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Efficient migration of CD4+ T cells into sites of infection/inflammation is a prerequisite to protective immunity. Inappropriate recruitment, on the other hand, contributes to inflammatory pathologies. The chemokine/chemokine receptor system is thought to orchestrate T cell homing. In this study, we show that most circulating human CD4+ T cells store the inflammatory chemokine receptors CXCR3 and CXCR1 within a distinct intracellular compartment. Equipped with such storage granules, CD4+ T cells coexpressing both receptors increased from only 1% ex vivo to ~30% within minutes of activation with PHA or exposure to the cyclooxygenase (COX) substrate arachidonic acid. Up-regulation was TCR independent and reduced by COX inhibitors at concentrations readily reached in vivo. The inducible inflammatory CXCR31/10/CXCR1+ phenotype identified nonpolarized cells, was preferentially triggered on CCR7+CD4+ T cells, and conferred increased chemotactic responsiveness. Thus, inducible CXCR3/1 expression occurs in a large fraction of CD4+ T cells. Its dependency on COX may explain a number of established, and point toward novel, effects of COX inhibitors. The Journal of Immunology, 2006, 177: 8806–8812.

The CD4+ T cells are central to many functions of the adaptive immune response. Through cytokine secretion and cell–cell interaction, CD4+ T cells provide help for CD8+ T cell responses and promote affinity maturation and isotype switching of Ag-specific B cells in B cell follicles and germinal centers (1–7). In nonlymphoid tissues, effector CD4+ T cells are thought to function largely through cytokine secretion to recruit and activate other populations of effector leukocytes such as CD8+ T cells, macrophages, neutrophils, and eosinophils (8–10).

The organization of the immune system is the result of tissue- and microenvironment-specific lymphocyte homing, which, in turn, is mediated by lymphocyte expression of surface adhesion and chemotactant receptors. Chemokines are major chemotactants acting on lymphocytes and are required for the appropriate tissue and microenvironmental organization of these cells (7, 11–18). Differential expression of chemokine receptors is thought to dictate, to a large extent, the migration properties of the various CD4+ T cell subsets.

In addition to peptide chemokines, lipid mediators (leukotrienes and PGs) have recently been identified (19–22) to directly mediate homing of specific T cell subsets. PGs are synthesized by activated lymphocytes and are required for the appropriate tissue and microenvironmental organization of these cells (7, 11–18). Differential expression of chemokine receptors is thought to dictate, to a large extent, the migration properties of the various CD4+ T cell subsets.

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Isolated of CD4+ T cells

Anticoagulated blood was drawn from healthy donors after written informed consent and Institutional Review Board approval. PBMCs were prepared by centrifugation through a density gradient on Histopaque-1077 (Sigma-Aldrich). Monocytes were depleted, and CD4+ cells positively selected by means of anti-CD14 and anti-CD4 magnetic beads, respectively (both from Miltenyi Biotec). Purity of CD4+ T cells, as assessed by flow cytometry, was typically >97% (data not shown).

Activation and cell surface staining of CD4+ T cells

Isolated CD4+ T cells were resuspended in medium and incubated with purified PHA (1–20 μg/ml, as indicated) (Phaselos spp.; REMEL) or arachidonic acid (60–150 μM, as indicated). Toxicity of short-term exposure of CD4+ T cells (2 min) to PHA and arachidonic acid was assessed in cell viability assays. Viability was identical and ≥ 98% in either PHA or arachidonic acid–exposed and nonexposed samples (data not shown). After each incubation, cells were spun down, resuspended in PBS/1% BSA, and labeled for 30–45 min at 4°C with appropriate Abs. After labeling, cells were washed twice in PBS/1% BSA, and data were acquired with a FACSCalibur flow cytometer (BD Biosciences) and analyzed using CellQuest software (BD Biosciences). When indicated, CD4+ T cells were preincubated for 2 h on ice with 1 μg/ml cytochalasin D (CytD) or 5 μg/ml cyclohexamide (CHX) (both from Sigma-Aldrich), or for 15 min at room temperature with ibuprofen (stock solution 1 mg/ml in DMSO; Glatt), indomethacin (stock solution 1 mg/ml in 0.9% NaCl solution; Merck), the
selective COX1 inhibitors FR122047 and SC560, the selective COX2 inhibitor SC791, or the 5-lipoxygenase-specific inhibitor nordihydroguaiaretic acid (stock solution 5 mg/ml in DMSO; Calbiochem). The final concentration of DMSO never exceeded 1%, and the dilution medium was controlled for in each experiment. For TCR cross-linking experiments, isolated CD4+ T cells were resuspended in RPMI 1640/10% heat-inactivated normal human serum and plated in 24-well plates (Nunc) that had either been precoated with anti-CD3 mAb (20 μg/ml) plus anti-CD28 mAb (20 μg/ml) or along with soluble anti-CD3 and anti-CD28 mAbs at the same concentrations.

**CD4+ T cells sorting/intracellular cytokine staining**

CD4+ T cell subset sorting was performed on a DakoCytometry MoFlo (DakoCytometry) cell sorter. The following populations of CD4+ T cells were sorted: bulk CD4+ T cells, CXCR3highCXCR1+CD4+ T cells ex vivo, and CXCR3dim&high CXCR1− CD4+ T cells after PHA activation (PHA at 10 μg/ml for 2 min). For each cell population, 10,000–200,000 cells were sorted. Purity was evaluated by postsort flow cytometry and was >85% (data not shown). Sorted cells were incubated overnight in RPMI 1640 supplemented with 10% FBS (R10) in the presence of 1.8 μg/ml PHA and 1 μl/ml GolgiPlug (BD Biosciences). After overnight activation, cells were fixed and permeabilized using the Cytofix/Cytoperm kit according to the manufacturer’s instructions and labeled with anti-IFN-γ and anti-IL-4 mAbs (BD Biosciences).

**Confocal microscopy**

Isolated CD4+ T cells (nonactivated or activated, as indicated) were permeabilized for 20 min at 4°C in PBS supplemented with 2% BSA, 5 mg/ml sandoglobulin (provided by the Red Cross, Blood Donor Center, Bern, Switzerland), and 0.2% saponin, a permeabilization buffer (Sigma-Aldrich). Subsequently, primary Abs (anti-EEA1-FITC, anti-GM130-FITC, anti-β2-microglobulin-FITC, anti-CD63-FITC, anti-CXCR3-FITC, anti-CXCR1-biotin, or appropriate isotype control mAb) were added to the buffer and incubated for 20 min on ice. After each incubation step, cells were washed three times and resuspended in permeabilization buffer. Biotinylated anti-CXCR1 mAb was detected by incubating with streptavidin-Alexa467 (Molecular Probes) for 20 min at 4°C. Following labeling, cells were placed on a 10-chamber slide (chamber diameter, 6 mm; Semadeni), mounted with Vectashield fluorescence mounting medium (Vector Laboratories) and analyzed using the LSM 510 META confocal laser scanning microscopy system (Zeiss). A Zeiss Plan Neofluar 63×/1.25 numeric aperture oil (n=1.47) objective was used, and images were imported into and arranged with Adobe Photoshop software (Adobe Systems).

**Chemotaxis assays**

Isolated CD4+ T cells were resuspended in RPMI 1640/1% BSA, activated with PHA (10 μg/ml for 2 min) as indicated, washed, and loaded in duplicate into 8-μm pore size polycarbonate Transwell inserts for 24-well cell culture dishes (Corning). IL-8 (CXC8), IFN-γ-inducible protein 10 (IP-10) (CXC10), and MIP-1α (CCL3) (all from PeproTech) were added to the lower wells at various concentrations. Cells were spun onto the membrane for 1 min at 50 × g. After incubation for 2 h at 37°C, transmigrated membrane-anchoring adherent cells were spun down by centrifugation for 8 min at 130 × g, and transmigrated cells were counted using a Neubauer counting chamber system (Milian).

**Statistical analyses**

Two-tailed Student’s t test analyses were performed with the JMP version 3 software (SAS Institute). Values of p < 0.05 were considered statistically significant.

**Results**

**A distinct population of PHA-activated human CD4+ T cells rapidly up-regulates CXCR3 and CXCR1**

We assessed expression of inflammatory chemokine receptors (CXCR3, CXCR1, CCR4, CCR5, and CX3CR1) on nonactivated VS PHA-activated CD4+ T cells by flow cytometry (Fig. 1A). Baseline chemokine receptor expression was as follows: CXCR3dim, 2.6 ± 1.7%; CXCR3high, 26.9 ± 9.1%; CXCR1, 3.3 ± 2.6%; CCR4, 12.3 ± 0.7%; CCR5, 5.7 ± 1.7%; CX3CR1, 1.3 ± 1.7%. After 2 min of activation with PHA (10 μg/ml), 32.9 ± 10.3% CD4+ T cells displayed a CXCR3high and a 35.3 ± 13.0% CXCR1high phenotype. By contrast, the percentage of CXCR3dim, CCR4+, CCR5+, and CX3CR1−CD4+ T cells did not significantly increase or even decreased upon activation (CXCR3dim, 17.4 ± 6.0%; CCR4+, 7.8 ± 0.5%; CCR5+, 2.6 ± 0.8%, and CX3CR1−; 2.7 ± 1.0%; n = 14 for CXCR3 and CXCR1; n = 3 for CCR4, CCR5, and CX3CR1). As shown in Fig. 1B, the appearance of CXCR3 and CXCR1 on CD4+ T cells of was dose dependent, detectable within seconds of activation, and transient, with expression of either receptor reapproaching baseline levels within 10 min of activation. Four-color flow cytometry identified the CXCR3highCXCR1+ phenotype to define a single and distinct CD4+ T cell population both before activation (1.4 ± 1.1%; Fig. 1C, upper panel; n = 14) as well as after activation (29.9 ± 11.1%; Fig. 1C, lower panel; n = 14).

**CD4+ T cells store preformed CXCR3 and CXCR1 in a distinct intracellular compartment**

Based on the kinetics with which CXCR3 and CXCR1 appeared on the cell surface, we assumed up-regulation from a preformed pool of protein. The assumption was tested by repeating PHA activation experiments in the presence of 1) CHX, inhibiting de novo protein synthesis, and 2) CytD, inhibiting actin polymerization (25, 26). As shown in Fig. 2A, inhibition of protein synthesis did not change the expression level of either CXCR3 or CXCR1 on nonactivated and PHA-activated CD4+ T cells: CXCR3dim&high nonactivated, 34.6 ± 12.5%; CXCR3dim&high activated, 85.0 ± 4.5%; CXCR3dim&high nonactivated and CHX, 33.5 ± 12.3%; and CXCR3dim&high activated and CHX, 84.6 ± 5.5%. CXCR1 nonactivated, 3.9 ± 2.2%; CXCR1 activated, 84.4 ± 11.7%; CXCR1 nonactivated and CHX, 2.9 ± 1.5%; CXCR1 activated and CHX, 84.8 ± 13.4% (n = 3). By contrast, and further indication of an intracellular pool of preformed CXCR3 and CXCR1, inhibiting actin polymerization abolished/reduced chemokine receptor up-regulation: CXCR3dim&high nonactivated, 28.7 ± 8.4%; CXCR3dim&high activated, 62.0 ± 12.6%; CXCR3dim&high nonactivated and CytD, 28.4 ± 8.3%; CXCR3dim&high activated and CytD, 18.1 ± 11.8% (i.e., 131.0% mean inhibition of CXCR3 up-regulation; range, 116–139%); CXCR1 nonactivated, 1.3% (±0.6%); CXCR1 activated, 51.8 ± 12.6%; CXCR1 nonactivated and CytD, 1.1 ± 0.4%; CXCR1 activated and CytD, 15.5 ± 9.9% (i.e., 71.5% mean inhibition of CXCR1 up-regulation; range, 60–82%) (n = 3) (Fig. 2A). The fact that activation-induced up-regulation of CXCR3 and CXCR1 occurred in the presence of inhibitors of protein synthesis, but not after inhibiting actin cytoskeleton rearrangement, was strongly suggestive of a regulated intracellular pool of preformed protein. Using confocal microscopy, we next aimed at better defining the CXCR3/CXCR1-storing compartment(s) with respect to each other, as well as with respect to established markers of the Golgi compartment (GM130), the secretory pathway (β2-microglobulin), the endosomal compartment (EEA-1), and the lysosomal compartment (CD63) (Fig. 2B). In line with the FACScan analyses shown in Fig. 1A, CXCR3 but no CXCR1 cell surface staining was seen on about one-third of all CD4+ T cells (data not shown). In addition, a granular intracellular staining pattern of variable intensity for both CXCR3 and CXCR1 was seen in most cells. Dual-color labeling and overlay analyses established near-total colocalization of CXCR3 and CXCR1, indicative of a single compartment storing both receptors (Fig. 2B, left panel). When analyzing activated CD4+ T cells, by contrast, most of the granular staining pattern of CXCR3 and CXCR1 was lost. Instead, a more diffuse (cell surface) staining-pattern became apparent (Fig. 2, middle panel). Note that up-regulation of CXCR3/1-storing granules translated into a parallel up-regulation of both receptors on the cell surface (Fig. 1B) and the appearance of a single

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The chemokine receptor CCR7 identifies CD4+ T cells that preferentially circulate between the blood and secondary lymphoid structures in search of cognate Ag (27). We compared cell surface expression of CCRX3 and CCRX1 on nonactivated and PHA-activated CCR7+ naive (CD45RA+) and central memory (CD45RA−) and on CCR7− effector memory (CD45RA−/−) cells (27, 28). Expression was assessed by subset gating of bulk CD4+ T cells. PHA activation per se had no significant impact on the percentage distribution of the CCR7+ vs CCR7− CD4+ T cell subsets (CCR7− nonactivated, 76 ± 11%; CCR7+ activated, 26 ± 9%). Intriguingly, although up-regulation was detected on both CCR7+ as well as CCR7− CD4+ T cell subsets, CCR7− CD4+ T cells preferentially acquired a CCR3bhighCCR4low phenotype (Fig. 3A). Therefore, regulation of the CCR3/3 storage compartment was subset selective, with preferential up-regulation occurring on CD4+ T cells with a CCR7− phenotype. To reveal the cytokine profile of CCRX3bhighCCR4low T cells, we sorted 1) bulk CD4+ T cells, 2) ex vivo CCRX3bhighCCR4low T cells, and 3) PHA-activated CCRX3bhighCCR4low T cells (PHA at 10 μg/ml for 3 min). Production of IFN-γ and IL-4 by sorted CCR4− cells was assessed via intracellular cytokine staining and flow cytometry. Compared with bulk CD4+ T cells, the frequency of CD4+ T cells producing IL-4 or IFN-γ tended to be higher among cells with a CCRX3bhighCCR4low phenotype. However, this difference did not reach statistical significance. No preferential (polarized) production of IFN-γ or IL-4 was observed in CCRX3bhighCCR4low T cells (Fig. 3B, n ≥ 4).

**FIGURE 1.** Expression of inflammatory chemokine receptors on nonactivated vs PHA-activated human CD4+ T cells. A, Expression of CCRX3, CCRX1, CCR4, CCR5, and CX3CR1 was assessed before (n/a, nonactivated) and after 2 min of activation (a, activated) with PHA (10 μg/ml). Upper panel, Each line represents a separate experiment and donor (n = 14 for CCRX3 and CCRX1, n = 3 for CCR4, CCR5, and CX3CR1); lower panel, representative histograms are shown (filled histogram, expression ex vivo; open histogram, expression after activation). Note that a significant population of cells was induced to express a CCRX3bhigh and a CCRX1low representative histograms are shown (filled histogram, expression ex vivo; open histogram, expression after activation). B, Time course and dose response of CCRX3bhigh and CCRX1 expression on PHA-activated CD4+ T cells (n = 3). Data are presented as mean ± SD. C, Representative FACs dot plot of CCRX3 and CCRX1 expression on CD4+ T cells. Upper panel, Expression ex vivo; lower panel, expression after 2 min of activation with PHA (10 μg/ml). Note the rapid, transient, and selective up-regulation of CCRX3bhigh and CCRX1 on a significant and distinct population of CD4+ T cells.
CXCL10 (IP-10, CXCR3 ligand), CXCL8 (IL-8, CXCR1 ligand), and CCL3 (MIP1α, CCR5 ligand). A robust increase in chemotactic activity of activated vs nonactivated CD4^+^ T cells was observed in gradients of CXCL10 and CXCL8. By contrast, activation did not increase chemotaxis toward CCL3, excluding activation per se, rather than cell surface up-regulation of CXCR3 and CXCR1, to be responsible for the observed increase in chemotactic activity (Fig. 4).

**FIGURE 2.** Evidence of intracellular CXCR3 and CXCR1 storage in CD4^+^ T cells. A, Up-regulation of both CXCR3 (left panel) and CXCR1 (right panel) occurred independently from protein synthesis (CHX), but was largely abolished by inhibiting actin polymerization (CytD) (n = 3). Data are presented as mean ± SD. B, After permeabilization, CD4^+^ T cells were double-labeled for CXCR3 and CXCR1, or for CXCR1 and GM130 (Golgi), β2-microglobulin (constitutive secretory pathway), CD63 (lysosomal compartment), and EEA-1 (endosomal compartment). CXCR3 and CXCR1 almost entirely colocalized in a subcellular compartment characterized by a granular staining pattern (left panel). When analyzing activated CD4^+^ T cells, most of the granular staining pattern of CXCR3 and CXCR1 was lost (middle panel). CXCR3/1-containing granules were further characterized relative to the endosomal compartment (marker protein: EEA-1), the constitutive secretory pathway (marker protein: β2-microglobulin), the Golgi apparatus (marker protein: GM130), and the lysosomal compartment (marker protein: CD63). Although partial colocalization of CXCR1 (used to mark CXCR3/1-containing granules) with GM130 and β2-microglobulin was observed, no relevant colocalization with EEA-1 or CD63 was detected. No staining was seen when incubating permeabilized CD4^+^ T cells with appropriate isotype control Abs (data not shown). n/a, Nonactivated.

CXCL10 (IP-10, CXCR3 ligand), CXCL8 (IL-8, CXCR1 ligand), and CCL3 (MIP1α, CCR5 ligand). A robust increase in chemotactic activity of activated vs nonactivated CD4^+^ T cells was observed in gradients of CXCL10 and CXCL8. By contrast, activation did not increase chemotaxis toward CCL3, excluding activation per se, rather than cell surface up-regulation of CXCR3 and CXCR1, to be responsible for the observed increase in chemotactic activity (Fig. 4).

**FIGURE 3.** Phenotypic characterization of CXCR3^high^CXCR1^+^CD4^+^ T cells. A, Before and after activation with PHA (2 min, 10 μg/ml), CD4^+^ T cells were labeled for CCR7, CD45RA, and CXCR1 or CXCR3 (n ≥ 6). Both CXCR3^high^ as well as CXCR1 was preferentially up-regulated on subsets of CD4^+^ T cells expressing the lymph node-homing chemokine receptor CCR7. B, Production of IFN-γ and IL-4 was assessed in CXCR3^high^CXCR1^+^CD4^+^ T cells that were sorted ex vivo or after activation, and was compared with bulk CD4^+^ T cells. The frequency of cells producing IL-4 or IFN-γ tended to be higher in CXCR3^high^CXCR^−^ cells as compared with bulk CD4^+^ T cells, although the difference did not reach statistical significance. No polarization toward preferential production of IFN-γ or IL-4 became evident in CXCR3^high^CXCR^−^CD4^+^ T cells (n ≥ 4). Data are presented as mean ± SD.
Mobilization of the CXCR3/1 storage compartment is regulated independently from TCR signaling by COX activity

Activation of CD4+ T cells can be grouped into Ag dependent (i.e., TCR dependent) vs Ag independent. PHA activation does not discriminate the two activation modalities because it has potent TCR-dependent as well as TCR-independent activating properties (29). To begin to understand the signaling pathways involved in regulating the CXCR3/1-storing compartment of CD4+ T cells we first performed classic TCR cross-linking experiments. As shown in Fig. 5A, activation of CD4+ T cells did not trigger up-regulation of the chemokine receptor storage compartment (left panel), whereas up-regulation of the early activation marker CD69 was readily induced (right panel). Lipid mediators, such as leukotrienes and PGs, are known to influence T cell function at various levels and have recently been identified to directly act as T cell chemoattractants. COX is expressed in most cells (including T cells), catalyzing the rate-limiting step in the biosynthesis of PGs from arachidonic acid (19, 21, 22, 30–32). Intriguingly, in CD4+ T cells pretreated with the selective and potent COX1 inhibitor FR122047, PHA-induced up-regulation was inhibited by a mean of 79% (range, 73–82%; n = 3). COX1-dependent regulation of CXCR3/1-storing granules was further confirmed using an additional COX1-selective as well as a COX2-selective inhibitor (SC560 (COX1 inhibitor); mean inhibition, 39%; range, 23–67%; n = 4, SC791 (COX2 inhibitor); mean inhibition, 2%; range, 0–12%; n = 6) (Fig. 5B, left panel). The 5-lipoxygenase-specific inhibitor nordihydroguaiaretic acid (60–120 ng/ml) left up-regulation of CXCR3/1-storing granules unaffected (data not shown).

FIGURE 4. Functional significance of CXCR3 and CXCR1 mobilization to the cell surface of CD4+ T cells. PHA-induced up-regulation of both CXCR1 and CXCR3 was linked to increased chemotaxis toward ligands of the respective receptors (left and middle panels, n = 3). The effect of PHA activation on chemotaxis was controlled for by comparing chemotactic activity of nonactivated vs PHA-activated CD4+ T cells in a gradient of CCL3 (right panel, n = 3). Data are presented as mean ± SD. ** Values of p < 0.005 comparing chemotaxis of activated vs nonactivated cells.

FIGURE 5. TCR-independent/COX-dependent up-regulation of CXCR3 and CXCR1. A, No impact on cell surface expression of CXCR3 and CXCR1 was observed in anti-CD3 and anti-CD28 Ab-treated CD4+ T cells, whereas CD69 expression was readily induced (n = 3). B, The COX1-selective inhibitors FR122047 and SC560 reduced PHA-induced up-regulation of both CXCR3 and CXCR1 by a mean of ~80 and ~40%, respectively (n = 3–4). By contrast, the COX2-selective inhibitor SC791 had no relevant effect (n = 6). At concentrations readily reached in vivo, the two widely used COX-inhibitors ibuprofen and indomethacin also inhibited up-regulation of CXCR3 and CXCR1 (n ≥ 3). This effect was found to be dose dependent and sustained (data not shown), whereas leaving up-regulation of the early activation marker CD69 unaffected (right panel, n = 4). C and D, Incubation of CD4+ T cells with arachidonic acid (AA) (i.e., the substrate of COX) induced rapid up-regulation of both CXCR3 and CXCR1 (C; representative of n = 9) in a time- and dose-dependent manner (D; n = 4–8). Data are presented as mean ± SD. *, Values of p < 0.05; **, a p value of <0.005 compared with baseline expression.
Importantly, also in CD4+ T cells pretreated with the widely used COX inhibitors ibuprofen and indomethacin at concentrations readily reached in vivo, cell surface mobilization of CXCR3/1-containing granules was consistently inhibited in a prolonged and dose-dependent manner (Fig. 5B, left panel; n = 7–8 and data not shown). By contrast, induction of the early activation marker CD69 was not affected by either indomethacin or ibuprofen (Fig. 5B, right panel; n = 4). Short-term exposure of CD4+ T cells to the COX substrate arachidonic acid also induced rapid up-regulation of CXCR3/1 storage granules (baseline, 1.0 ± 0.4%; arachidonic acid exposure for 2 min, 150 μM; 29.3 ± 12.5%) (Fig. 5C; n = 9). Arachidonic acid-mediated up-regulation of CXCR3 and CXCR1 was both time and dose dependent (Fig. 5D). Although not excluding additional, COX-independent effects mediated by arachidonic acid, these data are in line with a model of COX-regulated chemokine receptor expression.

Discussion

T cells are key to the resolution of a wide range of infections but also contribute to inflammatory diseases. The profile of chemokine receptors expressed on CD4+ T cells dictates to a large extent their homing properties and is intimately linked to their function. The capability to swiftly modify migrational behavior is therefore likely connected to the efficiency of CD4+ T cell-dependent immunity. In this study, we found that 1) circulating CD4+ T cells store the chemokine receptors CXCR3 and CXCR1 in a distinct intracellular compartment; 2) a mean of ∼30% of CD4+ T cells up-regulate functional CXCR3 and CXCR1 to the cell surface within minutes of activation; and 3) the COX pathway is important in this rapid regulation of chemokine receptor expression. In vivo, T cells are exposed to a network of chemotactic gradients, providing them with a plethora of rapidly changing migrational cues (33). To avoid conflicting signals and maintain appropriate homing, cellular responsiveness thus needs to be subjected to strict and rapid regulation. Protein expression in T cells is controlled each at the transcriptional level, via translational mRNA silencing, as well as by protein sorting into regulated secretory pathways (25, 34–39). Up-regulation of preformed protein, as shown in this study for CXCR3 and CXCR1, allows for the most efficient and rapid expression in T cells after acute infection or inflammation under inflammatory conditions.

Both CXCR3 and CXCR1 guide CD4+ T cells into sites of inflammation/infestation, defining them as inflammatory chemokine receptors (41–44). The ability of CD4+ T cells to promptly respond to microenvironmental changes by rapid, yet transient, up-regulation of preformed CXCR3 and CXCR1 (inducing a CXCR3/1 double-positive population) may be an important mechanistic feature to ensure adequate responsiveness of human CD4+ T cells under inflammatory conditions. Efficient homing in response to inflammatory stimuli might contribute to more efficient elimination of infectious agents. Excessive and/or undesired accumulation of inflammatory cells, in contrast, is a hallmark of clinical conditions such as chronic inflammatory diseases, allograft rejection, or ischemia/reperfusion injury (43, 45–49). In each of these pathologies, infiltrating CD4+ T cells may themselves release harmful proinflammatory mediators and/or recruit and activate other populations of effector leukocytes. Under these circumstances, blocking up-regulation of CXCR3/1 granules may prevent disproportionate recruitment of inflammatory homing CD4+ T cells while leaving default homing unaffected. It will now be interesting to assess the precise immunological/-inflammatory context in which CXCR3/1-storing granules are translocated to the cell surface in vivo and the mediators and receptors are involved.

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Disclosures

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