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Homeostatic Lymphoid Chemokines Synergize with Adhesion Ligands to Trigger T and B Lymphocyte Chemokinesis

Agnieszka N. Stachowiak, Yana Wang, Yen-Chen Huang, and Darrell J. Irvine

Homeostatic chemokines such as CCL19, CCL21, and CXCL13 are known to elicit chemotaxis from naïve T and B cells and play a critical role in lymphocyte homing to secondary lymphoid organs (SLO). Here we tested whether CCL21 and CXCL13 modulate murine lymphocyte motility in the absence of concentration gradients, using videomicroscopy to directly observe the migration of single cells. CCL21 treatment of T cells induced rapid polarization and sustained random migration with average speeds of $5.16 \pm 2.08 \, \mu \text{m/ sec}$; B cell migration (average velocity $4.10 \pm 1.58 \, \mu \text{m/sec}$) was similarly induced by CXCL13. Migration required the presence of both chemokine and adhesion ligands and was sustained for $>24 \, \text{h}$. Furthermore, in vitro assays modeling the relative infrequency of Ag-specific T cell-dendritic cell (DC) encounters during primary immune responses, we found that CCL21 addition to T-DC cocultures accelerated the kinetics of CD69 up-regulation and enhanced by 2-fold the proliferation of Ag-specific T cells in a manner dependent on G-protein-coupled receptor signaling in T cells. These results suggest that homeostatic chemokines could substantially impact the dynamics and priming of lymphocytes within SLO even in the absence of significant concentration gradients. The Journal of Immunology, 2006, 177: 2340–2348.

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2 Address correspondence and reprint requests to Dr. Darrell J. Irvine, Massachusetts Institute of Technology, Room 8-425, 77 Massachusetts Avenue, Cambridge, MA 02139. E-mail address: djirvine@mit.edu
3 Abbreviations used in this paper: SLO, secondary lymphoid organs; DC, dendritic cell; FN, fibronectin; iDC, immature DC; OVA-mDC peptide-bearing mature DC; PTX, pertussis toxin.

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Materials and Methods

Cell isolation

All animal work was approved by the MIT Committee on Animal Care, in accordance with federal, state, and local regulations. C57BL/6, OT-II, and GFP BL/6 transgenic mice were obtained from The Jackson Laboratory. DC were derived from the bone marrow of C57BL/6 mice after the procedure of Inaba et al. (15), cultured with 5 ng/ml GM-CSF (R&D Systems), and used on day 7. CD4+ T cells were isolated from spleens or lymph nodes of 6- to 12-wk-old mice by MACS negative selection (CD4+ T cell isolation kit; Miltenyi Biotec), yielding >90% CD4+ cells, >60% CD62LhighCD44low naive T cells. For some experiments, cells were further purified: CD4+ T cells were separated into CD62L+ and CD62L- fractions by MACS positive selection (CD4+CD62L+ T cell isolation kit; Miltenyi Biotec) or were sorted into CD62L+CD44low and CD62L+CD44high populations on a FACSAria (BD Biosciences). B cells isolated by MACS negative selection (B cell isolation kit; Miltenyi Biotec) were typically >85% B220+MHCII+. Cells were cultured in RPMI 1640 medium supplemented with 10% FCS, 10 mM HEPES, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM l-glutamine, and 50 μM 2-ME.

Cell treatments

Cells were incubated with 1–10,000 ng/ml recombinant mouse CCL19 or CCL21 (R&D Systems) for 20 min before initiation of imaging to allow the chemokine to take full effect. PMA treatment (Sigma-Aldrich) was performed at 50 ng/ml, with imaging likewise initiated at 20 min. For LFA-1 blocking studies, anti-LFA-1 clone M17/4 or isotype control Ab was used; anti-I-Ab clone AF6–155 was initiated at 20 min. For LFA-1 blocking studies, anti-LFA-1 imaging to allow the chemokine to take full effect. PMA treatment was also used for B cells. For Fc block treatment, B cells were depleted with 2.5 μg/ml recombinant ICAM-1/Fc or VCAM-1/Fc fusion proteins (R&D Systems) overnight at 4°C, before addition of other reagents. All Abs were obtained from BD Pharmingen and used at 10 μg/ml. In videomicroscopy studies utilizing pertussis toxin (PTX), cells were treated with 100 ng/ml PTX or PTX B oligomer (Sigma-Aldrich) for 10 min at 37°C, washed twice with warm medium, and rested for 90–120 min before use. In coculture studies, both a high dose (100 ng/ml) and a low dose (2 ng/ml) of PTX were tested; results followed the same trends for both doses. For chemokine removal experiments, cells were washed in 10 ml warm medium, and rested for 90–120 min before use. In coculture studies, both a high dose (100 ng/ml) and a low dose (2 ng/ml) of PTX were tested; results followed the same trends for both doses. For chemokine removal experiments, cells were washed in 10 ml warm medium, and rested for 90–120 min before use. In coculture studies, both a high dose (100 ng/ml) and a low dose (2 ng/ml) of PTX were tested; results followed the same trends for both doses. For chemokine removal experiments, cells were washed in 10 ml warm medium, and rested for 90–120 min before use. In coculture studies, both a high dose (100 ng/ml) and a low dose (2 ng/ml) of PTX were tested; results followed the same trends for both doses.

Videomicroscopy tracking of T cell polarization and chemokinesis

Eight-well chambered coverslips (Lab-Tek; Nalge Nunc) were incubated with 5 μg/cm² fibronectin (FN) for 2 h at 21°C, or 10 μg/ml recombinant ICAM-1/Fc or VCAM-1/Fc fusion proteins (R&D Systems) overnight at 4°C, before addition of cells (2–3 × 10⁸) and chemokine. A fraction of CD4+ T cells from C57BL/6 mouse spleens (−20%, for ease of automated tracking) were labeled with 2.5 μM fura 2-AM (Molecular Probes) for 25 min at 37°C. Similarly, splenic CD4+ T cells from GFP mice used for chemokine response longevity studies were diluted to 20% with C57BL/6 CD4+ T cells. Fura (for C57BL/6 mice, exc 380 nm, em 510 nm) or green (for GFP mice, excitation 488 nm, emission 510 nm) fluorescence and bright-field images were acquired at 30 s intervals for 40 min on a Zeiss Axiovert 200 epifluorescence microscope equipped with environmental stage (37°C, 5% CO2) with the aid of Metamorph software (Universal Imaging).

Image analysis

Cell polarization and migration were analyzed with the aid of Metamorph and Volocity (Improvision) software packages. Data were sharpened with the no-neighbors two-dimensional deconvolution algorithm in Metamorph to facilitate cell tracking using Volocity. For each field, 26–44 cells were analyzed (depending on fluorescent cell density) based on intensity thresholding and shape exclusion (to rid a small fraction of large cell contaminants); the dead cell population that developed during longevity experiments was excluded from analysis. A cell was scored as polarized at a single time point if its shape factor (4π area/perimeter²) did not exceed 0.85. To score average population behaviors, polarized cells were defined as those with a shape factor ≤0.85 in ≥15% of observed time points. Migrating cells were defined as polarizing cells with time-averaged velocities ≥4 (T cell) or 3.5 (B cell) μm/min.

T-DC cocultures

All experiments were performed in 96-well round-bottom plates (BD Falcon). To assay CD69 up-regulation, duplicate samples of 2 × 10⁵ total cells were prepared at an overall T/DC ratio of 1:1. A portion of day 6 DC were matured with 1 μg/ml LPS (Sigma-Aldrich) and pulsed with OVA peptide (SQAVHAA-HAINEAGR, 500 nM; AnaSpec Corporate) for 16 h, whereas others were left untouched; mixtures of peptide-loaded (and washed) mature DC (OVA-mDC) and immature DC (iDC) were then prepared at a 1:20 ratio. OT-II CD4+ T cells were also diluted 1:9 with C57BL/6 CD4+ T cells. T cells (with or without PTX pretreatment) and DC mixtures were aliquoted in FN-treated wells, briefly centrifuged (1200 rpm, 1–2 min), then placed in culture in the presence or absence of 1 μg/ml CCL21, which was replenished at 48 h. CD69 up-regulation was analyzed at times ranging from 24 to 96 h; duplicate samples were pooled, cells were stained with anti-CD69-FTTC, anti-Vα2-PE, and anti-CD4-biotin followed by streptavidin-allophycocyanin, and finally propidium iodide.

The effect of CCL21 on T cell proliferation was assessed by a CFSE dilution assay, using a similar coculture system: OT-II T cells were labeled with 10 μM CFSE for 10 min and washed 3× before mixing with C57BL/6 CD4+ T cells at a 1:9 ratio. Triplicate cocultures of 5 × 10⁵ total cells were prepared at an overall T/DC ratio of 9:1 and an iDC/OVA-mDC ratio of 1:1; 1 μg/ml CCL21 was added at time zero to CCL21-treated cultures. Proliferation in each well was assayed on day 4 by flow cytometry analysis of cultures stained with anti-Vα2-PE, anti-Vβ5-biotin (then streptavidin-allophycocyanin), and propidium iodide. All data was collected on a FACSCalibur (BD Biosciences) using CellQuest software and analyzed with FlowJo software, using Abs obtained from BD Pharmingen.

Results

CCL21 and CCL19 induce chemokinesis in resting CD4+ T cells exposed to an adhesive substrate

Many chemokines, including CCL19 and CCL21, are known to trigger polarization in resting T cells at uniform concentrations (16, 17). We confirmed that both CCL21 (Fig. 1, A and B) and CCL19 (data not shown) elicited pronounced polarization of ~75% of freshly isolated murine (C57BL/6 strain) CD4+ T cells within 5 min, whereas the majority of untreated resting cells were round. In experiments where CD62L+CD44low, CD62L+CD44high, and CD62L−CD4+ T cells were separated, all populations responded nearly equally to CCL21 (data not shown). When T cells were seeded on bare glass with soluble CCL21 and

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tracked by videomicroscopy, we observed not only rapid cell polarization, but also that a small fraction of cells (∼3%) appeared to actively migrate (Fig. 1B). Because polarization and adhesion are two prerequisites for T cell motility (6, 18), and CCL21 is known to induce adhesion to integrin ligands (19, 20), we tested whether motility would follow polarization if the cells were exposed to a suitable adhesive substrate.

CD4⁺ T cells were seeded at relatively high densities (crudely mimicking the dense cellularity of SLO) on glass substrates coated with recombinant ICAM-1, with or without chemokine. T cells had
low basal adhesion and motility when seeded on ICAM-1-coated substrates in the absence of chemokine; most cells moved only by convective drift and collisions with neighboring cells (Fig. 1B, data not shown). In contrast, addition of CCL21 stimulated migration in 60 ± 5% of T cells (Fig. 1B). As illustrated by single-cell migration paths (Fig. 1C), CCL21-treated cells migrated substantial distances on ICAM-1 with no preferred direction, whereas the majority of cells remained relatively stationary in the absence of chemokine or slowly convected. Convecting vs migrating cells were readily distinguished via single-cell velocity time courses (Fig. 1D) and histograms of instantaneous velocities (Fig. 1E); migrating T cells had peak speeds of up to 20 μm/min, whereas control cells had smoother velocity profiles with speeds rarely exceeding 10 μm/min. The time-averaged velocity of single cells exposed to CCL21 on ICAM-1-coated surfaces ($v_{av} = 5.16 ± 2.08 \mu m/min$) was likewise significantly higher ($p \leq 0.0001$) than that observed for cells on ICAM-1 with no chemokine (Fig. 1F).

Importantly, chemokinetic migration was dependent on the presence of both chemokine and adhesive ligand: when the T cell integrin LFA-1 was blocked with an Ab, T cells on ICAM-1 substrates polarized, but failed to migrate (Fig. 1G); isotype control Ab had no effect (data not shown). Treatment of T cells with PTX, which inhibits G-protein-coupled receptor signaling, reduced both polarization and migration ($p \leq 0.05$), whereas the PTX B oligomer (control) had no effect (Fig. 1G). Blocking LFA-1-ICAM-1 interactions with an anti-LFA-1 Ab or treating the cells with PTX significantly reduced average cell velocities as well (Fig. 1F). We tested whether the observed migration was a result primarily of increased adhesion to ICAM-1 rather than chemokinesis per se, by treating cells with the phorbol ester PMA, which increases T cell adhesion to ICAM-1 (21). PMA triggered polarization in a majority of cells, but did not induce migration on ICAM-1 (Fig. 1G). Thus, CCL21 elicits chemokinesis from CD4+ T cells, with migration dependent upon G-protein-coupled receptor signaling and exposure to an adhesive substrate such as ICAM-1.

Chemokinesis occurs at physiological CCL21 doses and supports long-lived cell migration

Naive T cells are estimated to spend 12–18 h in a single lymph node during their homeostatic recirculation between blood and secondary lymphoid tissues (9). For chemokinesis triggered by CCL21 to be relevant to T cells’ search for Ag in lymph nodes, the migratory response would need to be sustained throughout a similar timespan in response to chemokine doses present in the intact tissues. The concentration of CCL21 in SLO has been estimated at ~2 and ~10 μg/ml by ELISA and Western blotting of tissue supernatants, respectively (22–24). Using videomicroscopy, we found an onset of T cell motility induced by 10–100 ng/ml CCL21, whereas the percentage of cells polarizing and migrating began to plateau between 100 and 1000 ng/ml (Fig. 2A). However, the average time that any particular cell spent polarized and the average single-cell velocity continued to increase up to at least 10 μg/ml (data not shown). Notably, the onset and plateau response in chemokinetic migration measured here parallels the dose response reported for chemotactic migration triggered by murine CCL21 (25).

To assess the longevity of the observed chemokinetic response, T cells seeded on ICAM-1 surfaces were tracked by videomicroscopy 20 min after addition of CCL21, and again 24 h later. Live T cell migration in response to CCL21 did not appear to weaken at all over 1 day (Fig. 2, B–D), whereas only minor basal motility (<20%) developed in samples not treated with CCL21 (Fig. 2B). When we attempted to wash out the chemokine 24 h after addition (or 1 h; not shown), cell polarization was significantly reduced ($p \leq 0.0001$; Fig. 2C). Thus, CCL21-driven responses are sustained at physiological concentrations over time periods consistent with lymphocyte residence in a given lymph node and appear to be dependent on the persistent presence of chemokine.

![Figure 2](http://www.jimmunol.org/)
CCL21 triggers chemokinesis on several adhesion ligands present in secondary lymphoid tissues

To determine whether CCL21 could synergize with other adhesion ligands present within T cell areas of SLO to promote T cell migration, we observed CD4^+ T cell motility on VCAM-1 and FN-coated substrates for comparison to the response observed on ICAM-1 surfaces. T cell polarization induced by CCL21 was statistically identical on bare glass or in the presence of each adhesion ligand (Fig. 3A). For all four surfaces, the population time-averaged velocity was increased in the presence of CCL21 (Fig. 3B). (This was true even on bare glass where cells were non-adherent, due to increased convection caused by CCL21-induced polarization.) However, the CCL21-induced increase in mean velocity of T cells was significantly greater for cells cultured on ICAM-1, VCAM-1, and FN compared with bare glass, suggesting that CCL21 can synergize with multiple adhesion ligands to promote T cell migration (Fig. 3B; p ≤ 0.05). The exact velocity profiles of both chemokine-treated and untreated cells depended on the surface (Fig. 3C); this is to be expected, because cell migration rates are controlled by adhesion strength—which likely differs for each ligand at the single surface-coating densities tested here—as well as potential qualitative differences in the adhesion receptors involved (26). However, the responses measured here clearly indicate that chemokinesis induced by CCL21 in concert with an adhesive substrate is not limited to synergy with ICAM-1 alone.

CCL21 impacts CD4^+ T cell priming under conditions of rare Ag-specific T cell-DC encounters

To determine whether CCL21 could impact naive T cell priming under conditions modeling the rarity of Ag-specific encounters in vivo, we prepared T cell-DC cocultures where both Ag-specific T cells and peptide-bearing DCs were present at high dilution. We first assayed T cell activation kinetics via CD69 up-regulation, reasoning that chemokinesis triggered by CCL21 might alter the rate of early priming events. Peptide-loaded mature bone marrow-derived DCs (OVA-mDC) were mixed with peptide-free iDC at a ratio of 1:20 and plated in FN-coated round-bottom culture wells. A mixture of OVA peptide-specific transgenic CD4^+ T cells (OT-II) (27) and CD4^+ T cells from wild-type (C57BL/6) mice (at a 1:9 ratio) was then added for an overall 1:1 T/DC ratio. Wells received CCL21 (1 μg/ml) at both 0 and 48 h or were left untreated, and CD69 up-regulation was assessed as a function of time on equal numbers of live Ag-specific cells (identified as CD4^+ Vα2^+P^low cells, a gating that included some wild-type cells) (Fig. 4A). As shown in Fig. 4B for a representative experiment, the fraction of CD69^high Vα2^+ cells was higher for CCL21-treated samples than for untreated controls at all time points, by up to 1.5-fold at 48 h.

To distinguish between effects of CCL21 on T cells and DCs in the coculture, we also applied a PTX treatment strategy reported by Lo et al. (28) to block G-protein-coupled receptor signaling in a selected cell population for at least 26 h. PTX-treated T cells showed reduced CD69 up-regulation compared with untreated T cells in the presence of CCL21, indicating that the primary effect of CCL21 in these cocultures was mediated by T cells (Fig. 4B). The increase in the CD69^high population elicited by CCL21 was significantly greater for untreated vs PTX-treated cocultures across multiple experiments (p ≤ 0.05; Fig. 4C) and was most prominent at 48 h.

To examine the end-point effects of CCL21 on T cell priming, we performed a CFSE dilution assay to directly observe Ag-specific cell division. For this assay, OT-II T cells were again diluted at a ratio of 1:20 and plated in FN-coated round-bottom culture wells. A mixture of OVA peptide-specific transgenic CD4^+ T cells (OT-II) (27) and CD4^+ Vα2^+ cells, a gating that included some wild-type cells) (Fig. 4A). As shown in Fig. 4B for a representative experiment, the fraction of CD69^high Vα2^+ cells was higher for CCL21-treated samples than for untreated controls at all time points, by up to 1.5-fold at 48 h.
T/DC ratio of 9:1 was used, and the DC population was equal parts iDC and OVA-mDC. OT-II T cells in the culture were labeled with CFSE to track cell division by flow cytometry. At 85 h, equal volumes of total cells were analyzed, and the number of live OT-II T cells over time for control (CCL21), CCL21-treated (+CCL21), PTX-treated cells and without CCL21, and a control containing 50% T cells and 50% iDC are shown. A and B are data from one representative of three independent experiments. C. Percentage of increase in CD69high population for CCL21-treated samples (vs relevant control), comparing multiple similar experiments, we found that the fold increase in T cell recovery was consistently greater in the presence of Ag-bearing DCs than in their absence (2.1 ± 0.4-fold in the presence of Ag, 1.4 ± 0.1-fold in the absence of Ag, p < 0.05), thus showing an Ag-specific effect. The enhanced cell recovery in CCL21-treated cocultures was not due to a direct survival signal delivered to resting T cells by chemokine, as purified T cells cultured alone for 24 h with or without CCL21 were recovered in equal numbers (data not shown). Altogether, these results suggest that CCL21 signaling can impact the kinetics of early activation events and the ultimate number of expanded T cells while having a minor impact on the mean number of cell divisions, under conditions where Ag-specific T cells and peptide-bearing DCs are present at low frequency.

B cells exhibit chemokinesis in response to CXCL13

The data presented above show strong chemokinesis induced in CD4+ T cells by CCL21, a chemokine produced constitutively at high levels in the T cell areas of SLO (9). To determine whether chemokinesis is also a potential regulatory mechanism in B cell areas of SLO, we tested whether CXCL13, produced constitutively in follicles, would elicit an analogous response in B lymphocytes. B cells isolated from C57BL/6 mice were seeded on ICAM-1-coated substrates with or without CXCL13 and tracked by video-microscopy as above. Like CD4+ T cells, B cells displayed low motility on ICAM-1 surfaces, but cell polarization and migration increased dramatically on exposure to CXCL13 (v = 4.10 ± 1.58 µm/min) (Fig. 6A). PTX treatment blocked this response to CXCL13 completely, whereas treatment of cells with PTX B oligomer had almost no effect. Analogous to T cells, treatment with the nonspecific adhesion stimulus PMA caused polarization but did not induce migration. However, in contrast to T cell responses to CCL21, anti-LFA-1 treatment suppressed both CXCL13-induced migration and polarization. To rule out potential nonspecific effects of coating the cells with the blocking Ab or FcR signaling in the cells, we also compared the response of cells treated with an
isotype-matched anti-I-A\(^\beta\) (Fig. 6A); in this case B cell polarization and migration was not statistically different from untreated cells. (A non-binding isotype control Ab also failed to block CXCL13 responses; data not shown). These results suggest that in B cells, both polarization and migration on ICAM-1 are governed by a synergy between chemokine and LFA-1 signaling. Little movement of B cells from their point of origin was observed on ICAM-1 in the absence of chemokine, but single cell tracks revealed significant random motility upon addition of CXCL13, with peak instantaneous velocities \(>15\) \(\mu\)m/min and average velocities of \(4.10 \pm 1.58\) \(\mu\)m/min (Fig. 6, B and C). Thus, B cells respond to synergistic signaling from CXCL13 and ICAM-1 and exhibit chemokinetic migration in a manner largely analogous to the response of T cells to CCL21 and ICAM-1.

**Discussion**

Fewer than 1 in 1000 naive T cells express receptors specific for any given Ag (29, 30), and for a primary immune response to occur, these scarce Ag-specific T cells must come into physical contact with rare but strategically placed DCs (31, 32). Furthermore, during Ag-limited infections or immunizations, only a fraction of DCs may present the relevant Ag to T cells. Recent studies have revealed sphingosine-1 phosphate receptor down-modulation on entry into lymph nodes and slow re-expression during trafficking through the lymphoid tissue as a possible molecular timer influencing naive T cell residence times in lymph nodes (28). Rapid motility within lymph nodes may thus enable efficient detection of rare Ags before default trafficking of Ag-specific cells out of the organ. Here we tested whether chemokinesis could be one regulatory mechanism in SLO for inducing favorable motility during both T cell priming and homeostatic trafficking.

We first showed, using direct videomicroscopic observation, that the homeostatic chemokine most abundant in T cell areas of SLO——CCL21——causes pronounced T cell migration in the absence of specific gradients and that this response is critically dependent on the presence of appropriate ligands to promote cell adhesion, notably ICAM-1. In secondary lymphoid tissues where CCL21 (and CCL19) is constitutively produced, T cells are exposed to VCAM-1 on stromal cells and DCs (33), ICAM-1 on DCs (34) and potentially stromal cells (33), and possibly FN presented by fibroblastic reticular cells or exposed on the surface of a small fraction of reticular fibers not enveloped by reticular cells (35, 36).

We found that all three of these ligands could support some degree of cell migration. Highly favorable T cell migration on ICAM-1 may reflect the need for rapid investigation of DC cell bodies. Notably, histological studies reveal that \(\sim60\%) of T cells in lymph nodes are in contact with DCs at any given moment (31).

For chemokinesis to be relevant for T cell migration within SLO, it should be sustained for the typical cell residence time: \(12–18\) h in the case of lymph nodes (9). We found that CCL21-induced chemokinesis is indeed sustained for at least \(24\) h, consistent with the finding in the human system that, in contrast to many chemokine/receptor interactions, CCL21 binding to CCR7 does not trigger receptor down-modulation (37). The residual cell polarization we observed after washing out chemokine may be due to the high-affinity nature of CCL21-CCR7 binding and possible chemokine sequestration by cell surface proteoglycans (38, 39), which complicate full separation of the cells from ligand. Nevertheless, we observed that the long-term chemokinetic response was dependent at least in part on the continued presence of CCL21.

Based on these findings, we sought to examine whether chemokinesis triggered by CCL21 could impact T cell priming, using a culture system designed to model the infrequency of Ag-specific T-DC contacts in vivo. We found that addition of CCL21 to T cell-DC cocultures enhanced the initial kinetics of CD69 up-regulation and led to 2-fold greater numbers of Ag-specific OT-II T cells recovered after 4 days. Because activated/expanded T cells are constitutively motile (40–43), we might expect that the presence of CCL21 would only alter the initial number of (otherwise non-motile) naive T cells that would encounter a DC and begin proliferating. Naive cells that did make contact with DCs and divide (in the presence or absence of chemokine) would thus proceed at equal rates to begin dividing, assuming equal rates of further cell division and no differences in cell death (we found similar fractions of apoptotic cells in cultures with or without CCL21, data not shown). In agreement with this hypothesis, CCL21-treated T cells had similar cell division profiles as control cells—only the recovery of Ag-specific T cells changed. Surprisingly, we found that addition of CCL21 to peptide-free cocultures also increased the recovery of OT-II cells, though control experiments revealed no evidence for a direct survival signal given to T cells by CCL21 (data not shown). This could be due to CCL21-driven T cell motility increasing Ag-independent T-DC contacts, which promote T cell survival (44, 45). We found that the increase in T cell recovery was consistently greater in the presence of Ag-bearing DCs than in their absence, suggesting an Ag-specific effect apart from survival cues imparted to T cells by immature DCs.

These results suggested an influence of T cell chemokinesis on priming but were not definitive, since the receptor for CCL21, CCR7, is expressed by both naive T cells and mature DCs (46),
Cytosis is important for the generation of active T cells. T cells can efficiently migrate toward ELC, which is expressed by high endothelium, and is an agonist for the MIP-3beta receptor CCR7. J. Cell Biol. 141: 1035–1059.


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