RB in freshly purified populations ranged from 15 to 20% (data not shown). Since IL-8 binds IL8RA and IL8RB with equally high affinity, the estimated receptor number for IL-8 on T cells reflects the binding to both receptors. The actual number of binding sites on IL-8-responsive cells is presumably much higher than that estimated for the whole T cell populations, as only 15–20% of freshly purified T cells were stained by anti-IL8R antibodies. Although GRO/MGSA binds to neutrophils and triggers neutrophil chemotaxis, it induced less T cell migration than IL-8. This may be due to its preferential interaction with IL8RB on T cells, which accounts for only a portion of the total number of IL-8 receptors.

The basis for the down-regulation of IL-8 receptor expression by T cells incubated at 37°C with or without cross-linked anti-CD3 is unknown. It is unlikely that the lower receptor expression or responsiveness to IL-8 was due to a change in cell viability, because more than 90% of T cells remained alive as judged by trypan blue exclusion tests and T cells could still respond to other chemokines: RANTES or MIP1a (data not shown). Pleass and Camp [12] were able to obtain a positive migratory response to IL-8 with T cells after 18 h of culture at 37°C. However, these T cells, in our opinion, were probably cultured in the presence of some monocytes and B cells [12]. It is possible that cultured or anti-CD3-treated purified T cells may produce cytokines that are responsible for selectively down-regulating IL-8 receptor expression and responsiveness to IL-8. Alternatively, purified T cells may require a non-lymphocyte-derived factor(s) to maintain the receptor expression and responsiveness to IL-8. We are pursuing studies to elucidate the regulation of IL-8 receptor expression by T cells and the precise relationship of receptor expression to the capacity to exhibit chemotactic responses.

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