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Cutting Edge: T Helper 1 and T Helper 2 Cells Respond Differentially to Chemokines[1]

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T effector subsets, such as Th1 or Th2 cells, are key players in inflammatory reactions. It is not known whether chemokines are able to recruit these subsets differentially, as has been shown for memory vs naive T cells. Here we demonstrate that Th1 and Th2 cells differ in their intrinsic migratory properties and their chemotactic responsiveness toward distinct chemokines. While the CC-chemokines macrophage inflammatory protein (MIP)-1α, MIP-1β, and RANTES were efficient chemoattractants for Th1 cells, inducing a dose-dependent transmigration, Th2 cells were not attracted by these chemokines. Another CC-chemokine, JE/monocyte chemoattractant protein (MCP)-1, and a CXC-chemokine, stromal cell-derived factor (SDF)-1α, exerted chemotactic effects on both Th1 and Th2 cells, but differences in sensitivity and the percentage of responding cells were recorded between both subsets. These results indicate that chemokines play a distinct role in the regulation of local immune reactions by influencing the local balance between proinflammatory and antiinflammatory T cell subsets. ***The Journal of Immunology,*** **1998, 160: 550–554.**

Recirculation and homing are considered important components in the architecture of the immune system. More recently, our findings that Th1 effector cells are selectively recruited into inflamed tissues recognized a potential role of selective trafficking for the regulation of local immune responses (1).

Besides adhesion molecules such as selectins and integrins, chemokines are thought to be involved in the multi step process of extravasation by the triggering of integrins and chemotactic attraction of cell subsets (2, 3). The diversity of chemokines that potentially attract different cell populations makes them interesting candidates for the regulation of effector cell recruitment. Indeed, CC-chemokines especially are inducible and up-regulated in inflammatory lesions, suggesting a role for the recruitment of activated and proinflammatory T cell subsets into these sites.

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In vivo, a role for chemokines in B cell migration and germinal center formation (4), as well as for the colonization of lymphoid and nonlymphoid tissues of SCID mice by T cells, has been shown (5). Chemokines such as stromal cell-derived factor (SDF)[3]-1 (6), the recently described dendritic cell chemokine (DC-CK)-1 (7), and liver and activation-regulated chemokine (LARC) (8) have been shown to preferentially attract naive T lymphocytes, whereas others such as RANTES or monocyte chemoattractant protein-1 (MCP-1) were found to act predominantly on activated/memory stages of T cells (9, 10). In addition, differential effects on $CD4^+$ vs $CD8^+$ cells were reported for macrophage inflammatory protein (MIP)-1α and MIP-1β (11, 12).

Whether chemokines are involved in a differential recruitment of key effector subsets of T cells such as Th1 and Th2 is largely unknown. Recently, selective expression of the chemokine receptor CC-chemokine receptor (CCR)3 has been demonstrated in human Th2 cells (13). Here, we studied the ability of chemokines to differentially attract Th1 and Th2 cells in a chemotaxis assay. As the representativeness of long term lines or clones is questionable, we used primary cell populations generated by cytokine-induced differentiation in vitro (14, 15). Several members of the CC and CXC subgroups were analyzed. The data show a selective response of Th1 cells toward MIP-1α, MIP-1β, and RANTES, whereas other chemokines such as MCP-1 and SDF-1α were chemoattractant for both Th1 and Th2 cells, although with differing potency and efficacy.

Materials and Methods

Reagents

Purified recombinant chemokines were obtained from the following sources: murine MIP-1α, MIP-1β, JE/MCP-1, eotaxin, CRG-2/interferon-inducible protein-10 (IP-10), MIG, and KC from R&D Systems (Minneapolis, MN); human RANTES and SDF-1α from Peprotech (Rocky Hill, NJ); cytokines IL-2, IL-4, and IL-12 as well as unconjugated or phycoerythrin-/FITC-coupled anti-IFN-γ (clone XMG1.2) and anti-IL-4 (clone 11B11) from PharMingen (San Diego, CA). mAbs applied in the panning procedure were either used as supernatants or purified. Hybridomas were obtained from the American Type Culture Collection (Rockville, MD). Hamster anti-CD28 mAb (clone 37.51) was a gift of J. P. Allison through E. Schmitt (University of Mainz, Mainz, Germany). Murine fibronectin was obtained from Sigma (St. Louis, MO), BSA from Life Technologies (Paisley, U.K.), and rabbit anti-rat Ig from Dako (Glostrup, Denmark).

Cell preparation and culture

Th1 and Th2 cells were generated as described (1). In short, $CD4^+$ T cells were purified from peripheral and mesenteric lymph node cells of 8- to 15-wk-old specific-pathogen-free-reared female BALB/c mice by panning using anti-CD8 (53-6.72), anti-CD25 (PC 61 5.3), anti-FcR II/III (2.4G2),

[3] Abbreviations used in this paper: SDF, stromal cell-derived factor; MCP-1, monocyte chemoattractant protein 1; MIP, macrophage inflammatory protein; CCR, CC-chemokine receptor; IP-10, IFN-inducible protein-10.

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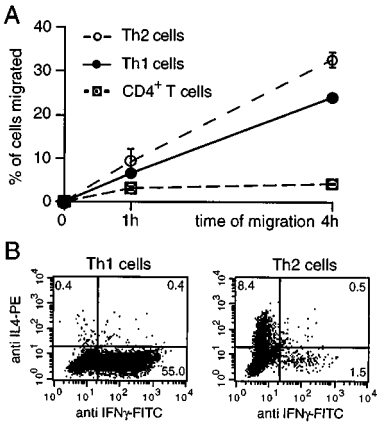

FIGURE 1. *A,* Spontaneous migration of Th1, Th2, and freshly prepared CD4$^+$ T cells. CD4$^+$ T cells (>90% of naive phenotype) and T effector cells (Th1/Th2) were generated as described in *Materials and Methods*. The percentage of total cells migrating into the lower chamber in the absence of chemokines within the indicated time is shown. The mean ± SD (triplicate determinations) for one representative experiment of several is given. *B,* Cytokine profile of generated Th1 and Th2 cell populations. Th1 and Th2 cells were double-stained for intracellular IFN-γ (FITC) and IL-4 (PE). Th1 cells show a high percentage of IFN-γ-producing cells and virtually no IL-4 production, whereas among the Th2 cell population, a significant fraction produces IL-4 and few cells produce IFN-γ.

and anti-MAC-1 (M1/70) mAbs and rabbit anti-rat Ig-coated plates (16). The purified cells were >96% CD4$^+$, >99% CD3$^+$, and largely (85–95%) of naive phenotype (L-selectin^{high}, CD45RB^{high}, IL-2R^{neg}). Cells were activated on six-well plates precoated with 3 μg/ml of anti-CD3 (145-2C11) in RPMI 1640 plus 10% FCS supplemented with either IL-12 (5 ng/ml), IFN-γ (20 ng/ml), and anti-IL-4 (1 μg/ml) for generation of Th1 cells, or IL-2 (5 ng/ml), IL-4 (10 ng/ml), and anti-IFN-γ (2 μg/ml) for Th2 cells, respectively. After 2 days, cells were transferred onto uncoated plates without a change of medium and cultured for an additional 4 days to allow the cells to return to a resting state.

Effector cells generated under these conditions express levels of L-selectin and CD45RB comparable with naive cells, increased levels of CD44 and LFA-1, and are predominantly in a resting state as judged by low IL-2R expression (specific fluorescence units 4–8, compared with 20–30 for fully activated CD4$^+$ T cells) and cell size. They actively home in vivo, in contrast to fully activated cells that circulate rather poorly (1, 16, 17).

Intracellular staining of Th1/Th2 subsets was performed after restimulation on plates precoated with 2 μg/ml of anti-CD3 and anti-CD28 overnight as previously described (1, 18). Stained cells were analyzed using a FACScan flow cytometer (Becton Dickinson, Heidelberg, Germany). In the generated Th1 cell population, 30 to 60% of the cells produced high amounts of IFN-γ and no IL-4. In the Th2 cell culture, a significant fraction of cells produced high levels of IL-4, whereas very few cells contained IFN-γ (Fig. 1*B*). In general, not all cells in such primary cultures are inducible; the number of cytokine-producing cells detected seems to be partially dependent on the conditions of restimulation, among other factors (18, 19).

Chemotaxis assay

For use in the transmigration assay, Th1 and Th2 cells were collected on day 6 and, after removal of dead cells by density centrifugation on 17% isotonic Nycodenz (Nycomed Pharma, Oslo, Norway), resuspended in assay medium (RPMI 1640 plus 0.5% BSA) at 5×10^6 cells/ml. Polyvinylpyrrolidone-free polycarbonate Transwell culture inserts of 6.5 mm diameter and 5 μm pore size (Costar, Cambridge, MA) were coated with 50 μl of distilled water containing 10 μg/ml fibronectin for 1 h in 5% CO_2 at 37°C. After removing the liquid, filters were dried for 2 h at 37°C. Either chemokines diluted in assay medium or assay medium alone was added to the lower chamber in a final volume of 600 μl. The filter inserts were placed in the wells, 5×10^5 of either Th1 or Th2 cells were added to the top chamber in a volume of 100 μl, and the chambers were incubated for 1 h in 5% CO_2 at 37°C. Cells that transmigrated into the lower chamber were suspended and counted using a Casy TT cell counter (Schärfe System, Reutlingen, Germany). At a minimum, all determinations were performed in triplicate. Statistical analysis was performed using Student's *t* test.

For an analysis restricted to the very producers of cytokines, Th1 and Th2 cells were mixed in a ratio of 1:2 and subjected to a chemotaxis assay toward either medium or 10 ng/ml MIP-1α. The cells from upper and lower compartments (30 chambers per sample were pooled) were washed, restimulated with PMA/ionomycin for 5 h, and then the absolute numbers of IFNγ or IL-4-producing cells were determined by intracellular staining.

Results and Discussion

Th1 and Th2 effector cells display a high but differential spontaneous migration

A surprisingly high level of intrinsic migratory activity was found for resting T effector cells compared with naive CD4$^+$ T cells. In the absence of chemokines, 5.3 ± 2.3% of Th1 cells and 7.9 ± 2.6% of Th2 cells (mean of 12 experiments, range 3–11%) had migrated after 1 h through fibronectin-coated filters, while freshly isolated CD4$^+$ T cells showed transmigration below 3% (Fig. 1*A*). Extension of the assay for 4 h resulted in continuous migration of the effector cell populations with up to 35% of the cells found in the lower well. In contrast, no additional significant increase was observed with fresh CD4$^+$ T cells, suggesting that it is predominantly the small fraction of effector/memory cells present among lymph node CD4$^+$ cells that migrates spontaneously. This observation is consistent with the correlation between migratory capacity and the T cell differentiation stage demonstrated previously (20, 21).

The differences between fresh CD4$^+$ cells and the effector cell populations were not simply due to different activation states; the used protocol results in largely resting Th1 or Th2 cells, as judged by low IL-2R and high L-selectin expression (16). Moreover, fully activated effector cells displayed a significantly lower migratory capacity than the resting population used here (not shown). This observation indicates that effector cells in this stage are destined to migrate through the tissue much more actively than naive cells.

When the spontaneous migration of Th1 and Th2 cells through fibronectin-coated filters was compared, clear differences could be observed (Fig. 1*A*). In 10 of 12 1-h experiments and all 4-h experiments, Th2 cells migrated to a higher degree than Th1 cells. After 1 h, from 1.2- to over 2-fold more Th2 than Th1 cells were found in the lower compartment, the difference being significant with $p < 0.001$ (12 experiments). Again, the effect could not be explained by differences in the activity state, because IL-2R and L-selectin expression of Th1 and Th2 cells were similar. Preliminary data point to a supportive role of fibronectin on spontaneous Th2, but not on Th1 cell transmigration. Studies on migration through endothelial monolayers revealed similar differences in the transmigratory activity of Th1 vs Th2 cells (J. T. Siveke and B. Engelhardt, unpublished data). Thus, differences in the intrinsic migratory activity between the Th1 and Th2 effector cell populations might contribute to their differential trafficking in vivo as reported previously (1).

Selective chemoattraction of Th1 cells by MIP-1α, MIP-1β, and RANTES

Chemotactic signals are assumed to be of critical importance for the recruitment of leukocyte subsets. We therefore investigated possible differences in the response of Th cell subsets toward different chemokines. Th1 cells were found to display a clear response to MIP-1α in the transmigration assay. As shown in Figure 2, the directed migration was dose dependent, reaching a maximum between 1 and 10 ng/ml. Optimal concentrations of MIP-1α induced more than a threefold increase in migration of Th1 cells (14–18% cells migrated) compared with spontaneous migration without MIP-1α of 4 to 7% in three experiments.

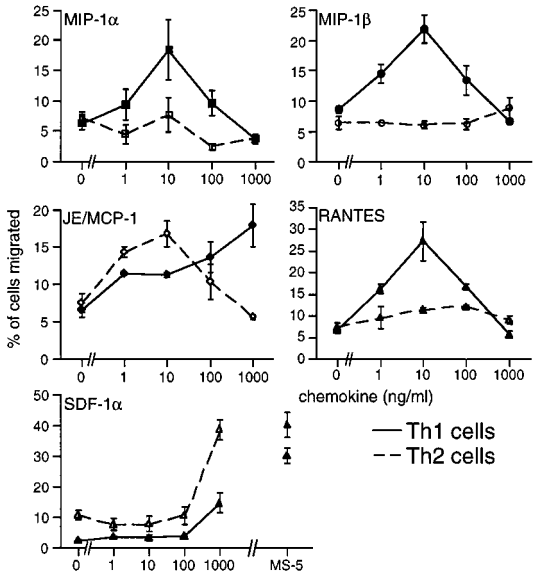

FIGURE 2. Transmigration of Th1 and Th2 cells. Th1 (solid line) and Th2 (dotted line) migrated in the chemotaxis assay toward the indicated chemokines or MS-5 (supernatant of a mouse stromal cell line containing SDF-1) over a time period of 1 h. The percentage of total cells that migrated to the lower chamber is given. The mean ± SD (triplicate determinations) for one representative of at least three independent experiments is given.

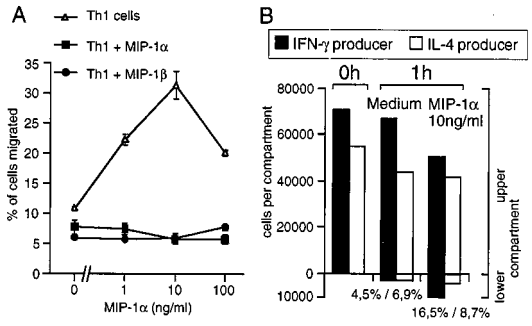

FIGURE 3. *A,* Checkerboard chemotaxis assay of Th1 cells toward MIP-1α and MIP-1β. 1-h transmigration of Th1 cells to MIP-1α in the presence of chemokines in the upper chamber. Cells were either untreated or 100 ng/ml of MIP-1α or MIP-1β were added to the cells 3 to 5 min before transfer into the chambers. The percentage of total cells that migrated to the lower chamber was measured. The mean ± SD (triplicate determinations) for a representative experiment is given. *B,* Migration of cytokine producers toward MIP-1α. Th1 and Th2 cell cultures were mixed and subjected to a chemotaxis assay toward either medium or 10 ng/ml MIP-1α. Cells from the upper and lower compartment were restimulated and stained for intracellular IFN-γ and IL-4 before and after a 1-h migration. The percentage of migrating cells among the respective subsets is given.

In contrast, Th2 cells were not significantly attracted toward MIP-1α. Interestingly, at the highest concentrations tested, the number of transmigrating Th2 cells was consistently diminished below background levels (Fig. 2). The reduction was significant in all three experiments (with $p < 0.002$, $p < 0.002$, and $p < 0.02$, respectively).

The differential response of Th1 vs Th2 cells toward MIP-1α was not dependent on the activation stage; similar responses (albeit at a lower level) were observed with fully activated Th1/Th2 cell populations (data not shown).

MIP-1β had largely similar effects to MIP-1α on Th1 cells. Migration was highest at 10 ng/ml with detectable chemoattractant activity at 1 and 100 ng/ml. As for MIP-1α, approximately threefold more cells migrated compared with background.

Previous reports have shown that MIP-1α, MIP-1β, and RANTES are ligands for murine CCR5 (22). Similar to MIP-1α and MIP-1β, human RANTES was found to induce a dose-dependent response in Th1 cells with an optimum response at 10 ng/ml.

In primary effector cell cultures, only a fraction of cells can be induced to produce cytokine. To confirm that the observed specific migration indeed applies to cells with a proven cytokine phenotype, we enumerated cells producing either IFN-γ (Th1) or IL-4 (Th2) after migration of a mixed population toward either medium or 10 ng/ml MIP-1α in the respective compartments. As shown in Figure 3*B*, the chemotactic response toward MIP-1α was almost completely restricted to IFN-γ producers.

To prove that migration was due to chemotaxis and not to chemokinetic effects, a checkerboard analysis was performed. As shown in Figure 3*A*, migration of Th1 cells toward MIP-1α could be completely blocked when 100 ng/ml MIP-1α or MIP-1β was added to the upper well. In contrast, migration toward MIP-1β could be blocked completely by MIP-1α (not shown). Thus, induced migration toward MIP-1α and MIP-1β is due to directed migration rather than to an increase in random motility of the cells. This directed migration, as well as the short duration of the assay used in this study, excluded indirect effects of the chemokines, e.g., by inducing the synthesis of other soluble factors.

A slight increase in the migration of Th2 cells at concentrations exceeding 100 ng/ml could be observed for MIP-1β, in contrast to the effects of MIP-1α (difference between 1000 ng and control values was significant with $p < 0.001$ in three experiments). At most, this increase was twofold above background but never did reach levels observed with Th1 cells (Fig. 2). Th2 cells also showed a slightly increased migration in the intermediate concentration range (1–100 ng/ml) toward RANTES.

The chemotactic response of Th2 cells to high concentrations of MIP-1β, but not to MIP-1α, points to differential biologic effects of these chemokines, although the checkerboard analysis suggested that both chemokines were competing for the same receptors under these conditions. In other studies, the two chemokines were found to have both partly overlapping and partly differing biologic activities in chemotaxis, activation, or hemopoiesis. Studies of T cells revealed preferential chemotactic migration of $CD8^+$ T cells toward MIP-1α, and of $CD4^+$ T cells toward MIP-1β (11, 12). A unique function of MIP-1α has been demonstrated in vivo (23). These data point to CCR5 as a good candidate for the selective chemoattraction of the Th1 subset by MIP-1α, MIP-1β, and RANTES. The small effects of MIP-1β (at high concentrations) and of RANTES (at intermediate concentrations) on Th2 cells both suggest the presence of other receptors such as CCR1 or CCR3 on Th2 cells. Low receptor affinity, density, or signaling of the receptors might restrict their biologic responsiveness in this subset.

The selective expression of CCR3 by human Th2 cells has recently been shown (13). This receptor binds eotaxin and RANTES. The small effects of both RANTES and eotaxin (not shown) on the chemotaxis of Th2 cells support this finding, but predict a rather low expression or function of this receptor among murine Th2 cells.

Both Th1 and Th2 cells are attracted by JE/MCP-1 and SDF-1α but show qualitative and quantitative differences in responsiveness.

To test whether other CC-chemokines are involved in a selective recruitment of Th1 or Th2 cells, JE, the murine homologue of human MCP-1, was tested in the chemotaxis assay. MCP-1 has been reported to be a major attractant for $CD4^+$ T cells of activated/memory phenotype (10, 24), and JE/MCP-1 has also been shown to bind to a receptor different from that of MIP-1α (25). As shown in Figure 2, both Th1 and Th2 cells were attracted by JE, yet the dose-response curve differed between the two populations. Th1 cells were effectively attracted (threefold higher) only at high concentrations. Thus, JE is as effective as MIP-1α and MIP-1β for Th1 cells but not as potent, because higher levels of chemokines were required for an optimal response. Th2 cells, on the other hand, were slightly less efficiently attracted and had a smaller response window with highest migration between 1 and 10 ng/ml, dropping to baseline levels at higher concentrations (Fig. 2). In three experiments performed, the migration of Th2 cells toward JE/MCP-1 at optimal conditions did not reach the maximal Th1 levels (maximal responses differed with $p < 0.05$). Thus, JE/MCP-1 is a chemoattractant for both populations with a preference for Th1 cells at high concentrations only.

As mentioned above, CCR3 has been found on human Th2, but not on Th1 cells (13). Eotaxin is a CC-chemokine binding selectively to CCR3. Indeed, we found no effects of eotaxin on the migration of Th1 cells, and its effects on Th2 cells were only very modest (data not shown).

As a classical CXC-chemokine, we tested KC, the suggested murine homologue of human GRO that binds to the mouse IL-8R type B (26). KC was not able to induce a significant increase in the migration of both Th1 and Th2 cells (data not shown), supporting previous reports showing that memory T cells respond to CC-chemokines, rather than to CXC-chemokines such as IL-8 and IP-10 (27). Other CXC-chemokines such as CRG-2 (IP-10) or MIG revealed a weak but significant chemotactic activity for Th1 cells at high concentrations (1000 ng/ml), whereas Th2 cells showed no significant increase in migration (data not shown).

SDF-1, recently described as being a highly efficacious chemokine for lymphocytes (6), induced the highest migration of all chemokines tested. At 1000 ng/ml, 15% of Th1 cells and 39% of Th2 cells had migrated (Fig. 2), while no effects were seen at low concentrations. Consistently, the response of Th2 cells to SDF-1α at 1000 ng/ml was higher than that of Th1 cells. Supernatant of the murine bone marrow stromal cell line MS-5, which contains high amounts of SDF-1 (6), induced the largest increase in Th1 migration, up to 35% (Fig. 2), whereas the response of Th2 cells toward MS-5 was similar to their response toward 1000 ng SDF-1α. Whether this difference between Th1 and Th2 cells is indeed due to a differential responsiveness toward SDF-1, whether additional factors secreted by the stromal cell line are the cause, or whether the very high migration of Th2 cells had already reached a maximal value limited by the type of assay must still be determined.

In our study, SDF-1α had the strongest effect of all chemokines tested and attracted Th1 as well as Th2 cells. In contrast to the inflammation-related CC-chemokines, it has been suggested that SDF-1 plays a role in recirculation and immune surveillance, rather than in inflammation, because of its wide expression in many tissues independent of inflammatory stimuli (28, 29) and because of the preferential expression of its receptor CXCR4 on naive T cells (30). SDF-1α was the only tested chemokine that exerted a stronger effect on Th2 than on Th1 cells. Previously, we found that Th2 cells had a greater preference for trafficking into the spleen (1). Preliminary observations also indicate that the migration within this tissue differs between Th1 and Th2 cells (our unpublished observations). It is tempting to suggest that SDF-1 might play a role in these migratory properties of Th2 cells.

The results of this work show a preferential responsiveness of the proinflammatory Th1 subset toward CC-chemokines. Many studies have demonstrated the eminent importance of the Th1 and Th2 subsets on the outcome of immune responses. The selective attraction of Th1 cells by CC-chemokines that are produced in sites of inflammation can be considered as a new mechanism that contributes to the local balance between these subsets and shapes the immune reaction within inflamed tissue. Our data suggest selective differences in the expression or function of chemokine receptors among Th subsets.

Interestingly, recent reports show a secretion of CC-chemokines in Th1 cells, but not in Th2 cells in humans (31, 32). Together, these findings could indicate the existence of a positive feedback loop that links the production of MIP-1α with a chemotactic response toward MIP-1α in Th1 cells.

In conclusion, this study provides evidence for a selective control of T effector cell recruitment mediated by chemokines. It confirms conclusions drawn from previous studies on in vivo migration and the selective use of adhesion molecules among Th1/Th2 cells by establishing an additional molecular level on which specific trafficking of lymphocytes can be regulated.

Acknowledgments

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