Dendritic Cells Regulate High-Speed Interstitial T Cell Migration in the Lymph Node via LFA-1/ICAM-1

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T lymphocytes vigorously migrate within the paracortex of lymph nodes (LNs) in search of cognate Ags that are presented by dendritic cells (DCs). However, the mechanisms that support T cells to exert the highest motility in a densely packed LN microenvironment are not fully understood. Two-photon microscopy using LN tissue slices revealed that LFA-1 and ICAM-1 were required for high-velocity migration (>10 μm/min) with relatively straight movement. Importantly, ICAM-1 expressed by myeloid lineages, most likely DCs, but not stromal cells or lymphocytes, was sufficient to support the high-velocity migration. Visualizing DCs in the LN from CD11c-EYFP mice showed that T cells traveled over thin dendrites and the body of DCs. Interestingly, DCs supported T cell motility in vitro in chemokine- and ICAM-1-dependent manners. Moreover, an acute lymphopenic environment in the LN significantly increased LFA-1 dependency for T cell migration, indicating that lymphocyte density modulates the use of LFA-1. Therefore, our results indicate that LFA-1/ICAM-1–dependent interactions between T cells and DCs play a crucial role not only in supporting firm arrest during Ag recognition but also in facilitating the Ag scanning processes.

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Chemokines trigger several modes of lymphocyte motility including integrin-dependent migration (12–14). Two-photon imaging of adoptively transferred T cells in LN explants revealed that a deficiency in the chemokines CCL19 and CCL21 or their shared receptor CCR7 reduced the T cell migration velocity by 20–35%. Furthermore, pertussis toxin (PTX), which inhibits Gi-coupled receptor signaling, reduced T cell migration velocity by ~50% (15–17). These data suggest that redundant chemokines and/or other migratory cues cooperatively regulate intranodal migration. Using the same approach, Woolf et al. (18) reported that a deficiency in β2 integrins or ICAM-1 reduced T cell velocities by 15 or 30%, respectively. However, the authors especially proposed that immobilized chemokines stimulate T cell migration without integrin activation in shear-free environments. The role of integrins in tissue interstitial migration was also challenged by a study showing that bone marrow (BM)–derived DCs that lacked almost all integrins could still migrate into and within the LN, and relatively slow amoeboid movement (~4 μm/min) was suggested to facilitate integrin-independent migration in collagen gel (19). However, T cells are able to migrate at much higher velocities than DCs with distinct migrating morphology. In addition, the same group recently showed that DCs also use integrin-dependent motility, provided that adhesive footholds are available nearby, and some studies have suggested that ICAM-1 plays significant roles in T cell migration in LNs (20–22). Thus, the role of LFA-1/ICAM-1 in intranodal T cell migration is still obscure and requires a detailed characterization.

Structure of lymphoid tissues is supported by the network of nonhematopoietic stromal cells, in particular fibroblastic reticular cells (FRCs), which have been suggested to play a role in T cell migration as a foothold (23). In fact, T zone FRCs express chemokines and integrin ligands (24–27). Given that T cells can directly access these trafficking molecules that are displayed on the stromal cell surface, it is reasonable to assume that FRCs are actually involved in T cell motility. However, the large numbers of DCs that are present in the paracortex (3, 28) may also be capable of supporting T cell motility, because these DCs express ICAM-1 and can bind chemokines on the surface (29, 30).

In this study, we tried to clarify the functional significance of LFA-1 and ICAM-1 in intranodal T cell migration and determine...
the tissue component that supports this adhesion machinery. We took advantage of two-photon imaging of LN slices by directly applying normal lymphocytes to the tissue parenchyma and evaluated the immediate effects of functional blocking using Abs or inhibitors. These approaches enabled us to demonstrate that LFA-1/ICAM-1 regulated high-velocity and relatively straight movements of T cells, and ICAM-1 expressed by DCs was likely key for LFA-1-dependent modulation of T cell migration in the LNs.

Materials and Methods

Mice

Mice were maintained and bred under specific-pathogen-free conditions in the animal facility of Kansai Medical University. C57BL/6 mice were purchased from CLEA Japan. ICAM-1−/− mice (B6.129S1-Icam1tm1(Jcgr)) and CD11c-EYFP mice were purchased from The Jackson Laboratory. CAG-EGFP mice were provided by Dr. M. Okabe (Osaka University, Japan). To generate BM chimera, mice were γ-irradiated with a single dose of 10.5 Gy from a cesium source and reconstituted with 1–5 × 10^6 BM cells. Two months after the BM transfer, the chimeric mice were used for experiments. All animal procedures were approved by the Committee on Animal Research at Kansai Medical University.

Abs

The following Abs labeled with or without fluorophores were used: anti-CD3 (145-2C11), anti-B220 (RA3-6B2), anti-CD11a (M17/4), anti-CD11b (M1/70), anti-CD11c (N418), anti-CD31 (390), anti-CD45 (30-F11), anti-CD80 (16-10A1), anti-CD86 (GL1), anti–ICAM-1 (Y1/N/1.74), anti–MHC class II (M5/114.15.2), anti-podoplanin (8.1.1) (E-Bioscience); ER-TR7 (ER-TR7) (BMA); anti-laminin (rabbit polyclonal) (LSL); anti-CCL21 (goat polyclonal), anti–VCAM-1 (goat polyclonal) (R&D Systems); normal rat IgG (Caltag Laboratories); and goat anti-rat or anti-rabbit AffiniPure Fluor-488, -594, and -633 (Invitrogen). mAbs against LFA-1 (KBA, FD441.8, and M17/4), VLA-4 (PS2), ICAM-1 (Y1/N/1), and CD62L (Mel-14) were purified from hybridoma supernatants.

T cell migration in LN tissue slices

Imaging of the LN slice was as described (31) with several modifications. Isolated LNs (inguinal, brachial, axillary, and cervical) were glued to a custom-made plastic pedestal with Vetbond (3M) and embedded in a 4% agarose gel (low gelling temperature, Type VII-A, Sigma-Aldrich) in PBS. The upper part of the organ (300–400 μm below the top) was cut off using a vibration slicer (Dosaka EM) at room temperature. T cells were isolated from the LNs and spleen by magnetic cell sorting using a Pan T cell isolation kit (Miltenyi Biotec) and labeled with 5 μM CMTMR or 2 μM CFSE (Invitrogen). The preparation of T cells always took place at room temperature using freshly prepared 1% FSC RPMI 1640 and 10 mM HEPES for 1 h at 37˚C. CD11c+ cells were isolated by two rounds of magnetic cell sorting using a microbead-conjugated anti-CD11c mAb (Miltenyi Biotec). The cells were incubated in the presence of 200 ng/ml Flt3L and 100 ng/ml TNF-α (PeproTech) for 24 h. Alternatively, DCs were isolated from BM progenitors as described (33). BM cells from wild-type (wt) or ICAM-1−/− mice were cultured with 200 ng/ml Flt3L for 9 d and stimulated with 10 ng/ml TNF-α during the final 24 h of culture. Differentiation of DCs was confirmed with the expression of CD11c, MHC class II, CD80, CD86, and ICAM-1 by flow cytometry.

After removing the dead cells with Lympholyte-M (Cedarlane Laboratories), 5 × 10^5 DCs were resuspended in 1% FCS Leibovitz’s L-15 medium and incubated in a drop on fibronectin-coated ΔT-dishes (Biotecs) for 1 h. CMTMR-labeled T cells (2 × 10^5) in 1% FCS Leibovitz’s L-15 medium with or without 100 nM CCL21 (R&D Systems) were gently loaded onto the condensed DCs. Phase-contrast and fluorescent images were obtained every 20 s at 37˚C using an LSM510 confocal microscope (Carl Zeiss) equipped with a heated stage adapter for two-photon microscopy. Control rat IgG was used as a negative control. To visualize the immediate effects of functional blocking using Abs or in vivo transduction with AAV vectors, mice were injected with 2 × 10^12 AAV-VEGF (MediA) and 200 ng of a-Ang2 or BDNF (BD Biosciences) and analyzed with CellQuest software (BD Biosciences).

Immunohistochecistry

Frozen LN sections (10 μm) were fixed with cold acetone and stained with Abs. The samples were examined using a confocal microscope (LSM510META; Carl Zeiss), and digital images were prepared using an LSM examiner (Carl Zeiss) and Adobe Photoshop CS5 software (Adobe Systems). Cell numbers in the images were estimated by dividing the total pixel intensities by the average pixel of a single channel.

Statistical analysis

GraphPad Prism software (GraphPad) was used for statistical analyses. The means of two groups were compared with a Student t test. Mann–Whitney U test was used to compare two nonparametric datasets.

Results

Optimal interstitial T cell migration in the LN requires LFA-1/ICAM-1

To determine the mechanisms underlying intranodal T cell migration, we established an imaging system using two-photon microscopy and modified LN slice technique (31). The upper part of LN was cut off using vibration slicing, and freshly isolated T cells were loaded onto the slice (Fig. 1A, 1B). T cells that entered the tissue exhibited active migration (median velocity: 11–14 μm/min;
FIGURE 1. Analysis of T cell migration using LN tissue slices. (A) Schematic representation of the experimental procedure using LN tissue slices. (B) Photomicrograph of a sliced inguinal LN that was glued onto a custom-made plastic pedestal and embedded in agarose gel at low gelling temperature. (C) Three-dimensional view of T cell migration trajectories in LN tissue slices that were treated with control rat IgG (left panel) or an anti–LFA-1 mAb (right panel). The images were reconstructed from a three-dimensional tracking analysis of time-lapse image datasets that were obtained by scanning every 20 s. The gridlines are squared by 15.5 μm. (D) T cell migration velocities in LN slices in the presence of rat IgG or an anti–LFA-1 mAb. The mean velocity of individual cells (circles) and the median (horizontal bars) are shown. (E) Mean displacement of cells plotted against the square root of time (seconds) in LN slices treated with rat IgG or an anti–LFA-1 mAb. Error bars, ± SEM. (F) Summarized plot of T cell migration with various treatments. The symbols represent the percentage of velocity in each experiment compared with the control, and the horizontal bars represent the mean. (G) Migration velocities of T cells in LN slices in the presence of rat IgG or an anti–ICAM-1 mAb. (H) Migration velocities of T cells in LN slices from wt or ICAM-1−/− mice. (I) Migration velocities of T cells pretreated with PTx or untreated (control). (J) Migration velocities of T cells treated with DMSO or latrunculin B (Lat. B). TP-LSM, Two-photon laser–scanning microscopy.
Fig. 1C–E). The advantage of this approach is that Abs or drugs can be directly administered into the parenchyma to examine their acute effects on T cell motility. In addition, because this method bypasses lymphocyte transmigration across high endothelial venules, the possible bias in the homing steps is limited.

The role of LFA-1/ICAM-1 was examined by adding blocking mAbs. As expected, inhibiting LFA-1 with an mAb (clone KBA) (35) significantly reduced migration velocity (Fig. 1C–F, Supplementary Video 1). Three different mAbs against LFA-1 showed similar inhibitory effects (Supplemental Fig. 1A), whereas mAbs against VLA-4 or CD62L showed little effect compared with the control (Fig. 1F, Supplementary Fig. 1B). Simultaneous treatment of anti–LFA-1 and anti–VLA-4 mAbs showed no further decrease of migration velocity compared with anti–LFA-1 mAb alone (Supplementary Fig. 1C), suggesting that VLA-4 does not compensate for the T cell motility under the LFA-1 blocked condition. Anti–ICAM-1 mAb also reduced T cell motility, and wt T cells added to LN slices from ICAM-1−/− mice consistently showed a slower migration than in ICAM-1–sufficient LNs (Fig. 1F–H, Supplementary Video 2). Pretreating T cells with PTX inhibited their motility by ~40%, indicating that Gi signaling is also required for high-speed motility (Fig. 1F, 1I, Supplementary Video 3). Stationary range of cell motility was estimated using latrunculin B, which disrupts the actin cytoskeleton, or an anti–CD3 mAb, which transmits Ag receptor–dependent stop signals (Fig. 1F, 1J, Supplementary Fig. 1D, Supplementary Video 4). These treatments dramatically suppressed T cell motility with median velocities of 3–5 μm/min. Therefore, LFA-1/ICAM-1 and Gi signaling contribute in part to interstitial T cell motility.

We also confirmed the importance of LFA-1/ICAM-1 in vivo. Mice that had been transferred with fluorescently labeled T cells were injected with an anti–LFA-1 mAb or control IgG, and 4 h after mAb administration, LN explants were subject to two-photon imaging (Fig. 2A). We detected a significant reduction in T cell motility with the anti–LFA-1 mAb compared with the control (Fig. 2B–E). The anti–LFA-1 mAb had similar inhibitory effects during intravital microscopy of the popliteal LN using the same protocol of adoptive transfer and Ab administration (Fig. 2F).

Moreover, when wt T cells were transferred to either wt or ICAM-1−/− mice, T cell motility was consistently lower in ICAM-1–deficient LN explants than an ICAM-1–sufficient condition (Fig. 2G). Together, these data show that LFA-1/ICAM-1 is necessary for optimal high-speed T cell migration.

The distribution profiles of mean velocities revealed that the majority of T cells migrates at a high speed, at >10 μm/min (Fig. 3). This fraction selectively disappeared upon LFA-1 or ICAM-1 inhibition, resulting in speeds ranging from 3–10 μm/min (Fig. 3A, 3C). In control experiments, the mean velocity inversely correlated with the mean turning angle, which reflects the straightness of the trajectory (Fig. 3B). T cells exhibiting a higher velocity showed a relatively smaller turning angle and the inhibition of LFA-1/ICAM-1 abrogated this fraction of motility (>10 μm/min and <50°) (Fig. 3B, 3D). PTX treatment led to migration patterns similar to those observed with LFA-1/ICAM-1 inhibition, whereas latrunculin B increased the turning angle with a severe reduction in velocity (Fig. 3E–H). Inhibition of VLA-4 did not influence on turning angle as well as velocity (data not shown). Therefore, T cells use the LFA-1/ICAM-1 axis for high speed and relatively straight movement in LN tissue.

**ICAM-1 expressed on DCs is necessary for high-speed intranodal T cell migration**

ICAM-1 is highly expressed by DCs, stromal cells, and a fraction of endothelial cells in the LNs, whereas lymphocytes and macrophages expressed ICAM-1 at one-order lower levels (Fig. 4A, 4B). Immunostaining of LNs from CD11c-EYTP mice (28) showed that in the parenchyma of the paracortex, DCs are closely associated with the VCAM-1+ ER-TR7+ FRCs, and together they form dense networks (Fig. 4C, 4D). Thus, both DCs and FRCs are in the location expected to support T cell migration.

To determine the contributions of DCs and FRCs in support of high-speed migration via providing ICAM-1, we generated hematopoietic chimeras using ICAM-1−/− mice and examined T cell motility. In the LN slices from wt mice reconstituted with ICAM-1−/− BM cells, the high-speed population was diminished with a significant decrease of median velocities, whereas LNs from ICAM-1−/− mice that received wt BM exhibited T cell motility comparable to the controls (Fig. 5A, 5B, Supplementary Video 5). These findings strongly suggest that hematopoietic ICAM-1, but not nonhematopoietic ICAM-1 including stromal cells in radioresistant components, is critical for high-speed migration of T cells. We next examined the relative contributions of ICAM-1 in lymphocytes and myeloid cells, including DCs. To this end, we made mixed chimeras of ICAM-1−/− mice as recipients with a mixture of BM cells from ICAM-1−/− mice and lymphocyte-deficient Rag2−/− mice (crossed with EGFP-transgenic mice) at a ratio of 1:4. Under this protocol, only Rag2−/− BM-derived myeloid cells, mainly DCs in the T zone, are expected to express ICAM-1, whereas lymphocytes and nonhematopoietic cells are not. Indeed, the majority of ICAM-1+ cells were CD11c+ DCs located in the paracortex of LNs from mixed chimeras (Fig. 5C, 5D). In contrast to ICAM-1−null LN environments, LNs from the mixed chimeras restored T cell motility to the control levels, and anti–ICAM-1 mAb inhibited the increase of velocity (Fig. 5E, 5F, Supplementary Video 6). These findings indicate that ICAM-1 in myeloid cells, most likely DCs, is required for LFA-1/ICAM-1–mediated high-speed T cell migration in the LN paracortex.

**Dynamic interaction between migrating T cells and DCs**

To characterize T cell migration supported by DCs, we performed live-cell imaging of LNs from CD11c-EYFP mice. Most of T cells migrated actively in association with the dense network of DCs (Fig. 6A, Supplementary Video 7). Migrating T cells constantly repeated the process of elongation and contraction of their cell bodies, with motions of the protruding front over the DC surface (Fig. 6B, 6C, Supplementary Video 8). When they came to the end of the cell body of DCs, T cells stretched their bodies and slid along the dendrites that were connected to the neighboring DCs (Fig. 6D, 6E). The quantitative analysis showed that most of the T cells (89.4 ± 6.7%, total of 463 T cells in 14 static three-dimensional images) were in contact with DCs, supporting the major role of DC for T cell migration. However, we noticed that in some areas where DCs were apparently sparse, T cells were still able to migrate (Supplementary Video 7), suggesting that FRCs might also support motility.

To evaluate the foothold area, the total perimeters and cross sections of DCs and FRCs were measured in immunostained LN sections from CD11c-EYFP mice. DCs exhibited perimeters and cross sections ~2-fold greater than FRCs, indicating that DCs have larger surface area than stromal cells (Fig. 7A). Immunostaining of LN sections from CD11c-EYTP mice (28) showed that in the paracortex, DCs are closely associated with the VCAM-1+ ER-TR7+ FRCs, and together they form dense networks (Fig. 4C, 4D). Thus, both DCs and FRCs are in the location expected to support T cell migration.
FIGURE 2. Inhibition of LFA-1/ICAM-1 reduces interstitial T cell migration within LNs. (A) Schematic representation of the experimental procedure for the LN explant method. (B) Three-dimensional view of T cell migration trajectories in LN explants from mice injected with control rat IgG (top panel) or an anti–LFA-1 mAb (bottom panel). The gridlines are squared by 16 μm. (C) T cell migration velocities in explanted LNs from mice pretreated with rat IgG or an anti–LFA-1 mAb. The left panel shows the mean velocity of individual cells (circles) and the median (horizontal bars). The right panel shows the median velocity for individual experiments (circles) and the mean of five experiments (horizontal bars). (D) Mean displacement of cells plotted against the square root of time (seconds). Error bars, ± SEM. (E) Distribution of the mean turning angle of individual cells. Arrowheads indicate the data group containing the median value. (F) Migration velocities of T cells in the popliteal LNs of mice that were injected with rat IgG or an anti–LFA-1 mAb examined by intravital microscopy. Symbols in the left panel represent the mean velocity of individual cells and the median (horizontal bars). (G) The migration velocities of T cells in explanted LNs from wt or ICAM-1−/− mice. Fluorescently labeled wt T cells were transferred to wt or ICAM-1−/− mice. The left panel shows the mean velocity of individual cells, and the right panel shows the median velocity of individual experiments. TP-LSM, Two-photon laser–scanning microscopy.
dicating that there are more DCs than stromal cells in the T zone. In addition, we detected the colocalization of CCL21 and ICAM-1 on DCs, even on their thin dendrites (Fig. 7C). Therefore, DCs likely present T cells with a surface decorated with ICAM-1 and CCL21.

To determine whether DCs can actually support high-speed T cell migration in vitro, we used primary DCs isolated from the spleen or cultured DCs from BM cells in the presence of Flt3L, seeded at a high-density on thermo-glass chamber dishes, in which the condensed DCs were piled up and formed a semi-three-dimensional environment (Fig. 8A, Supplemental Fig. 3A). Although T cells did not show active migration with DCs alone, the addition of CCL21 stimulated T cell migration at 9 to 10 μm/min with relatively straight trajectories (Fig. 8B–D, Supplemental Fig. 3B, Supplemental Video 9). The inhibition of ICAM-1 with mAb or ICAM-1 deficiency markedly reduced migration (Fig. 8C–F, Supplemental Fig. 3B, 3C, Supplemental Video 10). These findings demonstrate that DCs are able to support chemokine-
stimulated motility of T cells via ICAM-1 expressed on their cell surface.

A lymphopenic environment increases LFA-1–dependent T cell migration

A previous study showed that lymphocyte motility could be enhanced by confinement in vitro (36), whereas densely packed lymphocytes may compete with each other for DC surface. We finally asked the influence of cell density on T cell motility within the LN using an acute lymphopenic condition induced by blocking entry of lymphocytes. Twenty-four hours after i.v. injection of anti-CD62L mAb (37), total and T cell numbers in the LNs were dramatically decreased, whereas the compositions of T cells and B cells were unchanged (Fig. 9A, Supplemental Fig. 4A). Cell density in the center of the T zone was reduced to ~60%, whereas the FRC network seemed to be comparable (Fig. 9B, 9C, Supplemental Fig. 4B). Interestingly, DCs were increased by 2-fold relative to controls, and CCL21 expression in the paracortex was also augmented (Fig. 9A, 9B, Supplemental Fig. 4B, 4C).

We then measured T cell migration in normal or lymphopenic LN slices. Compared to the control LNs, the median velocity was consistently increased in lymphopenic LNs, with increased populations exhibiting velocities >15 μm/min (Fig. 9D–F). Treatment with anti–LFA-1 mAb markedly reduced the high motility fraction, whereas the residual motility was comparable or slightly reduced. As a consequence, the net fraction of LFA-1–mediated motility was increased (Fig. 9E). Although our experimental settings did not allow us to evaluate confinement-supported motility, the find-
FIGURE 5. ICAM-1 expressed by paracortical DCs is the key for high-speed T cell migration. (A) Migration velocities of T cells in LN slices from BM chimeric mice: wt BM cells into wt host (wt → wt), ICAM-1$^{-/-}$ BM cells into wt host (ICAM-1$^{-/-}$ → wt), and wt BM cells into ICAM-1$^{-/-}$ host (wt → ICAM-1$^{-/-}$). The mean velocity of individual cells (circles) and the median (horizontal bars) are shown. (B) Summarized plot of T cell migration in the LN slices from various BM chimeras. The symbols represent the median velocity for individual experiments (circles) and the mean of the experiments (horizontal bars). I$^{-/-}$, ICAM-1$^{-/-}$; R$^{-/-}$, Rag2$^{-/-}$; I$^{-/-}$ + R$^{-/-}$, mixed chimera (ICAM-1$^{-/-}$/Rag2$^{-/-}$ = 1:4). (C) ICAM-1 expression in LN cells from BM chimeric mice. LNs were digested with enzymes, and single-cell suspension was stained with Abs to ICAM-1 and CD11c for flow cytometric analysis. Note that ICAM-1 expression is restricted to CD11c$^+$ cells in the LN cells of mixed BM chimera (far right panel). (D) Reconstruction of the network of ICAM-1–expressing DCs in the paracortex of ICAM-1$^{-/-}$ LN. CMTMR-labeled T cells (red) were applied to LN slice of I$^{-/-}$ + R$^{-/-}$ mixed chimera and examined by two-photon microscopy. EGFP-expressing DCs derived from Rag2$^{-/-}$/EGFP-Tg BM cells were densely repopulated in the paracortex, but not in the follicle (F). The image is shown as a z-projection view. (E) T cell migration velocities in the LN slice from BM chimeric mice. High-speed T cell migration was restored in the LN slice from I$^{-/-}$ + R$^{-/-}$ mixed chimera compared with the I$^{-/-}$ chimera. (F) Migration velocities of T cells in the LN slice from mixed BM chimera in the presence of anti–ICAM-1 mAb.
ings revealed that lymphopenic LN conditions could promote LFA-1/ICAM-1–dependent motility, likely through easy access of T cells to ICAM-1 and CCL21 on the increased numbers of DCs.

Discussion

The LN paracortex is an immunologically specialized tissue compartment where T cells constitutively exhibit extremely high motility as they survey cognate Ags. Using several approaches, including the LN slice method, we showed that LFA-1 and ICAM-1 play a significant role in intranodal migration, especially for the motility component exceeding 10 μm/min with smaller turning angles. During the high-velocity and relatively straight movement, T cells travel across the three-dimensional thicket of DCs in the T zone with successive and transient contacts through LFA-1/ICAM-1 adhesion machinery. This modality is clearly different from integrin-independent slow motility observed in some leukocytes within a collagen gel or interstitium of peripheral tissues, by which cells can move forward in a dense matrix with complicated elongation and squeezing motions (14, 19, 38).

Previous studies reported the involvement of LFA-1 and ICAM-1 in immune cell trafficking in LN at various degrees (18, 21, 22). Woolf et al. (18) showed that CD18−/− T cells adoptively trans-
ferred into wt mice migrated within LNs at the average velocity that was reduced by 15% compared with the control. Interestingly, wt T cells transferred into ICAM-1−/− animals exhibited even less (∼30%) migration. There are several possibilities for the greater effect of an ICAM-1 deficiency compared with a CD18 deficiency on T cell migration. First, the requisite involvement of LFA-1 for entry into the LNs could skew a CD18−/− T cell population independent of LFA-1/ICAM-1 for interstitial migration. In contrast, because ICAM-2 is expressed in high endothelial venules, but only minimally expressed in the parenchyma, ICAM-2 could compensate for an ICAM-1 deficiency during the LFA-1–dependent homing of wt T cells to LNs, but not during the migration in the T zone (21). Second, a chronic LFA-1 deficiency could induce compensatory mechanisms such that T cells generated under this condition are capable of trafficking without LFA-1. It should be noted that CD18−/− mice exhibit confounded immunological abnormalities with increased effector/memory T cell populations (39). Thus, adoptive transfer experiments using lymphocytes from CD18−/− mice could cause the role of β2 integrin to be underestimated, and it is often difficult to evaluate the requirement of LFA-1 in interstitial migration under normal conditions when using T cells from LFA-1–deficient mice.

In this respect, the LN slice method offers several advantages over conventional adoptive transfer methods, notably the imaging of nonbiased resting T cells from wt animals directly applied into the LN parenchyma without homing steps. The LN slice method allows direct delivery of Abs or drugs that can immediately act on the cells. Evaluation of drugs with relatively short t1/2 or simultaneous

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**FIGURE 8.** DCs support ICAM-1–dependent T cell motility in the presence of CCL21 in vitro. (A) Schematic representation of the experimental procedure for T cell migration on densely packed DCs in vitro. (B) Phase-contrast/fluorescent-superimposed image sequence of CMTMR-labeled T cells (red) migrating on crowded DCs in the presence of 100 nM CCL21. The blue and red lines represent the trajectories of cell centroids. T cell migration on DCs. Migration trajectories (C, colored lines) and velocities (D) of T cells are shown. Migration trajectories (E) and the velocities (F) of T cells on Flt3L-induced BM-derived DCs from wt or ICAM-1−/− mice.
treatments of powerful inhibitors that potentially suppress homing process are technically difficult in conventional transfer experiments. Although a lack of blood and lymph supply and the transmigration process might be potential caveats for the analysis, T cell migration behaviors observed using tissue slices were comparable with those using intravital and explants with the similar inhibitory effect of anti-LFA-1 mAb, indicating that sliced LN can support normal interstitial T cell migration. Analysis under a lymphopenic tissue setting induced by anti-CD62 mAb, which severely inhibits entry of lymphocytes into the LNs, has been made possible only with this technique. In addition, this method is also valuable for in-depth imaging of the interaction of T cells with other cell components in the LN parenchyma. Therefore, LN slice method is suitable to address the detailed mechanism of interstitial T cell migration and broadens future applications.

It has been suggested that the stromal network may function as a guide rail for T cell migration in the paracortex (23), which implied the direct contact between T cells and FRCs. We expected that FRCs could support LFA-1–dependent T cell motility in vivo. However, BM chimeras showed that ICAM-1 expressed by myeloid cells rather than stromal cells was required to high-speed migration. Several findings also support the notion that DCs mediate T cell motility. First, the majority of T cells associated with DCs. Second, DCs have a large surface area presenting ICAM-1 and CCL21, a condition in which chemokine-triggered integrin-dependent motility could be stimulated. A fraction of CCL21 secreted from FRCs can be immobilized on the surface of DC via heparan sulfate proteoglycans (30). Third, DCs can support T cell motility through LFA-1/ICAM-1 and CCL21 in vitro. In addition, the relative contribution of DCs and adhesion molecules are likely

FIGURE 9. Velocity and LFA-1 dependency of T cell migration are increased in lymphopenic LNs. (A) Total cell number and the contents of cell populations in control and acute lymphopenic LNs. LNs were isolated from mice 24 h after they had received PBS or an anti-CD62L mAb. LN cells were counted and stained with Abs to CD3, B220, and CD11c for flow cytometry. The horizontal bars represent the means. (B) Microanatomy of the paracortex in control and acute lymphopenic LNs. LN cryosections were stained with the indicated Abs. Representative part of the paracortex that was stained for T cells (CD3), DCs (CD11c), and the reticular network (laminin). The nucleus was stained with DAPI to determine the total cell density as in (C). (C) The cell density in the paracortex of acute lymphopenic LNs was determined by calculating the average pixel density for DAPI staining. Data are shown as the mean of 10 images relative to the control (PBS). Error bars, +SD. (D) T cell migration velocities in LN slices from mice pretreated with PBS or an anti-CD62L mAb in the presence or absence of an anti-LFA-1 mAb during imaging. Bars represent the median velocity. (E) LFA-1–dependent fraction of T cell migration in LN slices prepared from mice treated with PBS or an anti-CD62L mAb. LFA-1 dependency is defined as the percent inhibition of T cell migration by the anti-LFA-1 mAb compared with the untreated control. Horizontal bars indicate the mean of individual experiments (circles). (F) Compiled bar graphs showing the frequencies of cells migrating with low (0–5 μm/min, blue), medium (5–10 μm/min, green), high (10–15 μm/min, red), or very high (>15 μm/min, dark red) mean velocities in each condition. Data were pooled from four independent experiments.
changed, as exemplified under acute lymphopenic conditions, in which the role of LFA-1 is augmented perhaps due to increased DCs and production of CCL21 as well as reduced competition of T cells for DCs. However, our results do not rule out the involvement of FRC in intranodal motility. It is clear that FRCs are important for producing chemokines, in particular CCL21, and for an anchorage scaffold for resident DCs. FRCs may also produce some other factors for LFA-1–dependent T cell motility. The lower migration velocities of T cells within the condensed DCs in vitro than LN tissues suggests that there are other components to support T cell motility in vivo.

Because DCs are nonstatic and the dendrites are constantly moving, T cells would not continuously contact with the same DCs during the course of migration. For this reason, LFA-1 should be fine-tuned to catch ICAM-1 transiently with a moderate affinity over a small part of the contact area, so that they can detach easily from the foothold without reducing velocity. Therefore, it is clear that the interaction between LFA-1 and ICAM-1 functions as an accelerator of T cell movement even in a densely packed LN environment. Given the limited resident time in the LN, the Ag scanning process by naive T cells likely requires high motility and so do stable contact with DCs for effective Ag recognition. In this context, LFA-1 serves as a versatile adhesion molecule that allows T cells to migrate rapidly and stop immediately upon encounter with cognate Ag presented on DCs. Thus, the inhibition of LFA-1/ICAM-1 axis is likely to influence on the efficiency of Ag scanning. There is also the possibility that transient contact with DCs could be strengthened by LFA-1/ICAM-1, which may provide other signals for the homeostasis as well as migration ability of T cells. Taken together, our study identified DCs as a T cell trafficking platform, and it should aid our understanding of the process of dynamic changes of adhesion modalities mediated by LFA-1 for efficient T cell priming.

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