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Chemokine Receptor Expressions and Responsiveness of Cord Blood T Cells

Katsuaki Sato,* Hiroshi Kawasaki,† Hitomi Nagayama,* Makoto Enomoto,*
Chikao Morimoto,† Kenji Tadokoro,‡ Takeo Juji,‡ and Tsuneo A. Takahashi¹*

Chemokines and their receptors play a critical role in the selective attraction of various subsets of leukocytes. We examined the chemokine receptor expressions and responsiveness of cord blood (CB) T cells. Flow-cytometric analysis revealed that peripheral blood (PB) T cells expressed CCR-1, CCR-2, CCR-5, CCR-6, CXC chemokine receptor-3 (CXCR-3), and CXCR-4, while CB T cells expressed only CXCR-4 on their surface. Chemotactic migratory response of CB T cells to macrophage-inflammatory protein (MIP)-1 α , monocyte chemoattractant protein-1, RANTES, MIP-3 α , monokine induced by IFN- γ , and IFN- γ -inducible protein-10 was significantly impaired compared with those of PB T cells. In contrast, the ability of CB T cells to migrate to MIP-3 β , 6Ckine, and stromal cell-derived factor-1 α was greater than that of PB T cells, and these events were correlated with the expression levels of CCR-7 and CXCR-4, respectively. Engagement of CD3 and CD28 specifically up-regulated CXCR-3 expression and chemotaxis to monokine induced by IFN- γ and IFN- γ -inducible protein-10, whereas this stimulation down-regulated CCR-7 expression and chemotaxis to MIP-3 β and 6Ckine in PB T cells, but not in CB T cells. These results suggest that PB T cells and CB T cells exhibit distinct chemokine responsiveness via different chemokine receptor repertoire. *The Journal of Immunology*, 2001, 166: 1659–1666.

Chemokines are members of a family of small molecular mass proteins (8–10 kDa) that regulate leukocyte migration and activation (1, 2). Chemokines have been implicated in regulation of normal immune response and inflammation as well as certain physiological and pathogenic processes, including hemopoiesis, angiogenesis, allergy, autoimmune diseases, and viral infectious diseases (1, 2). There are two major chemokine subfamilies (CC and CXC), depending on the number of cysteines and spacing between the first two cysteines (1, 2). Responses to chemokines are mediated by seven-transmembrane-spanning G protein-coupled receptors (1, 2). The chemokine receptors also comprise two major groups: CCR1–10 bind CC chemokines, and CXC chemokine receptor (CXCR)² 1–5 bind CXC chemokines (1, 2). In addition, distinct chemokines appear to act on more than one receptor type *in vitro* (1, 2).

Accumulating results suggest that the interaction between chemokines and their receptors is crucial to the selective migration of circulating peripheral blood (PB) T cells to sites of inflammatory reactions or secondary lymphoid organs, including lymph nodes and Peyer's patches (2–4). A pattern of chemokine responsiveness and chemokine receptor expressions in PB T cells is implicated to

be correlated with the properties of their subsets, including memory phenotype vs naive phenotype (3–10) and Th1s vs Th2s (11–14). However, the chemokine receptor expressions and responsiveness of cord blood (CB) T cells remain unclear.

In this study, we examined the expression of CCRs and CXCRs in CB T cells and their chemotactic migratory ability in response to their respective ligands.

Materials and Methods

Media and reagents

The medium used throughout was RPMI 1640 supplemented with 2 mM L-glutamine, 50 μ g/ml streptomycin, 50 U/ml penicillin, and 10% heat-inactivated FCS. IL-8, macrophage-inflammatory protein (MIP)-1 α , monocyte chemoattractant protein (MCP)-1, RANTES, eotaxin, MIP-3 α , MIP-3 β , 6Ckine, growth-related oncogene α , monokine induced by IFN- γ (MIG), IFN- γ -inducible protein-10 (IP-10), and stromal cell-derived factor (SDF)-1 α , and B cell-attracting chemokine-1 were purchased from Pepro-Tech (London, U.K.).

Preparation of mAbs to CCR-1 and CCR-3

The preparation of mAbs to CCR-1 (IgG1 κ , clone 141-2) and CCR-3 (IgG1 κ , clone 444-11) was performed as described previously (15, 16). The specificities of the mAbs and their lack of cross-reactivity were verified by the staining of CCR-1- and CCR-3-expressing transfectants (15, 16). Their specificities were also certified using anti-CCR-1 mAb (R&D Systems, Minneapolis, MN) and anti-CCR-3 mAb (R&D Systems).

Preparation and culture of T cells and their subsets

Samples of PB and umbilical CB were obtained according to institutional guidelines with informed consent from all healthy volunteers (each 40 individuals). Mononuclear cells (MNCs) from each sample were obtained by Ficoll-Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden) density centrifugation, and purification of T cells was performed by E-rosetting (17). T cell preparations were typically >97% pure, as indicated by anti-CD3 mAb staining (Becton Dickinson, Mountain View, CA). For preparation of CD45RO⁺ T cells and CD45RA⁺ T cells from PB T cells and CB T cells, cells were negatively selected with anti-CD45RA mAb (PharMingen, San Diego, CA) and anti-CD45RO mAb (PharMingen) in combination with anti-mouse IgG mAb-conjugated immunomagnetic beads (Dyna, Oslo, Norway). The purities of these preparations were more than 98%

Departments of *Cell Processing and †Clinical Immunology and AIDS Research Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan; and ‡Japanese Red Cross Central Blood Center, Tokyo, Japan

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¹ Address correspondence and reprint requests to Dr. Tsuneo A. Takahashi, Department of Cell Processing, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan. E-mail address: takahashi@ims.u-tokyo.ac.jp

² Abbreviations used in this paper: CXCR, CXC chemokine receptor; BM, bone marrow; CB, cord blood; CBT, CB transplantation; GVHD, graft-vs-host disease; IP-10, IFN- γ -inducible protein-10; MCP, monocyte chemoattractant protein; MIG, monokine induced by IFN- γ ; MIP, macrophage-inflammatory protein; MNC, mononuclear cell; PB, peripheral blood; SDF, stromal cell-derived factor.

by flow-cytometric analysis with FITC-conjugated anti-CD45RA mAb and PE-conjugated anti-CD45RO mAb (all from Becton Dickinson).

Stimulation of T cells

PB T cells and CB T cells were either unstimulated or stimulated with a combination of immobilized mAbs to CD3 and CD28 (all from PharMingen) for 3 days at 37°C (17), and cells were used for subsequent experiments.

Flow cytometry

For surface marker analysis, cells were treated with 0.5% mouse serum (Dako, Glostrup, Denmark) for 15 min at 4°C to block the FcR, and stained with the following mAbs conjugated to FITC or PE for direct fluorescence: CD3, CD45RA, and CD45RO (Becton Dickinson); CCR-2, CXCR-1, CXCR-2, CXCR-3, and CXCR-5 (R&D Systems); CD28, CCR-5, and CXCR-4 (PharMingen). Cells were also stained with the corresponding FITC- or PE-conjugated isotype-matched control mAb (all from Becton Dickinson). In indirect staining, cells were incubated with biotin-conjugated anti-CCR-1 mAb (clone 141-2) or biotin-conjugated anti-CCR-3 mAb (clone 444-11) for 30 min at 4°C, washed twice with cold PBS, and subsequently stained with FITC-conjugated avidin (Becton Dickinson) for 30 min at 4°C. Thereafter, cells were washed twice, and suspended in PBS containing 0.2 µg/ml propidium iodide (Sigma; St. Louis, MO) to exclude dead cells. Analysis of fluorescence staining was performed with a FAC-Scalibur flow cytometer (Becton Dickinson) and CELLQuest Software. The expression levels of CCR-1 and CCR-3 were also confirmed by flow cytometry with anti-CCR-1 mAb (R&D Systems) and anti-CCR-3 mAb (R&D Systems).

Semiquantitative RT-PCR

RNA from each sample (5×10^6) was isolated using Trizol LS reagent (Life Technologies, Gaithersburg, MD). The first strand cDNA kit (SuperScript Preamplification System; Life Technologies) was used to make cDNA (20 µl) from 5 µg of each RNA. Amplification of each cDNA (1 µl) was performed with a SuperTaq Premix kit (Sawady Technology, Tokyo, Japan) using the specific primers of CCR-7 (16) and CXCR-3 (18). Specific primers for β-actin (Toyobo, Osaka, Japan) were also used for amplification. To activate DNA polymerase, preheating (95°C for 5 min) was performed. The reaction mixture was then subjected to 30 cycles of PCR under the following conditions: CCR-7, 94°C for 1 min, 61.5°C for 2 min, and 72°C for 3 min; CXCR-3, 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s. Following these procedures, a final extension (72°C for 10 min) was performed. PCR products were analyzed by electrophoresis through 2% agarose gels and visualized under UV light after ethidium bromide staining.

Assay for chemotaxis

The in vitro migration of T cells and their subsets was assessed in a Transwell cell culture chamber (Costar, Cambridge, MA), as described previously (3–7, 12), with some modifications (15, 16). In brief, 5-µm-pore-size polycarbonate filters were precoated with 5 µg of gelatin (WAKO, Osaka, Japan) in a volume of 50 µl on the lower surface, and dried overnight at room temperature. The coated filters were washed in PBS and then dried immediately before use. Cells ($5 \times 10^5/100 \mu\text{l}$) were added to the upper compartment of the chamber. Chemokines (1–100 ng/ml) diluted in serum-free culture medium (600 µl) were loaded in the lower compartment. After a 2-h incubation at 37°C, the filters were fixed with methanol and stained with hematoxylin and eosin (all from WAKO). Cells on the upper surface of the filters were removed by wiping with cotton swabs. Cells that had migrated to various areas of the lower surface were manually counted under a microscope at a magnification of $\times 200$, and each assay was performed in triplicate. The data are expressed as number of migrated cells/high power field. We also performed checkerboard control assay for each distinct chemotaxis assay, and the migration was not observed in the absence of a chemokine gradient (data not shown), indicating that the migratory response to these chemokines by these T cells was chemotactic and not due to chemokinesis.

Results

Expression levels of CCRs and CXCRs in PB T cells and CB T cells

Accumulating results indicate that leukocyte migration from the circulation into inflammatory sites is mediated through chemotaxis, which is regulated by specific interactions between chemokine and their receptors (2–14). To better understand the chemokine receptor

expressions and responsiveness of CB T cells, PB T cells and CB T cells were isolated from PBMNCs and CBMNCs (Fig. 1A) (17), and the chemotactic properties of these T cells were examined. Flow-cytometric analysis showed that PB T cells expressed CCR-1, CCR-2, CCR-5, CCR-6, CXCR-3, and CXCR-4, but not CXCR-1, CXCR-2, and CXCR-5, on their surface (Table I and Fig. 1B), consistent with previous reports (2–9). In contrast, CB T cells expressed CXCR-4, but not any CCRs or other CXCRs, on their surface (Table I and Fig. 1B).

Sallusto et al. (9) recently reported that PB naive T cells and central memory T cells, but not effector memory T cells, expressed CCR-7. To determine whether CB T cells express CCR-7, the expression level of CCR-7 in PB T cells and CB T cells was examined by semiquantitative RT-PCR. Fig. 5 shows that the transcriptional expression of CCR-7 was higher in CB T cells than PB T cells (Fig. 5).

Chemotactic migratory responses of PB T cells and CB T cells to CC and CXC chemokines

To determine the chemokine responsiveness of PB T cells and CB T cells, the chemotactic migratory abilities of PB T cells and CB T cells in response to various CC and CXC chemokines were examined (Table II and Fig. 2). PB T cells exhibited chemotactic migratory abilities in responses to MIP-1α (for CCR-1 and CCR-5), MCP-1 (for CCR-2 and CCR-4), RANTES (for CCR-1, CCR-3, and CCR-5), MIP-3α (for CCR-6), MIP-3β (for CCR-7), 6CKine (for CCR-7), MIG (for CXCR-3), IP-10 (for CXCR-3), and SDF-1α (for CXCR-4). CB T cells exhibited significantly less of a chemotactic migratory response to inflammatory chemokines, including MIP-1α, MCP-1, RANTES, MIP-3α, MIG, and IP-10, than those of PB T cells. In contrast, the ability of CB T cells to migrate to secondary lymphoid tissue-related chemokines such as MIP-3β and 6CKine as well as SDF-1α, which is a chemokine secreted from bone marrow (BM) stromal cells, was higher than that of PB T cells. We also observed neither cell type migrated in response to eotaxin (for CCR-3), IL-8 (for CXCR-1 and CXCR-2), growth-related oncogene α (for CXCR-2), or B cell-attracting chemokine-1 (for CXCR-5).

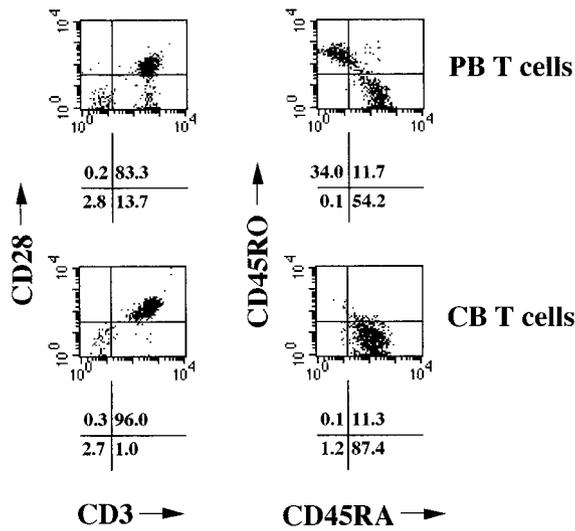
Expression levels of CCRs and CXCRs in CD45RO⁺ and CD45RA⁺ subsets of PB T cells and CB T cells

To address the feature of chemokine responsiveness of PB T cells and CB T cells, CD45RO⁺ and CD45RA⁺ T subsets were isolated (Fig. 3A), and the cell surface expressions of CCRs and CXCRs were examined (Table I and Fig. 3B). CD45RO⁺ PB T cells expressed CCR-1, CCR-2, CCR-5, CCR-6, CXCR-3, and CXCR-4, whereas CD45RA⁺ PB T cells expressed only CCR-1 and CXCR-4. In contrast, CD45RA⁺ CB T cells expressed CXCR-4, but no CCRs or other CXCRs, on the cell surface. We also observed that the level of the transcriptional expression of CCR-7 was lower in CD45RO⁺ PB T cells than CD45RA⁺ PB T cells and CD45RA⁺ CB T cells (Fig. 5).

Chemotactic migratory responses of CD45RO⁺ and CD45RA⁺ subsets of PB T cells and CB T cells to CC and CXC chemokines

We also investigated the chemotactic migratory responses of CD45RO⁺ subsets and CD45RA⁺ subsets of PB T cells and CB T cells to CC and CXC chemokines (Table II and Fig. 4). CD45RO⁺ PB T cells exhibited more potent chemotactic migratory ability in response to MIP-1α, MCP-1, RANTES, MIP-3α, MIG, and IP-10 than those of CD45RA⁺ PB T cells. Interestingly, the responsiveness of CD45RA⁺ CB T cells to MIP-1α and RANTES was less than that of CD45RA⁺ PB T cells. In contrast, the ability of CD45RO⁺ PB T cells to migrate to MIP-3β, 6CKine,

A



B

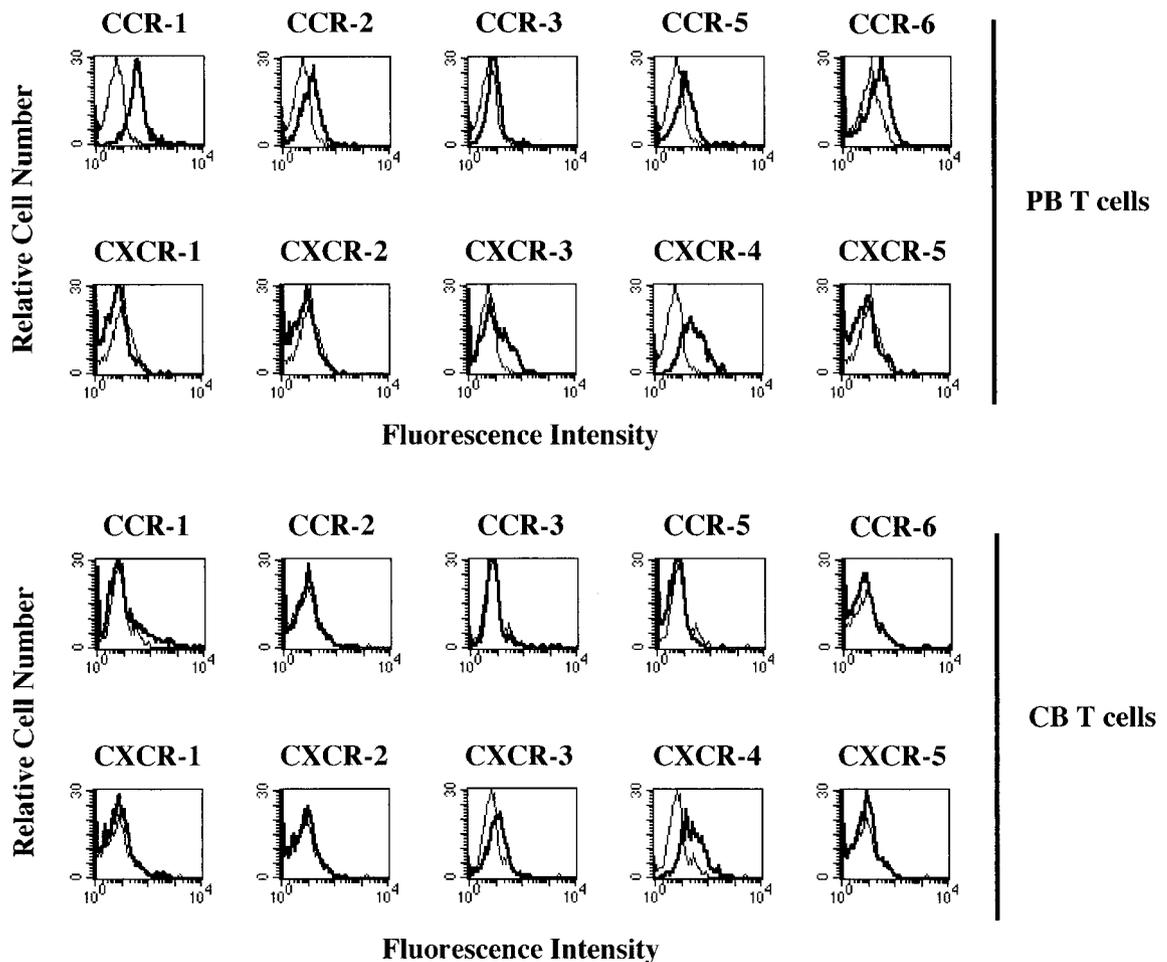


FIGURE 1. Expressions of CCRs and CXCRs in PB T cells and CB T cells. *A*, Characterization of PB T cells and CB T cells. PB and CB T cells were stained with the stated mAbs or isotype-matched mAbs, and their cell surface expressions were analyzed by FACS. Data are represented by a dot plot with a statistical analysis. *B*, Cell surface expressions of CCRs and CXCRs in PB T cells and CB T cells. PB and CB T cells were stained with the stated mAbs or isotype-matched mAbs, and their cell surface expressions were analyzed by FACS. Data are represented by a histogram. Cells were stained with stated mAbs (thick lines) or isotype-matched mAbs (thin lines). The results are representative of 20 experiments performed with similar results.

Table I. Effect of mAbs to CD3 and CB28 on the cell surface expressions of CCRs and CXCRs in PB T cells, CB T cells, and their subsets^a

Chemokine Receptors	Cell Surface Expression Levels (mean fluorescence intensity)/Positive Cells (%)				
	PB T cells			CB T cells	
	Total	CD45RO ⁺ T cells	CD45RA ⁺ T cells	Total	CD45RA ⁺ T cells
Unstimulation					
CCR-1	46 ± 7/87 ± 9	44 ± 5/87 ± 10	49 ± 5/89 ± 12	17 ± 3/13 ± 5	15 ± 5/9 ± 4
CCR-2	20 ± 6/18 ± 8	32 ± 6/35 ± 8	6 ± 4/<1	6 ± 3/<1	6 ± 2/<1
CCR-3	5 ± 2/5 ± 2	6 ± 2/2 ± 2	7 ± 3/<1	7 ± 3/<1	6 ± 3/<1
CCR-5	13 ± 5/14 ± 3	20 ± 5/28 ± 5	7 ± 4/<1	7 ± 4/<1	6 ± 2/<1
CCR-6	15 ± 6/16 ± 4	23 ± 5/35 ± 7	8 ± 3/<1	7 ± 3/<1	6 ± 3/<1
CXCR-1	8 ± 2/<1	8 ± 3/<1	7 ± 3/<1	6 ± 2/<1	6 ± 2/<1
CXCR-2	8 ± 3/<1	8 ± 3/<1	6 ± 3/<1	6 ± 2/<1	6 ± 2/<1
CXCR-3	14 ± 5/20 ± 5	21 ± 4/36 ± 7	7 ± 3/1 ± 1	7 ± 3/<1	6 ± 3/<1
CXCR-4	28 ± 6/64 ± 5	24 ± 4/55 ± 6	31 ± 5/79 ± 14	29 ± 6/76 ± 13	30 ± 6/78 ± 15
CXCR-5	7 ± 2/<1	8 ± 2/<1	7 ± 2/<1	7 ± 2/<1	7 ± 2/<1
Stimulation with mAbs to CD3 and CD28					
CCR-1	43 ± 6/86 ± 12	42 ± 6/85 ± 11	48 ± 6/85 ± 11	18 ± 4/12 ± 4	15 ± 5/9 ± 4
CCR-2	22 ± 5/16 ± 6	45 ± 7/34 ± 8	9 ± 4/<1	8 ± 3/<1	7 ± 3/<1
CCR-3	5 ± 2/5 ± 2	5 ± 2/2 ± 2	6 ± 3/<1	6 ± 3/<1	6 ± 3/<1
CCR-5	14 ± 6/13 ± 3	21 ± 4/27 ± 4	8 ± 2/1 ± 1	7 ± 3/<1	5 ± 4/<1
CCR-6	16 ± 6/15 ± 4	24 ± 6/34 ± 6	8 ± 3/<1	9 ± 3/<1	7 ± 3/<1
CXCR-1	7 ± 2/<1	7 ± 3/<1	7 ± 3/<1	6 ± 2/<1	6 ± 2/<1
CXCR-2	7 ± 3/<1	7 ± 3/<1	6 ± 3/<1	6 ± 2/<1	6 ± 2/<1
CXCR-3	30 ± 5/45 ± 4	47 ± 8/53 ± 10	18 ± 5/23 ± 6	6 ± 3/<1	5 ± 2/<1
CXCR-4	20 ± 5/51 ± 9	19 ± 3/45 ± 5	22 ± 6/65 ± 11	30 ± 5/77 ± 12	29 ± 4/77 ± 12
CXCR-5	7 ± 3/<1	8 ± 2/<1	6 ± 2/<1	7 ± 2/<1	7 ± 2/<1

^a PB T cells, CB T cells, and their subsets were either unstimulated or stimulated with mAbs to CD3 and CD28 for 3 days at 37°C. Cells were stained with stated mAbs or isotype-matched mAb, and their cell surface expressions of CCRs and CXCRs were analyzed by FACS. Cell surface expression levels of chemokine receptors were expressed as mean fluorescence intensity. The background staining of FITC- and PE-staining cells were <5. Data are given as mean ± SD of 20 individual experiments.

and SDF-1 α was lower than that of CD45RA⁺ subsets of PB T cells and CB T cells.

Effect of stimulation with mAbs to CD3 and CD28 on chemokine receptor expressions and responsiveness in PB T cells and CB T cells

We further examined the effect of mAbs to CD3 and CD28 on the chemokine receptor expressions and responsiveness in PB T cells and CB T cells. Treatment with mAbs to CD3 and CD28 caused enhancements of the expressions of cell surface product and transcript of CXCR-3 and chemotaxis to MIG and IP-10 in PB T cells

as well as CD45RO⁺ PB T cells and CD45RA⁺ PB T cells, while this stimulation failed to induce these events in CB T cells as well as CD45RA⁺ CB T cells as compared with unstimulated cells (Tables I and II, Fig. 5). In contrast, this stimulation reduced transcriptional expression of CCR-7 and chemotaxis to MIP-3 β and 6Ckine in PB T cells as well as CD45RO⁺ PB T cells and CD45RA⁺ PB T cells, with little or no effect on these events in CB T cells as well as CD45RA⁺ CB T cells when compared with unstimulated cells (Tables I and II, Fig. 5). Furthermore, ligation by mAbs to CD3 and CD28 slightly reduced cell surface expression of CXCR-4 and chemotaxis to SDF-1 α in PB T cells as well

Table II. Effect of mAbs to CD3 and CB28 on the chemotaxis of PB T cells, CB T cells, and their subsets^a

Chemokines	No. of Migrated Cells (Unstimulation/Stimulation with mAbs to CD3 and CD28)				
	PB T cells			CB T cells	
	Total	CD45RO ⁺ T cells	CD45RA ⁺ T cells	Total	CD45RA ⁺ T cells
None	7 ± 3/6 ± 2	5 ± 3/6 ± 2	5 ± 2/6 ± 3	4 ± 2/4 ± 1	4 ± 1/4 ± 1
MIP-1 α	56 ± 11/60 ± 14	82 ± 16/76 ± 14	18 ± 3/15 ± 3	8 ± 2/7 ± 3	7 ± 2/8 ± 3
MCP-1	43 ± 4/40 ± 5	64 ± 14/59 ± 13	15 ± 4/13 ± 3	9 ± 2/9 ± 4	10 ± 3/7 ± 2
RANTES	54 ± 10/58 ± 12	80 ± 16/79 ± 17	17 ± 5/14 ± 5	8 ± 2/6 ± 3	8 ± 3/6 ± 3
Eotaxin	6 ± 3/5 ± 1	5 ± 3/6 ± 2	8 ± 2/6 ± 4	6 ± 2/6 ± 1	7 ± 2/6 ± 2
MIP-3 α	39 ± 9/40 ± 10	63 ± 18/60 ± 14	7 ± 3/6 ± 3	7 ± 2/5 ± 1	5 ± 2/5 ± 2
MIP-3 β	42 ± 8/30 ± 7	18 ± 3/10 ± 4	53 ± 7/25 ± 8	56 ± 10/58 ± 11	63 ± 12/62 ± 13
6Ckine	38 ± 8/29 ± 7	16 ± 5/8 ± 4	51 ± 9/23 ± 7	63 ± 11/64 ± 14	72 ± 13/75 ± 15
IL-8	6 ± 3/4 ± 2	5 ± 2/5 ± 3	5 ± 3/6 ± 3	5 ± 2/4 ± 2	4 ± 2/5 ± 2
GRO α	5 ± 2/6 ± 2	5 ± 1/4 ± 2	5 ± 2/5 ± 3	5 ± 3/4 ± 1	6 ± 3/5 ± 1
MIG	65 ± 10/115 ± 18	103 ± 14/168 ± 18	20 ± 5/45 ± 8	7 ± 2/8 ± 3	5 ± 2/4 ± 3
IP-10	62 ± 9/112 ± 14	105 ± 12/162 ± 15	31 ± 8/53 ± 6	6 ± 3/5 ± 1	6 ± 3/5 ± 1
SDF-1 α	95 ± 14/80 ± 10	72 ± 11/60 ± 14	123 ± 13/98 ± 11	118 ± 10/116 ± 14	128 ± 14/126 ± 15
BCA-1	7 ± 2/5 ± 2	8 ± 6/6 ± 2	7 ± 3/6 ± 3	6 ± 2/4 ± 1	5 ± 2/4 ± 2

^a PB T cells, CB T cells, and their subsets were either unstimulated or stimulated with mAbs to CD3 and CD28 for 3 days at 37°C. Cells were seeded on the filters precoated on the lower surface with 5 μ g gelatin. Chemokines (100 ng/ml) were added to the lower chamber. After a 2-h incubation, the migrated cells on the lower surface were visually counted. Data are given as mean ± SD of 20 individual experiments.

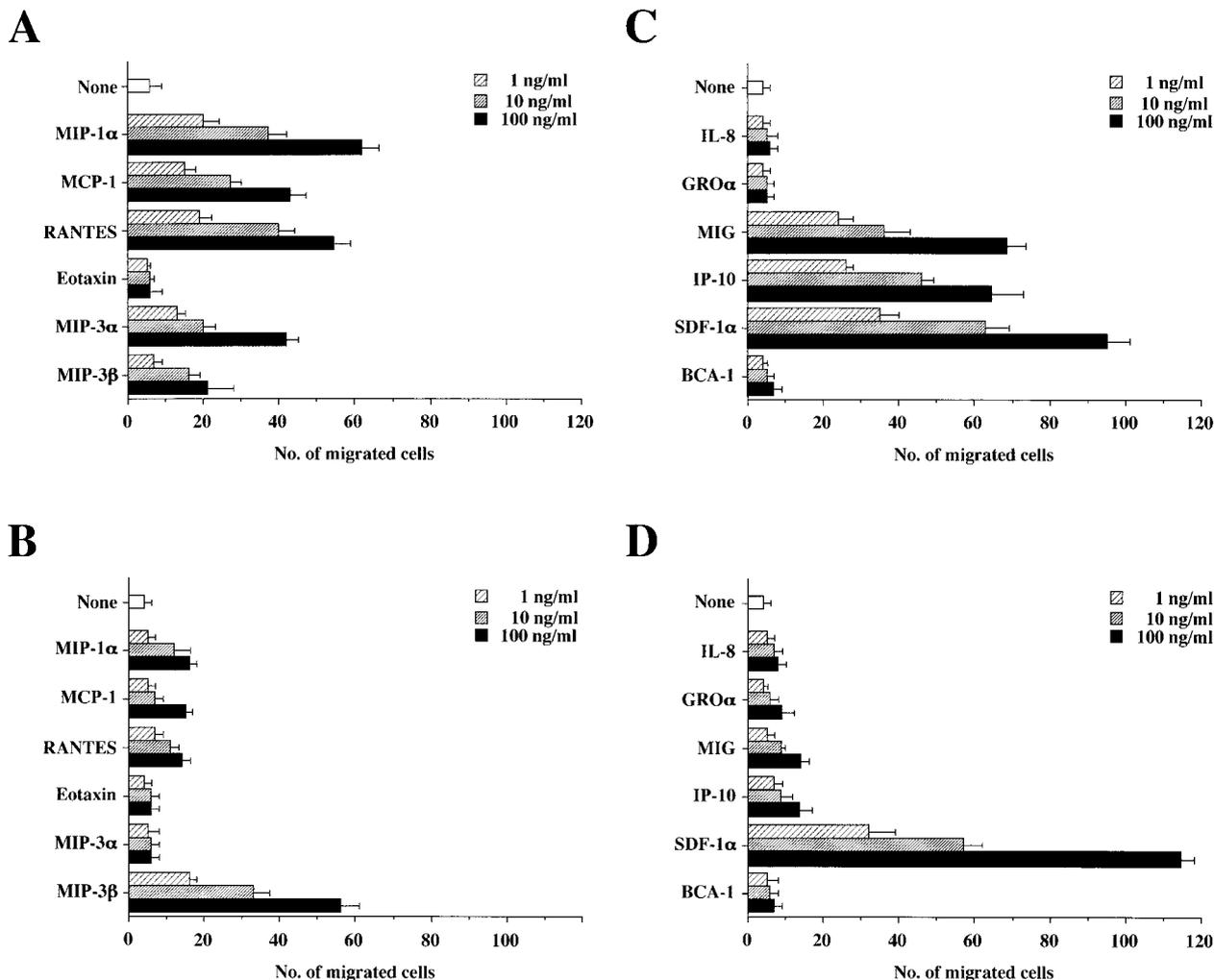


FIGURE 2. Chemotactic migratory abilities of PB T cells and CB T cells in response to CC and CXC chemokines. PB T cells (A, C) and CB T cells (B, D) (5×10^5) were seeded on filters precoated on the lower surface with $5 \mu\text{g}$ of gelatin. CC (A, B) and CXC chemokines (C, D) (0.1–10 ng/ml) as chemoattractants were added to the lower chamber. After a 2-h incubation, cells that migrated to the lower surface were visually counted. The results are representative of 20 experiments performed with similar results.

as CD45RO⁺ PB T cells and CD45RA⁺ PB T cells, but not in CB T cells as well as CD45RA⁺ CB T cells (Tables I and II). We also observed that stimulation with mAbs to CD3 and CD28 had little or no effect on expressions of other chemokine receptors as well as chemotaxis to the respective ligands in these cell types (Tables I and II).

Discussion

In this study, we demonstrated that PB T cells and CB T cells exhibit different chemokine responsiveness via selective expressions of chemokine receptors.

We showed that the ability of CB T cells to migrate to certain inflammatory chemokines was lower than that of PB T cells, while CB T cells exhibit more potent migratory response to secondary lymphoid tissue- and BM-related chemokines than PB T cells (Table II and Fig. 2). Furthermore, these events were associated with the expression levels of the respective receptors (Table I; Figs. 1B and 5). These results suggest that the distinct chemotactic behavior between PB T cells and CB T cells may involve different expression pattern of chemokine receptors.

The pattern of chemokine responsiveness and chemokine receptor expressions of T cells is reportedly correlated with the properties of their subsets (2–14). Consistent with previous reports (2–

10), CCR-2, CCR-5, CCR-6, and CXCR-3 were expressed on CD45RO⁺ PB T cells, but not on CD45RA⁺ PB T cells (Table I and Fig. 3). Furthermore, their chemokine receptor repertoire were correlated with their chemotactic migratory responses of these cell types to MCP-1, MIP-3 α , MIG, and IP-10 (Table II and Fig. 4). In contrast, the cell surface expression of CCR-1 was comparable between CD45RA⁺ PB T cells and CD45RO⁺ PB T cells (Table I and Fig. 3) (8). We also showed that the chemotactic migratory response of CD45RA⁺ PB T cells to MIP-1 α and RANTES, which are ligands for CCR-1 and CCR-5, was significantly decreased as compared with that of CD45RO⁺ PB T cells (Table II, Fig. 4) (4, 8). Indeed, we observed that MIP-1 α - and RANTES-induced chemotaxis in PB T cells as well as CD45RO⁺ PB T cells were mainly mediated by CCR-1, although these events are partly mediated by CCR-5 (Sato et al., manuscript submitted for preparation). Furthermore, MIP-1 α and RANTES exhibit more potent binding affinities to CCR-1 than CCR-5 (19). These results indicate that the expression level of CCR-1 does not simply correlate with the chemokine responsiveness to its respective ligands in CD45RA⁺ PB T cells. These phenomena imply that an inactivation of the downstream of CCR-1-mediating signaling events may account for the reduced chemokine responsiveness of CD45RA⁺ PB T cells to

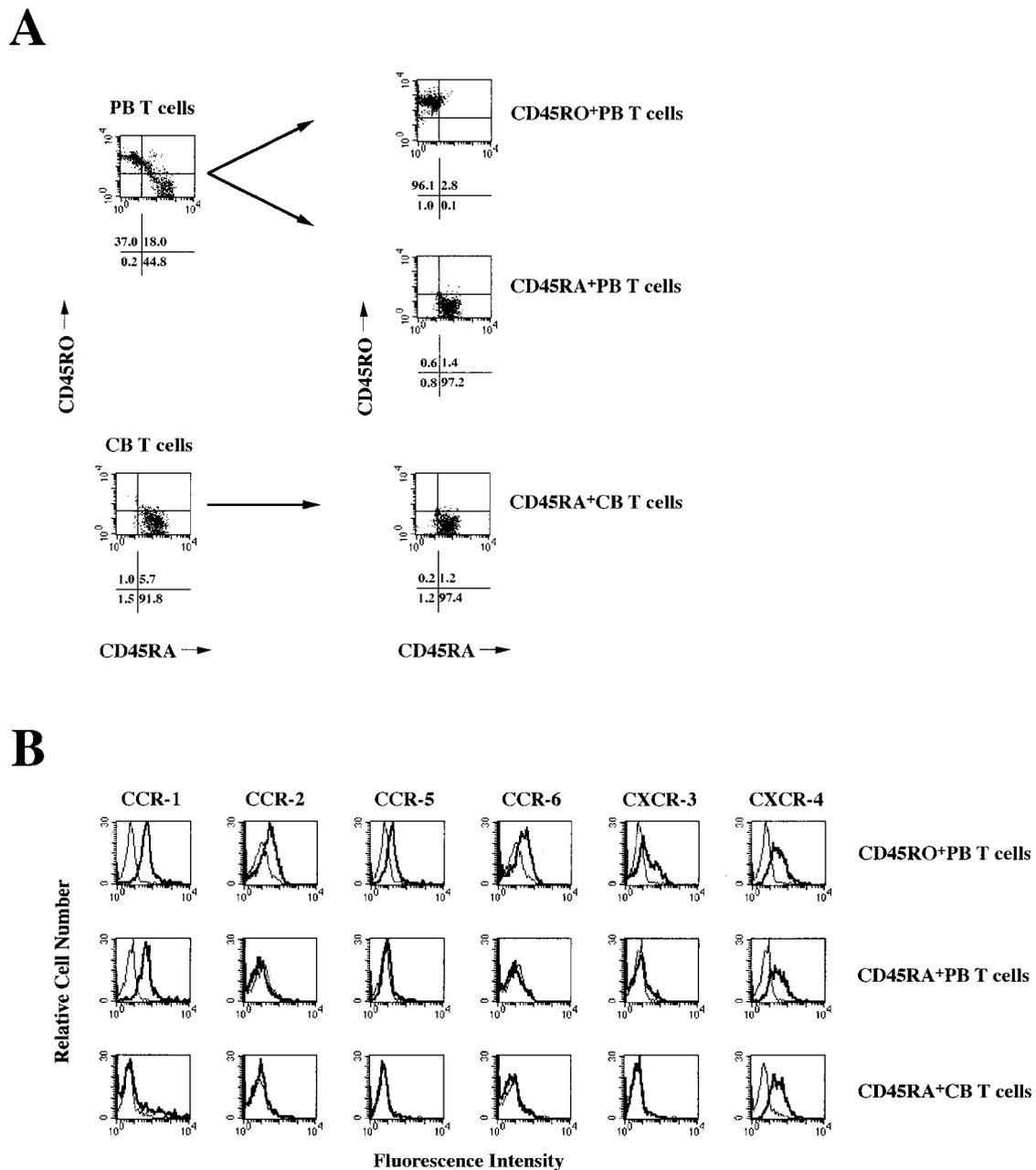


FIGURE 3. Expressions of CCRs and CXCRs in CD45RO⁺ and CD45RA⁺ subsets of PB T cells and CB T cells. *A*, Characterization of CD45RO⁺ and CD45RA⁺ subsets of PB and CB T cells. Cells were stained with the stated mAbs or isotype-matched mAbs, and their cell surface expressions were analyzed by FACS. Data are represented by a dot plot with a statistical analysis. *B*, Cell surface expressions of CCRs and CXCRs in CD45RO⁺ and CD45RA⁺ subsets of PB and CB T cells. CD45RO⁺ PB T cells, CD45RA⁺ PB T cells, and CD45RA⁺ CB T cells were stained with the stated mAbs or isotype-matched mAbs, and their cell surface expressions were analyzed by FACS. Data are represented by a histogram. Cells were stained with stated mAbs (thick lines) or isotype-matched mAbs (thin lines). The results are representative of 20 experiments performed with similar results.

MIP-1 α and RANTES as compared with that of CD45RO⁺ PB T cells. Further study will be needed to test this possibility.

We showed that CD45RA⁺ CB T cells as well as CD45RA⁺ PB T cells did not express CCR-2, CCR-5, and CCR-6 on the cell surface, and failed to migrate to their respective ligands (Tables I and II, Figs. 3 and 4). In contrast, these cell types exhibited more potent expressions of CCR-7 and CXCR-4 and migration to their respective ligands than CD45RO⁺ PB T cells (Tables I and II, Figs. 4 and 5). Interestingly, unlike CD45RO⁺ PB T cells and CD45RA⁺ PB T cells, CD45RA⁺ CB T cells did not exhibit cell surface expression of CCR-1 and chemotaxis for MIP-1 α and RANTES (Tables I and II, Figs. 3 and 4). These results suggest

that, although CD45RA⁺ PB T cells and CD45RA⁺ CB T cells exhibit the naive phenotype, they may exhibit distinct properties in terms of chemotactic properties.

There are conflicting reports about the role of CD3- and CD28-mediated signaling events in chemokine receptor expressions and responsiveness of mature T cells (3, 6–8, 20). In accordance with some of the results in the previous reports (6–8), ligation by CD3 and CD28 reciprocally regulated receptor expressions of CCR-7 and CXCR-3 and chemotaxis to their respective ligands in PB T cells as well as CD45RO⁺ PB T cells and CD45RA⁺ PB T cells (Tables I and II and Fig. 5). These phenomena imply that antigenic stimulation may allow PB naive T cells and central memory T cells

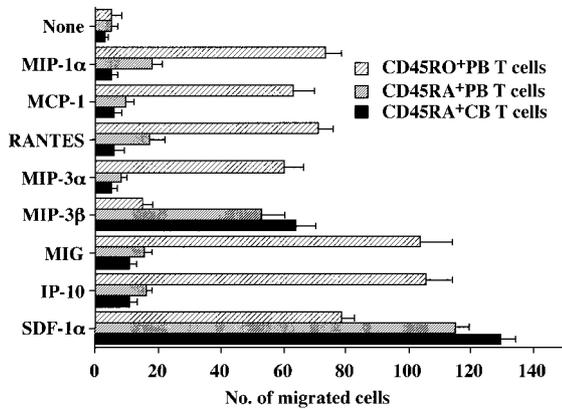


FIGURE 4. Chemotactic migratory abilities of CD45RO⁺ and CD45RA⁺ subsets of PB T cells and CB T cells in response to CC and CXC chemokines. CD45RO⁺ PB T cells, CD45RA⁺ PB T cells, and CD45RA⁺ CB T cells (5×10^5) were seeded on filters precoated on the lower surface with 5 μ g of gelatin. CC and CXC chemokines (10 ng/ml) as chemoattractants were added to the lower chamber. After a 2-h incubation, cells that migrated to the lower surface were visually counted. The results are representative of 20 experiments performed with similar results.

in secondary lymphoid tissues to traffick from these sites to peripheral inflammatory sites, while this stimulation may enhance the migration of PB effector memory T cells from circulation and other sites to inflammatory regions. In contrast, the discrepancies in the effect of mAbs to CD3 and CD28 on chemotactic properties of mature T cells in several groups including ours may be due to the cell culture condition and experimental design.

The molecular mechanism underlying the inability of mAbs to CD3 and CD28 to regulate the chemokine receptor expressions and responsiveness of CB T cells remains unclear. We (17) and others (21) have reported that protein tyrosine kinase-dependent cascades are activated in CD45RA⁺ PB T cells as well as CD45RO⁺ PB T cells following stimulation with mAb to CD3 and CD28, while this stimulation failed to activate these signaling events in CD45RA⁺ CB T cells. Although the molecular mechanism responsible for the regulation of the chemotactic properties of T cells is still unknown, CD3- and CD28-mediated signaling events involving protein tyrosine kinase-dependent cascades may be involved in this regulation. Thus, our results suggest that the inactivation of these signaling events be responsible for CD3- and CD28-mediated unfunctional regulation of chemokine receptor expressions and responsiveness in CD45RA⁺ CB T cells.

There appears to be a reduced incidence and severity of acute or extensive chronic graft-vs-host disease (GVHD) in CB transplantation (CBT) when compared with results obtained using PB and BM (22–24). Accumulating results suggest that reduced incidence and severity of GVHD in CBT may involve an inactivation of donor-derived CB T cells with respect to the secretion of proinflammatory cytokines as well as killing activity due to the expressions of perforin and Fas ligand (17, 25–28). Clinical manifestations suggested that transplanted T cells derived from BM and PB inoculum may be activated, while transplanted CB T cells may exhibit inactivated status following stimulation with allogeneic Ag and various cytokines (17, 22–24, 29). Recently, chemotaxis of donor-derived T cells was also implicated as crucial in the initiation and persistence of GVHD in certain animal models (30). It has been shown that certain chemokine receptor expressions and responsiveness were up-regulated in PB T cells by stimulation with several cytokines such as IL-2 and IL-12 (3, 13) as well as mAbs to TCR/CD3 complex and costimulatory molecules (6–8). In con-

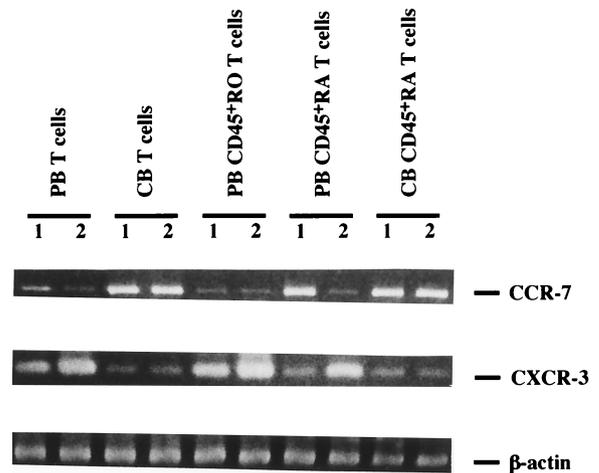


FIGURE 5. Effect of mAbs to CD3 and CD28 on the transcriptional expressions of CCR-7 and CXCR-3 in PB T cells and CB T cells. PB T cells, CB T cells, and their subsets were either unstimulated (1) or stimulated with mAbs to CD3 and CD28 (2) for 3 days at 37°C. RNA was extracted from the resultant cells of each three individuals, and the mRNA expressions of CCR-7 and CXCR-3 were evaluated by semiquantitative RT-PCR. PCR products for CCR-7 (1067 bp), CXCR-3 (293 bp), and β -actin (645 bp) are shown. The results of RT-PCR for β -actin demonstrate the loading of equal amounts of DNA on the gel. The results are representative of 10 experiments performed with similar results.

trast, CB T cells exhibit lower expressions of certain cytokine receptors, including IL-2R and IL-12R, than PB T cells (17, 29). Furthermore, stimulation with allogeneic Ag causes less activation in CB T cells than PB T cells (17). These phenomena imply that allogeneic Ag- and cytokine-stimulated PB T cells and BM T cells may more vigorously infiltrate into peripheral tissues mediated through up-regulation of CXCR-3, whereas these cell types fail to home into secondary lymphoid tissues via down-regulation of CCR-7, following with BM transplantation and PB stem cell transplantation. Conversely, CB T cells may preferentially home into secondary lymphoid tissues via CCR7 after CBT, while these cells failed to home into peripheral inflammatory regions in response to inflammatory chemokines via their respective receptors. These phenomena imply that the inability of CB T cells to migrate to certain inflammatory chemokines may partially account for lower incidence and severity of acute or extensive chronic GVHD of CBT when compared with BM transplantation as well as PB stem cell transplantation.

In summary, our results suggest CB T cells exhibited inability to migrate to certain inflammatory chemokines, while this cell type preferentially migrates to secondary lymphoid tissue- and BM-related chemokines via selective expressions of their respective receptors. A recent series of experiments revealed that the pattern of expression of chemokine receptors in T cell subsets is modulated by various proinflammatory cytokines, and flexible programs of chemokine receptor expression control the tissue-specific migration of effector T cells (3, 12). Although the change of the chemokine receptor expressions and responsiveness of CB T cells following various extracellular stimulation remains unclear, defining this property of CB T cells may clarify the immunological feature of CB.

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