Interleukin-2 Regulates CC Chemokine Receptor Expression and Chemotactic Responsiveness in T Lymphocytes

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Summary

Several studies have shown that CC chemokines attract T lymphocytes, and that CD45RO$^+$, memory phenotype cells are considered to be the main responders. The results, however, have often been contradictory and the role of lymphocyte activation and proliferation has remained unclear. Using CD45RO$^+$ blood lymphocytes cultured under different stimulatory conditions, we have now studied chemotaxis as well as chemokine receptor expression. Expression of the RANTES/MIP-1α receptor (CC-CKR1) and the MCP-1 receptor (CC-CKR2) was highly correlated with migration toward RANTES, MCP-1, and other CC chemokines, and was strictly dependent on the presence of IL-2 in the culture medium. Migration and receptor expression were rapidly downregulated when IL-2 was withdrawn, but were fully restored when IL-2 was added again. The effect of IL-2 could be partially mimicked by IL-4, IL-10, or IL-12, but not by IL-13, IFNγ, IL-1β, TNF-α, or by exposure to anti-CD3, anti-CD28 or phytohemagglutinin. Activation of fully responsive lymphocytes through the TCR/CD3 complex and CD28 antigen actually had the opposite effect. It rapidly downregulated receptor expression and consequent migration even in the presence of IL-2. In contrast to the effects on CC chemokine receptors, stimulation of CD45RO$^+$ T lymphocytes with IL-2 neither induced the expression of the CXC chemokine receptors, IL8-R1 and IL8-R2, nor chemotaxis to IL-8. The prominent role of IL-2 in CC chemokine responsiveness of lymphocytes suggests that IL-2–mediated expansion is a prerequisite for the recruitment of antigen-activated T cells into sites of immune and inflammatory reactions.

Several chemokines of the CC subfamily are critically involved in the regulation of phagocyte and lymphocyte recruitment in many pathological situations (1, 2). The effects of CC chemokines on monocytes (3–5), basophil, and eosinophil leukocytes (6, 7) are well defined and generally accepted. The situation is more complex for lymphocytes. T lymphocytes were shown to migrate toward RANTES, the macrophage inflammatory proteins MIP-1α and MIP-1β (8–10) and, more recently, to respond to the monocyte chemotactic proteins MCP-1, MCP-2, and MCP-3, which are highly effective on CD4$^+$ and CD8$^+$ T cells (11–13). All these CC chemokines induced a transient cytosolic free Ca^{2+} rise in T cell clones, which was prevented by *B. pertussis* toxin (11) suggesting the involvement of G protein-coupled receptors (14). Neither Ca^{2+} changes nor chemotaxis, however, were observed in freshly isolated blood lymphocytes, suggesting that activation is necessary.

Several chemokine receptors were identified by cloning of their corresponding cDNAs from phagocyte-derived libraries (15–22). CXC chemokine receptors are expressed primarily in neutrophils (15, 16, 23), whereas CC chemokine receptors (CC-CKR)[1] are generally found on other leukocytes (17–22). No corresponding cDNA has been obtained from lymphocytes, however, and little is known about the expression of chemokine receptors in these cells. We have now studied the migration responses to RANTES, MCP-1, and other chemokines, and the expression of the relevant receptors, CC-CKR1 (RANTES/MIP-1α receptor) and CC-CKR2 (MCP-1 receptor), in CD45RO$^+$ blood lymphocytes exposed to different stimulatory conditions. The data presented in this paper show that lymphocyte responses to chemokines closely correlate with the expression of transcripts for chemokine receptors, and that receptor expression and consequent ability to migrate is strictly dependent on IL-2. Our results suggest that stimulation by IL-2 make T lymphocytes competent for recruit-

[1] *Abbreviations used in this paper:* CC-CKR, CC chemokine receptor; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein.

ment by chemokines into sites of immune and inflammatory reactions.

Materials and Methods

Cytokines. All chemokines used, MCP-1, MCP-2, MCP-3, MIP-1α, MIP-1β, RANTES, and IL-8, were synthesized by solid-phase methods using the tertiary butyloxycarbonyl and benzyl protection methods (24). Human recombinant IL-10, IL-12, and IL-13 were obtained from R&D Systems Ltd., Minneapolis, MN. Other human recombinant cytokines were kindly provided by Dr. A. Lanzavecchia (Basel Institute for Immunology, Basel, Switzerland; IL-2), Chiron Corp. (Emeryville, CA; IL-4, Hoffmann-La Roche Ltd. (Basel, Switzerland; TNFα), Ciba-Geigy Ltd. (Basel, Switzerland; IL-1β), and Bioferon (Laupheim, Germany; INFγ).

Antibodies. A monoclonal antibody (mAb) to CD28 (9.3) was provided as ascites by Dr. J. Ledbetter (Bristol-Myers Squibb, Seattle, WA) and mAbs to CD3 (TR66, IgG1), CD8 (OKT8, IgG2a), CD16 (B-73, IgG1), and CD45RO (UCHL-1, IgG2a) by Dr. A. Lanzavecchia. mAbs to CD4 (13B8.2, IgG1), CD14 (RM052, IgG2a), and CD20 (B9E9, IgG2a) were purchased from Immunotech (Marseille, France); mouse isotype controls (IgG1, IgG2a) from Sigma Chemical Co. (St. Louis, MO); FITC-conjugated goat anti-mouse IgG from Dako Corp. (Carpinteria, CA); and goat anti-mouse IgG microbeads from Miltenyi Biotec (Bergisch-Gladbach, Germany).

Cell Preparation and Culture. Human peripheral blood lymphocytes (PBL) were isolated from donor blood buffy coats by centrifugation on Ficoll-Paque followed by Percoll (25). For the isolation of $CD45RO^+$ lymphocytes, the PBL were incubated for 25 min at 6–10°C with anti-CD45RO, washed twice and incubated for 15 min at 6–10°C with MACS goat anti-mouse IgG microbeads (26). After washing twice, the microbead-coupled lymphocytes were magnetically separated. The cells obtained were cultured in RPMI 1640 supplemented with 1% glutamine, non-essential amino acids, sodium pyruvate and kanamycin, 5×10^{-5} M 2-mercaptoethanol (all from Gibco, Paisley, Scotland) and 5% human serum (Swiss Red Cross Laboratory, Bern, Switzerland). When indicated, human recombinant IL-2 (400 U/ml) was added to this medium. Throughout the whole culture period the cell density was kept between 1 and 2.5×10^6 cells/ml by diluting the proliferating cells with medium. The phenotype of $CD45RO^+$ lymphocytes conditioned with IL-2 (Table 1) was determined by flow cytometry (FACScan®; Becton Dickinson, Mountain View, CA) after staining with mouse mAbs and FITC-conjugated goat anti-mouse IgG.

Treatment with Anti-CD3 and Anti-CD28. Plates precoated with 10 μg/ml of TR66 were used for stimulation with anti-CD3, whereas anti-CD28 (9.3) was added in soluble form (1:250 dilution). In these experiments, $CD45RO^+$ lymphocytes were always used at the density of 2×10^6 cells per ml.

Lymphocyte Chemotaxis. Cell migration was measured in 48-well chemotaxis chambers (Neuro Probe Inc., Cabin John, MD) (11). Chemokines in Hepes-buffered RPMI 1640 supplemented with 1% pasteurized plasma protein (Swiss Red Cross Laboratory) were added to the lower and 100,000 cells in the same medium to the upper wells. Polyvinylpyrrolidone-free polycarbonate membranes with 3-μm pores coated with type IV collagen were used. After incubation for 1 h at 37°C, the membrane was removed, washed on the upper side with PBS, fixed and stained. Migrated cells were counted microscopically at ×1000 magnification in five randomly selected fields per well. All assays were performed in triplicate.

Table 1. *Phenotypic Analysis of Cultured $CD45RO^+$ Lymphocytes*

Surface marker	Percent staining	Range
CD3	95.8 ± 4.9	86.2–98.9
CD4	30.3 ± 5.8	24.6–38.4
CD8	43.3 ± 13.0	23.4–58.4
CD14	1.0 ± 0.4	0.6–1.5
CD16	3.5 ± 4.0	0.6–9.6
CD20	0.6 ± 0.2	0.0–2.8
CD45RO	98.6 ± 8.9	94.4–99.8

$CD45RO^+$ lymphocytes cultured for 10 to 16 d in the presence of 400 U/ml IL-2 were stained with the appropriate mouse monoclonal antibodies followed by FITC-conjugated goat anti-mouse IgG. The percent of cells expressing the indicated antigen was determined by a FACScan® flow cytometer. Mean values ± SD obtained with cells from seven different donors.

Northern Blot Analysis. Total lymphocyte RNA was extracted by the acid guanidinium thiocyanate phenol-chloroform method (27) or the RNAzol B method as recommended by the supplier (Tel-Test Inc., Friendswood, TX). Samples of 10 μg RNA were fractionated on 0.8% denaturing agarose-formaldehyde gels (28), vacuum-transferred onto Nytran membranes and immobilized. The following hybridization probes were used: A 478-bp BstI-ClaI fragment for CC-CKR2 (19), a 360-bp PCR fragment corresponding to an internal region in CC-CKR1 (17, 18), a 562-bp BstI-BglII fragment for IL-8R1/2 (23), and a 308-bp SacI-PstI fragment for the IL-2 receptor alpha chain (29). The probes were labeled with [α-^{32}P]dATP using a random primer labeling kit (Boehringer Ltd., Mannheim, Germany) and used for hybridization at $>2 \times 10^6$ cpm/ml in the presence of 50% formamide at 42°C for 24 h (28). The membranes were washed to a stringency of 0.25 × SSC, 0.1% SDS at 65°C and exposed to screens which were subsequently analyzed using a phosphorimager (Molecular Dynamics Inc., Sunnyvale, CA) and the ImageQuant software. Integrated counts of the ^{32}P-labeled chemokine receptor RNA were expressed relative to the corresponding ethidium bromide-stained 28S RNA. After each hybridization the probe was removed by washing in 50% formamide, 0.1 × SSC, 0.1% SDS at 65°C for 30 min.

Results

Induction of Chemotactic Responsiveness by IL-2. Freshly isolated $CD45RO^+$ lymphocytes did not migrate toward MCP-1, RANTES or IL-8, and did not acquire migratory properties after culturing for up to 10 d in medium alone (Fig. 1 *A*). When the culture medium was supplemented with IL-2, however, the cells became responsive to MCP-1 and RANTES, as shown by the progressive increase in migration from day 4 through day 15, but not to IL-8. Since lymphocyte activity may vary with the immune state of the donor, the effect of conditioning with IL-2 was tested on

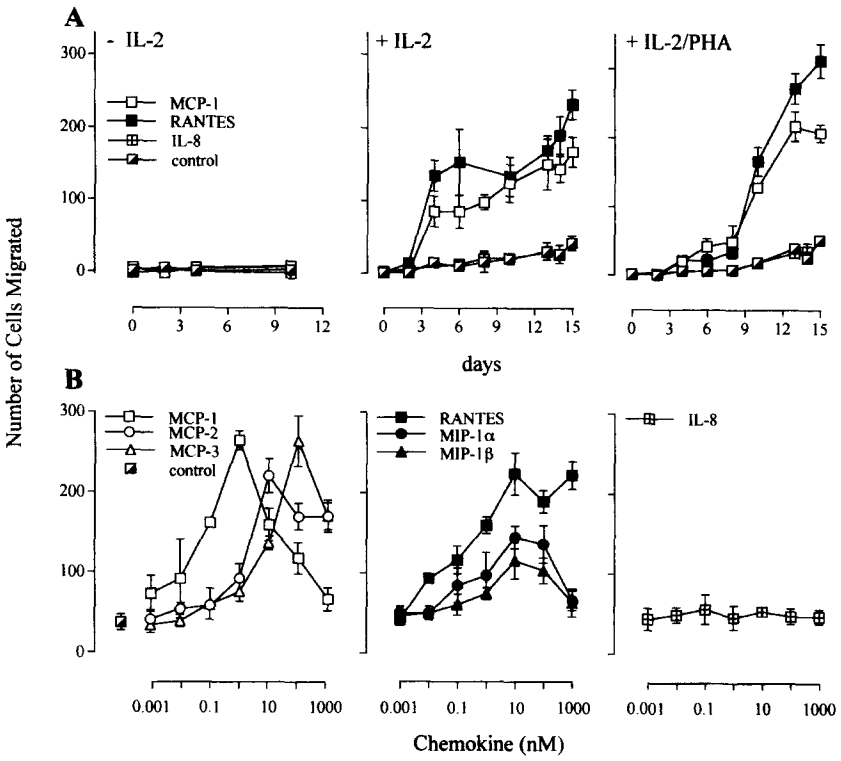

Figure 1. Induction of lymphocyte responsiveness to chemokines by IL-2. (*A*) Time course. 1.5×10^6 CD45RO$^+$ lymphocytes/ml were cultured in the medium described in Materials and Methods without additions (*-IL-2*) or with 400 U/ml IL-2 (*+IL-2*). 1 μg/ml PHA was added at day zero (*+IL-2/PHA*). Shown is the number of migrating cells (mean ± SD per five high-power fields in triplicate wells) in the presence of 1 nM MCP-1, 10 nM RANTES (maximum effective concentrations), and 10 nM IL-8. The data are representative for four independent experiments. (*B*) Concentration-dependent migration of CD45RO$^+$ lymphocytes cultured for 12 d in the presence of IL-2. Mean numbers of migrating cells are shown as above. The data are representative of three independent experiments.

CD45RO$^+$ cells from 15 different individuals. While fresh cells consistently failed to respond, treatment with IL-2 elicited migration toward MCP-1 and RANTES in all cases. Addition of the mitogenic lectin phytohemagglutinin delayed the effect of IL-2, and migration toward MCP-1 and RANTES was observed only after 9 instead of 4 d of culture (Fig. 1 *A*). These results indicate that chemotactic responsiveness was induced by IL-2 but was not necessarily related to blast formation and proliferation of the T cells.

As shown in Fig. 1B, IL-2 conditioned CD45RO$^+$ lymphocytes responded not only to MCP-1 and RANTES but also to MCP-2, MCP-3, MIP-1α, and MIP-1β, while IL-8 was inactive. Except for RANTES, for which a plateau was obtained above 10 nM, a typically bimodal concentration dependence between 0.001 nM and 1 μM was observed for all CC chemokines. In three independent experiments, the highest numbers of migrated cells counted in five high-power fields (mean ± SEM), were 209 ± 34 for MCP-1, 161 ± 32 for MCP-2, 181 ± 41 for MCP-3, 204 ± 23 for RANTES, 140 ± 10 for MIP-1α and 126 ± 7 for MIP-1β, whereas random migration (no chemokine added) amounted to 36 ± 1. Based on the concentration at which maximum migration was reached, MCP-1 was more potent than MCP-2, RANTES, MIP-1α, and MIP-1β which were superior to MCP-3. Overall, the responses of CD45RO$^+$ lymphocytes conditioned with IL-2 were similar to those described previously for cloned CD4$^+$ and CD8$^+$ T cells (11).

Functional Response Correlates with Chemokine Receptor Expression. The effect of conditioning with IL-2 on migration toward MCP-1, RANTES and IL-8 was compared with the expression of the mRNA for the relevant receptors, CC-CKR1 (RANTES/MIP-1α-R), CC-CKR2 (MCP-1-RA/B), and IL-8R1/2. As shown in Fig. 2, chemotactic responses to MCP-1 and RANTES increased with the level of expression of the respective receptors. Transcripts for CC-CKR1 and CC-CKR2 were undetectable by Northern analysis in freshly isolated CD45RO$^+$ lymphocytes, which did not migrate, but became readily measurable after about 4 d when the cells began to show chemotaxis, and increased markedly with time as did the numbers of migrating cells. A similar IL-2–dependent increase in receptor message and function was observed in unfractionated blood lymphocytes (data not shown). The time course of receptor expression showed some differences: the message of ~3.5 kb for CC-CKR2, which was barely detectable after 4 d, increased continuously in intensity until day 12, while the 3 kb message for CC CKR1 was already marked at day 4, reached a maximum at day 6 to 10, and decreased to an intermediate intensity at day 12. No transcripts for the IL-8 receptors were detected in CD45RO$^+$ lymphocytes at any time during the treatment with IL-2, which fully agrees with the lack of IL-8–mediated chemotaxis. IL-8 receptor message, however, was readily found in neutrophils which are the main target cells for IL-8. Neutrophils and monocytes, which were routinely examined for chemokine receptor expression as controls, were also positive for CC-CKR1 transcripts, whereas expression of CC-CKR2 was only found in monocytes.

As a possible correlate of the IL-2–dependent induction of lymphocyte responsiveness to chemokines, we assessed the expression of the alpha subunit (Tac antigen) that is re-

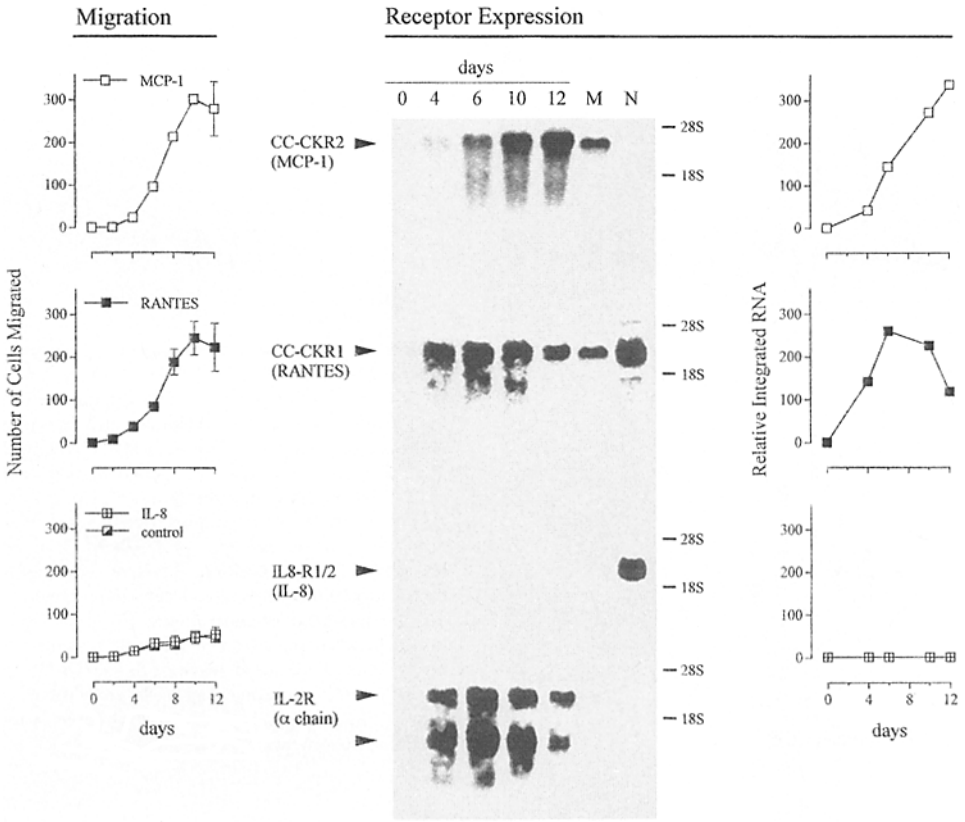

Figure 2. Chemotaxis correlates with chemokine receptor expression. CD45RO$^+$ lymphocytes were cultured in the presence of 400 U/ml IL-2 for up to 12 d, and tested for in vitro migration and chemokine receptor expression. Chemotaxis to 1 nM MCP-1, 10 nM RANTES, 10 nM IL-8, and random migration (control) in dependence of IL-2 exposure time is shown (mean number ± SD of migrating cells per five high power fields in triplicate wells). Total RNA from IL-2 conditioned CD45RO$^+$ lymphocytes and freshly isolated monocytes (*M*) and neutrophils (*N*) was analyzed by Northern blotting (10 μg per lane). The blot was sequentially hybridized with ^{32}P-labeled cDNA probes for the MCP-1 (CC-CKR2), RANTES (CC-CKR1), IL-8 (IL8-R1/R2), and IL-2 (α chain) receptors. Counts of the ^{32}P-labeled chemokine receptor RNA and the ethidium bromide-stained 28S RNA were integrated using the ImageQuant software and expressed as relative RNA levels. The position of the 18S and 28S ribosomal RNA is indicated. The data are representative of four independent experiments.

quired for formation of the heterotrimeric, high-affinity IL-2 receptor complex (30, 31). As shown in Fig. 2, stimulation of CD45RO$^+$ lymphocytes with IL-2 induced the expression of the 1.5- and 3.5-kb mRNA species that are typical for the alpha chain (29, 32) suggesting that IL-2 elicits its effects through the high-affinity IL-2 receptor.

Modulation of Chemokine Responsiveness by IL-2. The essential role of IL-2 is illustrated in Fig. 3. Lymphocytes that were fully responsive to MCP-1 and RANTES after a 10-day exposure to IL-2 rapidly lost the ability to migrate when IL-2 was removed. Chemotaxis toward MCP-1 or RANTES decreased to ~30% within 8 h, and fell below 10% in 48 h. When IL-2 was supplied again chemotactic responses were largely restored within the following 48 h. This modulatory effect of IL-2 was concentration dependent, as shown in Fig. 4 *A*, and was observed in 10 separate experiments performed with cells from different donors (Table 2). The loss and recovery of chemotactic responsiveness was paral-

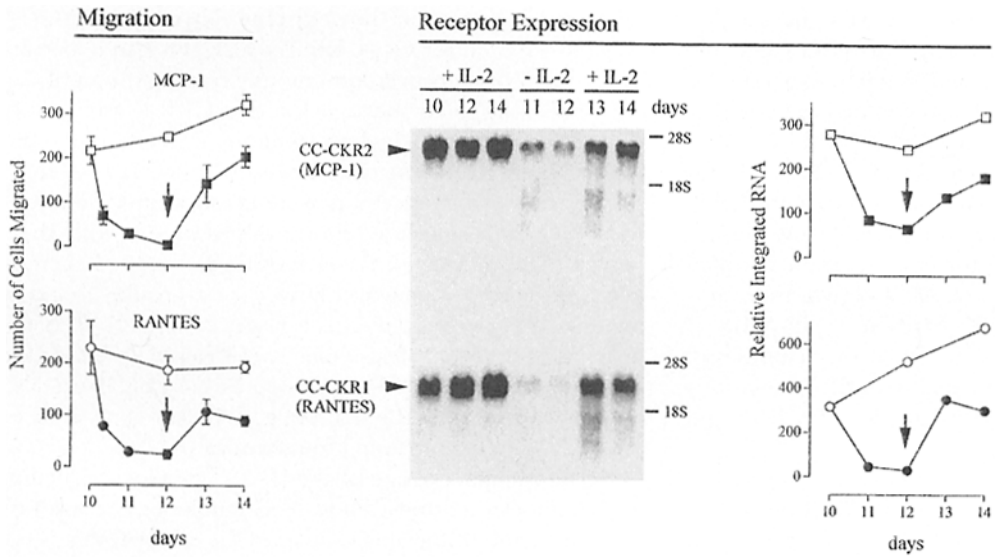

Figure 3. Continuous presence of IL-2 is required for chemokine responsiveness. CD45-RO$^+$ lymphocytes were cultured for 10 d in the presence of 400 U/ml IL-2, washed three times with PBS and further cultured with or without IL-2 (open and closed symbols, respectively). After 2 d, 400 U/ml IL-2 was added to the IL-2-free cultures (arrow). Chemotactic responses to 1 nM MCP-1 or 10 nM RANTES are shown as in Fig. 2. Total RNA was analyzed by Northern blotting (10 μg per lane). The blot was sequentially hybridized with ^{32}P-labeled cDNA probes for CC-CKR1 and CC-CKR2 and hybridization was quantitated as described in Fig. 2. The data are representative of three independent experiments.

Table 2. *Loss and Recovery of Chemotactic Responsiveness Is Regulated by IL-2*

	Time (h)*	MCP-1‡	RANTES‡
−IL-2	8	40.5 ± 15.0	47.7 ± 17.6
	24	10.8 ± 8.0	18.2 ± 13.2
	48	5.6 ± 3.9	12.3 ± 8.1
+IL-2	24	62.4 ± 11.8	72.3 ± 21.4
	48	89.7 ± 28.0	88.0 ± 27.2

*$CD45RO^+$ lymphocytes were cultured for 10 to 13 d in the presence of 400 U/ml IL-2, washed three times with PBS, and further cultured for up to 48 h without IL-2. After this time, the medium was supplemented with 400 U/ml IL-2 and incubation was continued for 24 or 48 h. ‡Chemotactic responses to 1 nM MCP-1 and 10 nM RANTES are expressed as percent migration with respect to the controls (cells cultured in the presence of IL-2). Mean values ± SD of 10 independent experiments.

leled by changes in receptor expression. CC-CKR1 and CC-CKR2 transcripts fell rapidly upon withdrawal of IL-2 and almost regained the initial values after renewed addition of IL-2 (Fig. 3). Although the response was similar for both receptors, the changes in CC-CKR1 mRNA were always more pronounced.

The effect of IL-2 was compared with that of several cytokines known to act on T lymphocytes. $CD45RO^+$ cells conditioned with IL-2 were kept for 2 d in IL-2–deficient medium and were then exposed to IL-2 or another cytokine. As shown in Fig. 4 *B*, the chemotactic responsiveness to MCP-1 and RANTES, which was lost upon withdrawal of IL-2, was partially restored by IL-4, IL-10, and IL-12, but remained virtually unaffected by treatment with IL-13, IFNτ, IL-1β, and TNF-α. IL-12 was the most effective substitute which led to the recovery of 15 and 40% of the chemotactic activity of MCP-1 and RANTES, respectively (Fig. 4 *B*).

Lymphocyte Activation with Anti-CD3 and Anti-CD28. The state of activation of lymphocytes is considered to affect their responsiveness to chemokines, and it was reported that anti-CD3 antibodies enhanced the migration of

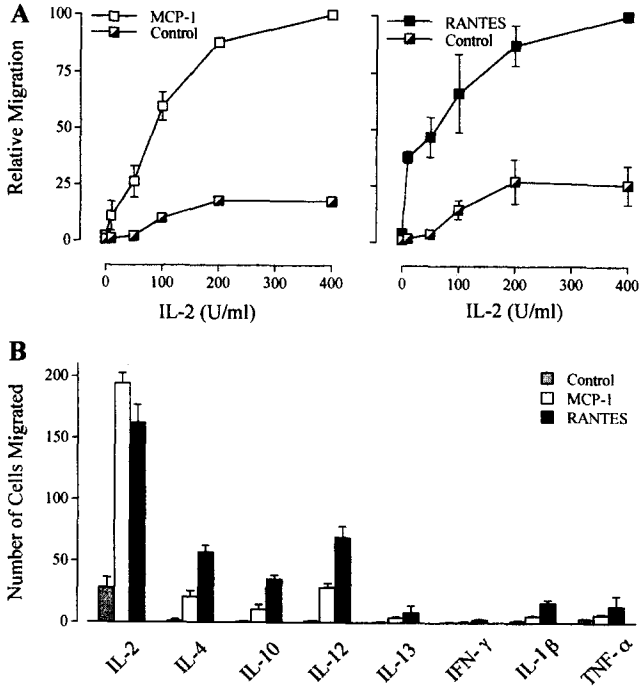

Figure 4. Recovery of chemokine responsiveness. Dependence on IL-2 concentration and effects of different cytokines. $CD45RO^+$ lymphocytes were cultured for 12 d in the presence of 400 U/ml IL-2, washed three times with PBS, further cultured for 2 d without IL-2, and then the medium was supplemented with increasing concentrations of IL-2 (*A*) or different cytokines (*B*). These were 400 U/ml IL-2, 100 U/ml IFNγ, and 100 ng/ml of IL-4, IL-10, IL-12, IL-13, IL-1β, or TNFα. After two days the chemotactic responses to 1 nM MCP-1 or 10 nM RANTES were tested. Mean numbers of migrating cells per five high-power fields in triplicate wells were determined. Values in (*A*) were normalized by setting to 100% the response obtained with cells exposed to 400 U/ml IL-2. Mean values ± SD from three independent experiments.

T cells toward MIP-1α, MIP-1β, and IP10 (9, 33). As shown in Fig. 5, neither chemotaxis toward MCP-1 or RANTES nor the expression of the corresponding chemokine receptors could be induced in freshly isolated $CD45RO^+$ lymphocytes by exposure to anti-CD3. These results suggest that signaling through TCR/CD3 complex is not sufficient for induction of responsiveness to chemo-

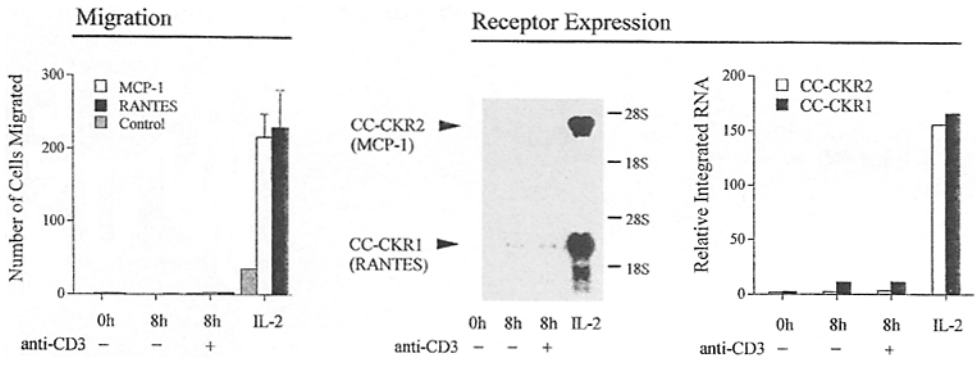

Figure 5. Effect of anti-CD3. Freshly isolated $CD45RO^+$ lymphocytes were used as such (0 h) or after culturing for 8 h on plates with or without coating with anti-CD3 in the absence of IL-2. Migration in response to 1 nM MCP-1, 10 nM RANTES, or medium alone (control), and chemokine receptor expression were then tested as described in Fig. 2. $CD45RO^+$ lymphocytes that were cultured for 13 d in the presence of 400 U/ml IL-2 were used for comparison. The data are representative of three independent experiments.

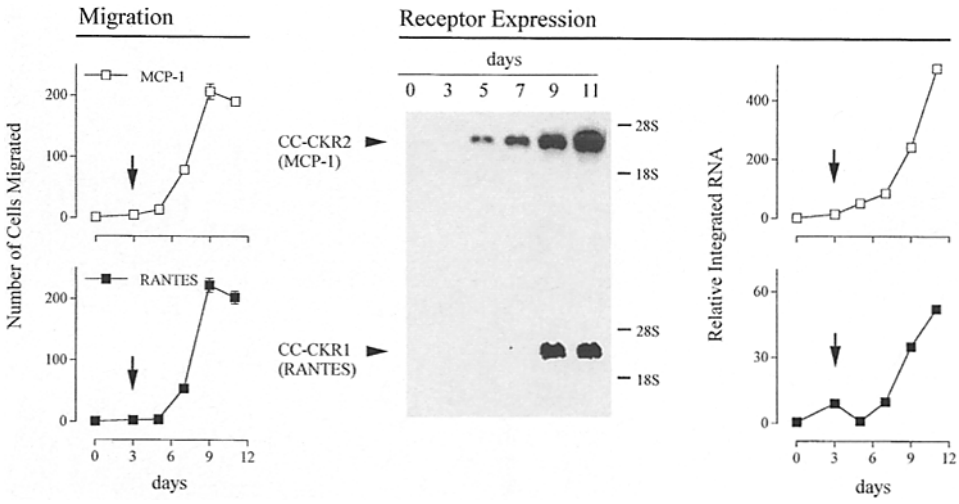

Figure 6. Effect of combined treatment with anti-CD3 and anti-CD28. Freshly isolated CD45RO$^+$ lymphocytes were cultured for 3 d on plates coated with anti-CD3 in a medium containing soluble anti-CD28 but no IL-2. Medium containing 50 U/ml IL-2 was then added (*arrow*) and the culture was continued for up to 11 d. Migration in response to 1 nM MCP-1 or 10 nM RANTES, and chemokine receptor expression were tested at different times as described in Fig. 2. One out of two independent experiments is shown.

kines, and that co-stimulation such as activation of the CD28 antigen (34, 35) may be required.

To test this hypothesis we examined the co-stimulatory effect of anti-CD28 in combination with anti-CD3. Freshly isolated CD45RO$^+$ lymphocytes were activated for 3 d with immobilized anti-CD3 and soluble anti-CD28 antibodies and then diluted into medium containing IL-2. Although they underwent blast formation and massive proliferation, the cells did not become responsive to chemokines unless IL-2 was added (Fig. 6). Similar effects were obtained with another mitogen, phytohemagglutinin (Fig. 1 *A* and data not shown). These observations suggested that anti-CD3 and anti-CD28 are not involved in the induction of chemokine receptor expression, but actually appear to counteract the effect of IL-2. As shown in Fig. 7, this appears to be the case: CD45RO$^+$ lymphocytes made responsive to MCP-1 and RANTES by treatment with IL-2 rapidly lost receptor expression and the capacity to migrate after exposure to anti-CD3 and anti-CD28 although IL-2 was still present. Chemotaxis toward MCP-1 and RANTES fell to 10–20% within 3 h, and was undetectable after 24 and 72 h. Concomitantly, a rapid and persistent decrease of chemokine receptor expression was observed.

Discussion

In recent years numerous laboratories have examined the responses of lymphocytes to chemokines, but the results have often been contradictory. Work with lymphocyte subpopulations and analysis of the phenotype of the migrating cells showed that CC rather than CXC-chemokines act on lymphocytes and that CD45RO$^+$, memory phenotype cells are the main responders (8, 12). Using blood-derived CD45RO$^+$ lymphocytes, we have studied the mechanisms

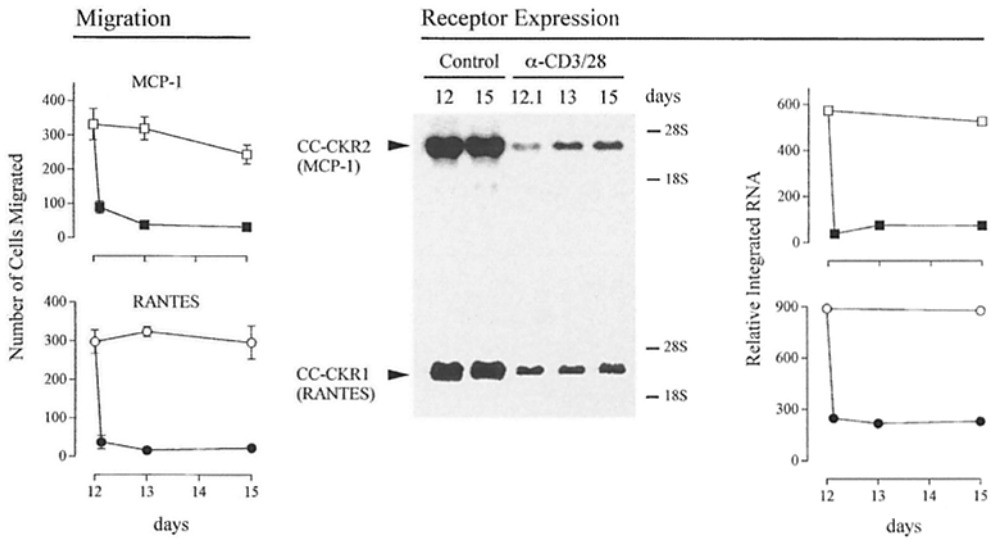

Figure 7. Down-regulation of chemokine responsiveness by anti-CD3 and anti-CD28. CD45-RO$^+$ lymphocytes cultured for 12 d with 400 U/ml IL-2 were cultured further under the same conditions (*open symbols*) or on plates coated with anti-CD3 in the presence of IL-2 (400 U/ml) and soluble anti-CD28 (*closed symbols*). Migration in response to 1 nM MCP-1 or 10 nM RANTES, and chemokine receptor expression were tested at different times as described in Fig. 2. The data are representative of three independent experiments.

that control chemokine responsiveness. In this paper we show that IL-2 induces the expression of two CC chemokine receptors, CC-CKR1 and CC-CKR2, which are selective for RANTES and MCP-1, respectively, and independently signal for migration.

In contrast to IL-2, other stimuli that also induce the activation and proliferation of $CD45RO^+$ cells, like antibodies against the TCR/CD3 complex and the CD28 antigen or phytohemagglutinin, did not elicit chemotactic activity and CC chemokine receptor expression, but actually delayed the effect of IL-2. This observation may be taken to suggest that T cells become responsive to recruitment by chemokines produced at inflammatory sites after IL-2 mediated expansion and not already during antigen-dependent activation in the draining lymph nodes. In agreement with the present results, Gao et al. (18) reported that lymphocytes exposed to phytohemagglutinin do not express CC-CKR1 mRNA, and work from our own laboratory showed a marked reduction of migration of cloned human T cells after treatment with anti-CD3 (11). An opposite effect, however, was reported by Taub et al. (9) on blood lymphocytes.

While progressively enhancing migration toward MCP-1, RANTES and the other CC chemokines, exposure to IL-2 did not induce the expression of IL-8 receptors and did not elicit chemotactic responsiveness of $CD45RO^+$ lymphocytes to IL-8. The present results are in agreement with our previous observations with cloned T cells (11) and NK-cells (36), which only responded to CC chemokines. A lack of chemotactic activity of IL-8 as well as IP10 for T lymphocytes was also reported by Roth et al. (13) who studied transendothelial migration. Although the controversy about lymphocyte responsiveness to IL-8 may remain (1), the demonstration of high-level CC-CKR1 and CC-CKR2 expression in the absence of detectable transcripts for the IL-8 receptors adds substantial new evidence against a role of IL-8 in lymphocyte recruitment.

The close correlation between the numbers of migrating cells in response to MCP-1, MCP-2, MCP-3, RANTES, MIP-1α, and MIP-1β, and the levels of CC-CKR1 and CC-CKR2 expression suggests that these receptors are of major importance for lymphocyte recruitment. CC-CKR1 was originally described as a RANTES/MIP-1α receptor (17, 18), and CC-CKR2, which occurs in two alternatively spliced forms, was shown to bind MCP-1 and MCP-3 but not RANTES or MIP-1α (19, 37, 38). Two additional CC chemokine receptors were reported recently, CC-CKR3, which binds eotaxin (20), and CC-CKR4 which has high affinity for RANTES and MIP-1α (22). CC-CKR4 was cloned from a human basophilic cell line and reported to occur at low (RT-PCR detectable) levels in resting and IL-2–stimulated blood T lymphocytes (21). Since RANTES and MCP-1 are major attractants for T cells (11, 13) and are selective for CC-CKR1 and CC-CKR2, respectively, these receptors were best suited for assessing expression as a correlate of migration.

IL-2 has pleiotropic regulatory effects on leukocytes and was shown to enhance various T cell responses including antigen-specific proliferation and cytotoxicity (39). The beta and gamma chains of the IL-2 receptor are constitutively expressed in resting blood lymphocytes (40, 41), whereas the alpha chain, which is required for formation of the high-affinity receptor, is expressed only after T cell activation (42, 43). In agreement with previous reports (30, 31), IL-2 treatment of $CD45RO^+$ lymphocytes induced the expression of the alpha chain. The time dependence of this effect was closely correlated with the expression of CC-CKR1 and CC-CKR2, suggesting that chemokine receptors are induced by signaling through the high affinity IL-2 receptor. It will be interesting to study the role of signal transducers and activators of transcription (44) in the regulation of chemokine receptor expression.

It is well known that chemokine receptors are subjected to agonist-dependent desensitization and that chemokine-challenged leukocytes rapidly become refractory to repeated stimulation (1). As shown for C5a, desensitization is related to the phosphorylation of serine residues in the carboxy-terminal region of the receptor (45–47). Desensitization could regulate the progress of a response like chemotaxis which is guided by increasing chemokine concentrations along a gradient. Receptor sequestration or internalization is another possible way to influence responsiveness. It has been shown that ligand-bound IL-8 receptors on neutrophils are rapidly endocytosed and recycled (48, 49), but no corresponding information is available on CC chemokine receptors. Receptor numbers can also be regulated at the level of gene expression. Changes in IL-8R expression were observed in neutrophils after treatment with G-CSF and LPS (50), and a decrease in the number of MCP-1–binding sites and responsiveness to MCP-1 was reported during differentiation of THP-1 cells by phorbol esters (51). By comparison, the effects of IL-2 on $CD45RO^+$ lymphocytes are much more pronounced. The strict dependence of receptor expression and chemokine responsiveness on IL-2 suggests that lymphocytes may be selected for immigration into sites of inflammation and immune intervention through activation and expansion by IL-2, which must be regarded as a major regulatory factor of lymphocyte migration in vivo. The IL-2-like effects of IL-4, IL-10, and IL-12 could be explained by the sharing of receptor subunits or similarities in the signal transduction pathways.

In this paper we have shown that IL-2 enhances CC chemokine receptor expression and chemotactic responsiveness of T lymphocytes. The upregulation of CC-CKR1 is a prerequisite for the activities of RANTES, MIP-1α, and MIP-1β, the three chemokines that were recently reported to suppress HIV replication in $CD4^+$ T cells (52). If this effect is mediated by receptors and signal transduction mechanisms known to elicit migration, it can be assumed that treatment with IL-2 may enhance the antiviral activity of T lymphocytes.

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