Altered migration of gut-derived T lymphocytes after activation with concanavalin A

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Altered migration of gut-derived T lymphocytes after activation with concanavalin A. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G763–G772, 1999.—Although activation of lymphocytes is known to be associated with profound changes in homing behavior, it remains unclear how activation alters migration of gut-derived lymphocytes in lymphoid and nonlymphoid organs. The objectives of this study were (1) to compare migration of naive and concanavalin A (ConA)-activated T lymphocytes into the gut mucosa, spleen, and liver and (2) to define the role of specific adhesion molecules in this homing process. Fluorescently labeled T lymphocytes collected from rat intestinal lymph were injected into the jugular vein, and the kinetics of appearance of the infused lymphocytes were monitored in ileal Peyer’s patches, patches, spleen, and liver. The migration of naive and ConA-activated T lymphocytes into microvessels were compared using an intravital microscope. ConA stimulation significantly increased the rolling velocity of T lymphocytes in postcapillary venules of Peyer’s patches, and ConA-stimulated lymphocytes exhibited a loss of the selective adherence properties in Peyer’s patches that is normally observed with naive T cells. ConA activation also suppressed the accumulation of T cells in the spleen. On the other hand, the adherence of T cells to hepatic sinusoidal endothelium was significantly increased after ConA activation, especially in the periportal area, and this increase was attenuated by an anti-intercellular adhesion molecule (ICAM)-1 antibody. Flow cytometry analysis revealed a decline in L-selectin expression and an increase in CD11a expression and ICAM-1 on the surface of ConA-treated T cells. In conclusion, activation of gut-derived T lymphocytes with ConA significantly alters their migration pattern by extravasating through the endothelium of specialized postcapillary or high endothelial venules (HEVs) (11). In several species, a variety of lymphocyte adhesion molecules, namely L-selectin (10), CD44 (19), and α4β7 (2, 12, 17), are considered to play a role as organ-specific homing receptors. Recently, we have reported that α4β7 integrins make a critical contribution to the rolling and sticking of T cells and their subsequent transendothelial migration in postcapillary venules (PCVs) of Peyer’s patches exposed to physiological shear rates (22).

There is evidence that the homing behavior of lymphocytes can be profoundly altered on activation and differentiation. The migration properties of activated lymphocytes appear to be both more selective and more diverse than those of naive lymphocytes; indeed, the concept of “organ-specific” homing originated from studies on lymphoblast traffic. Some migration properties of “memory” lymphocytes more closely resemble those of activated lymphocytes (7, 15, 21, 31). Most memory and effector lymphocytes probably traffic through lymphoid organs, but, unlike naive cells, they also access and recirculate through extralymphoid immune effector sites such as the intestinal lamina propria or inflamed skin and joints (9, 21, 30). In humans, for example, CD4 cells that express both cutaneous lymphocyte-associated antigen and L-selectin preferentially accumulate in inflamed skin (28). Although large numbers of gut-derived lymphocytes are known to traffic through the liver and spleen, where they contribute to immunologic defense, it remains unclear whether the nature and intensity of lymphocyte-endothelial cell adhesion in these vascular beds are altered following lymphocyte activation (15). Moreover, it is not known how activation of lymphocytes affects their trafficking within microvessels supplying blood to either lymphoid (Peyer’s patch) or nonlymphoid (lamina propria) regions of the gut mucosa.

Concanavalin A (ConA), a plant lectin from jack beans, is known to mitogenically activate T lymphocytes via the antigen receptor. This lectin, along with intravital microscopic procedures for monitoring the dynamic process of lymphocyte migration, was employed in the present study to address three major objectives: (1) to compare the migration of naive and
ConA-activated gut T lymphocytes into lymphoid (Peyer’s patches) and nonlymphoid regions of the gut mucosa, 2) to assess the influence of ConA activation on the recruitment of lymphocytes in the vascular beds of the spleen and liver, and 3) to determine the contribution of $\alpha_\gamma$-integrin, LFA-1$\alpha$, intercellular adhesion molecule (ICAM)-1, and L-selectin to the adhesive interactions between activated lymphocytes and endothelial cells in the gut, spleen, and liver. We found that activation of gut-derived T lymphocytes with ConA significantly alters their migration path, with a diminished localization to Peyer’s patches and spleen and a preferential accumulation in hepatic sinusoids, and found that this altered migration pattern likely results from changes in the expression of leukocyte adhesion molecules including L-selectin and CD11a.

METHODS

Experimental setup for microvascular studies. Male Wistar rats weighing 250–300 g were maintained on standard laboratory chow (Oriental Yeast, Tokyo, Japan). The care and use of laboratory animals were in accordance with the National Institutes of Health guidelines. Under anesthesia with 50 mg/kg of pentobarbital sodium, the abdomen was opened via a midline incision. Twelve centimeters of the ileal segment ending at the cecal valve were chosen for observation and placed on a plastic plate. The intestine was kept warm and moist by continuous superfusion with physiological saline warmed to 37°C. Two small incisions in the bowel wall were made with a microcurette. Krebs-Ringer solution (pH 7.4) was infused into the lumen to flush away food residue. The luminal pressure of the gut loop was maintained at 15 cmH$_2$O with warm Krebs-Ringer solution, which was instilled into loops through vinyl tubes from the proximal end to obtain appropriate resolution and to minimize intestinal motility. Suitable areas of the microcirculation in Peyer’s patch and villus mucosa were observed through the serosa by an inverted type fluorescence microscope (Diaphot TMD-25, Nikon, Tokyo) equipped with a silicon-intensified target image tube camera with a contrast-enhancing unit (C-2400-08, Hamamatsu Photonics, Shizuoka, Japan) via a ×10 or ×20 objective lens. In separate groups, the microcirculation of intestinal villi was also observed from the mucosal surface after the intestine was cut along its antimesenteric border. The adjacent intestinal segment and mesentery were covered with absorbent cotton soaked with Krebs-Ringer solution. The behavior of fluorescently labeled lymphocytes was visualized on a television monitor through a fluorescence microscope according to a previously described method (22). Epi-illumination was achieved with filters of excitation at 470–490 nm and emission at 520 nm.

In another set of experiments, the liver and spleen were placed on a plastic stage with a nonfluorescent coverglass and carefully adjusted to minimize respiratory movements. The exposed portions of the liver or spleen were immediately covered with a transparent plastic film. In vivo microcirculatory images of the surface of the liver and spleen were observed after lymphocyte injection through a fluorescence television microscope as described above. After observation of lymphocyte adherence, FITC-labeled BSA (5 mg/ml; Sigma Chemical, St. Louis, MO) was injected (as a bolus) from the jugular vein to evaluate the distribution of microvascular blood flow in the liver and spleen.

The femoral artery was cannulated for measurement of systemic arterial pressure, using a Statham P23A pressure transducer (Oximel, CA). Systemic arterial pressure was then continuously recorded with a Grass polygraph recorder (Grass Instruments, Quincy, MA). The femoral vein was also cannulated for administration of monoclonal antibodies (MAbs).

In some experiments, an optical light rod (3.2 mm outer diameter, Sugita, Tokyo, Japan) connected with a xenon cold light source (Olympus, Tokyo, Japan) was inserted into the intestinal loop through the output vinyl tube. A high-speed video camera system equipped with a recorder (Kodak Ekta Pro 1000) was used for measurement of the velocity of red blood cells (RBCs) as reported previously (23). The observed area was recorded using a high-speed video recording system on an S-VHS videotape at a speed of 1,000 frames/s. The videotape was replayed at 30 frames/s. Changes in the RBC velocity of each microvascular branch were determined as time course changes with a video-measuring gauge (For A, Houei, Tokyo, Japan).

Collection and separation of lymphocytes. After an intraperitoneal injection of pentobarbital sodium (50 mg/kg), the main mesenteric lymphatic duct was cannulated as described by Bollman et al. (5). When the surgical procedure was completed, animals were maintained in Bollman’s cages and saline was infused intravenously from the jugular vein at a flow rate of 2.4 ml/h to replenish the fluid and electrolyte loss associated with lymphatic drainage. Lymph samples were collected in ice-cold vials containing 6 U/ml heparin, fetal bovine serum, and RPMI 1640 medium (pH 7.4; Gibco, Grand Island, NY). Lymphocytes from mesenteric lymph were washed three times with working medium [RPMI 1640 (pH 7.4), penicillin and streptomycin (Gibco), and 0.1% BSA (Sigma)] before separation and labeling.

A T cell-rich fraction of mesenteric lymphocytes was first obtained using a nylon-wool column. The whole cell population of 1 × 10$^8$ lymphocytes in 20 ml of RPMI 1640 medium with 1% fetal bovine serum was incubated in 1 g of nylon-wool (Kanto Kagaku, Tokyo, Japan) in a column, and the pass-through fraction was designated as the T cell-rich fraction after incubation for 1 h at 37°C. The T cell-rich suspension was then passed through an IgG-coated Immunex (Bectecx, Houston, TX) column to obtain negatively selected T cells. These manipulations had no significant effect on lymphocyte viability as assessed by trypan blue exclusion. The purity of T cells was evaluated by a fluorescence-activated cell sorter (FACS; Becton-Dickinson, Mountain View, CA). An MAb against rat CD3 (W3/13) (Serotec, Kidlington, UK) was used for FACS analysis. The purity of the isolated T cells was shown to be at least 95%.

Lymphocyte stimulation and labeling with carboxyfluorescein diacetate succinimidyl ester. T cells were cultured at a concentration of 2 × 10$^6$ cells/ml in RPMI 1640 medium supplemented with 10% fetal bovine serum, 5 × 10$^{-5}$ M 2-mercaptoethanol, penicillin, and streptomycin. The T cells were stimulated with several concentrations of ConA (Sigma) and incubated in 96-well flat bottom microtiter plates (0.2 ml/well) for 48 h at 37°C in 5% CO$_2$-95% air. To see the T cell activation, 1 μCi of [H]thymidine (ICN Biochemicals, Costa Mesa, CA; 6.7 Ci/mM) was added to each well 6 h before the end of incubation. Cultures were harvested onto glass filter papers (LKB, Gaithersburg, MD) using a Skatron cell plate counter (LKB). T cells were washed extensively to remove the activating stimulus before use in the presence of 25 mM methyl a-d-mannoside (Sigma) to block any residual ConA that may have been transferred with the lymphocytes. Cultured lymphocytes not exposed to ConA were used as controls.

Carboxyfluorescein diacetate succinimidyl ester (CFDSE; Molecular Probes, Eugene, OR) was dissolved in DMSO to
15.6 mM, and a small aliquot (300 µl) was stored in a cuvette sealed with argon gas at −80°C until the experiments. Lymphocytes (1 × 10⁶) in 20 ml of RPMI 1640 were incubated with 20 µl of CFSE solution for 30 min at 37°C. After hydrolysis by cytoplasmic esterases, CFSE forms a stable fluorochrome carboxyfluorescein succinimidyl ester (CFSE). Intracellular fluorophores react with lysine residues of intracellular proteins and remain within cells as long as the membrane is intact (32).

Data analysis of lymphocyte behavior. Lymphocytes (3 × 10⁷ cells in 1 ml RPMI 1640) were injected into the jugular vein of recipient rats over a 3-min period. The traffic of CFSE-labeled T lymphocytes in the microvasculature of Peyer’s patches and villus mucosa was continuously monitored and recorded on S-VHS video tape for 60 min after infusion of the cells. The locomotive behavior of individual lymphocytes was evaluated by replaying the video recording on a four-head video cassette recorder with a shuttle wheel single frame forward and reverse control (GT-4W, NV-FS70, Panasonic). To determine the distribution of lymphocytes at different depths, the focusing plane was placed at 5-µm intervals from the surface of the intestine. Lymphocytes adhering to the venules with occasional movement along the wall were defined as “rolling” lymphocytes. Those adhering to the wall without movement after exhibiting transient rolling were defined as “adherent” lymphocytes. They remained in the same position throughout each observation period (30 s). The average lymphocyte rolling velocity was plotted for at least 50 lymphocytes in more than six independent distinct venous (total of > 300 lymphocytes).

Because T lymphocytes are known to selectively adhere to 25- to 500-µm-diameter PCVs of Peyer’s patches, which correspond to second- or third-order branches of large interfollicular veins (22), we assessed T lymphocyte behavior using microvessels in this portion of Peyer’s patches. In the villus mucosa, the submucosal arteriolar and venular branches were identified and lymphocyte adherence was studied along third-order branches of venules. Villus tip capillaries with arcade vessels were identified by observation from the mucosal side. The number of adherent cells in PCV of Peyer’s patches and in unbranched straight venules and capillaries of villus mucosa was normalized to a 1-mm² observation field.

The migration of CFSE-labeled T lymphocytes through the microvasculature of the liver or spleen was continuously monitored and recorded on video tape for up to 50 min after injection. In the liver, suitable images including several units of hepatic lobules were selected and the lobular landmarks, the terminal portal venules (TPVs) and terminal hepatic venules (THVs) were identified when the replayed images of the FITC-labeled hepatic microangiograph were evaluated. The location of migrated lymphocytes was arbitrarily divided into three zones of the liver acinus, namely, periporal, midzonal, and perivenular. The number of T lymphocytes draining into THVs of liver was also compared between control and ConA-treated cells and expressed as lymphocyte flux per 10-min observation period.

Agents studied. A mouse IgG2a MAb functionally blocking the rat integrin α₄β₁-chain (MoR4₄,1) was purified from ascites on a protein G column as described previously (36). WT-3, an MAb that functionally blocks CD11a (the leukocyte function-associated molecule–1α; LFA–1α) was obtained from Seikagaku Kogyo (Tokyo, Japan). F(ab)² fragments were obtained by incubation of 2.0 µg of pepsin/1.0 mg of IgG for 2 h at 37°C, pH 3.5 (24). The digestate was then dialyzed to neutral pH, and samples were examined on gel electrophoresis for characterization of the fragments. CFSE-labeled T lymphocytes were preincubated with MoR4₄,1 or WT-3 in vitro for 20 min (3 × 10⁷ lymphocytes in 10 ml RPMI 1640 containing 0.5 mg F(ab)² fragments of antibody) and then infused into the recipient rats via the femoral vein. The antibody functionally blocking L-selectin (HRL3, IgG1) was purchased from Seikagaku Kogyo. In the case of HRL3, lymphocytes (3 × 10⁷) were preincubated with 0.6 mg of HRL3 in 10 ml of calcium-free Hanks’ for 20 min. In control animals, lymphocytes were preincubated with nonbinding antibodies (P6H6 or HRL4) for 20 min before infusion. P6H6 was provided by Cytel (San Diego, CA), and HRL4 was obtained from Seikagaku Kogyo. In some experiments, animals were pretreated (30 min before lymphocyte infusion) with an MAb directed against ICAM-1 (1A-29, 2 mg/kg). 1A-29 was purchased from Seikagaku Kogyo. As controls, nonbinding antibody P6H6 was used, and the same protocol was used.

In a separate series of experiments, gadolinium III chloride hexahydrate (GdCl₃; Aldrich Chemical, Brussels, Belgium), which depletes Kupffer cells and inhibits their function (16), was administered intravenously (4 mM solution in 0.9% NaCl (1.0 ml)/250 g body wt 24 h before lymphocyte infusion, and the same protocol was employed in liver microcirculation. Analysis of cell surface adhesion molecules of T cells. Selected single cell suspensions of T cells from intestinal lymph were washed in Hanks’ balanced salt solution containing 0.2% BSA and 0.1% NaN₃. This medium was used throughout the staining procedure. All incubations with antibodies were performed at 4°C for 30 min. For immunofluorescence staining, 1 × 10⁶ lymphocytes were first incubated with mouse anti-rat MABs to characterize and quantify α₄-integrin and CD11a expression. After incubation, the cells were washed in 400 µl of Hanks’ balanced salt solution and centrifuged three times at 1,500 for 30 s. The cells were then incubated with 1 ml of FITC-labeled anti-IgG. Cells were washed twice and resuspended for analysis. The antibody against L-selectin (HRL3, IgG1) was detected by an FITC-conjugated anti-hamster antibody (Cappel, West Chester, PA). For controls, lymphocytes were preincubated with isotype-matched, irrelevant antibodies. The lymphocytes were analyzed with an EPICS Elite flow cytometer (Coulter, Hialeah, FL). Data were obtained using CONSORT software on viable cells, as determined by forward light scatter intensity. Representative data from at least four individual measurements are shown.

Statistics. All results are expressed as means ± SE of 6 rats. Differences among groups were evaluated by ANOVA and Fisher’s post hoc test. For the comparison of histogram profiles of lymphocyte rolling velocity between different groups, the mean value and distribution were statistically evaluated by a nonparametric Mann-Whitney U test and Kolmogorov-Smirnov test, respectively. Statistical significance was set at P < 0.05.

RESULTS

Lymphocyte stimulation and surface expression of adhesion molecules on T lymphocytes. Activation of T lymphocytes during blastogenesis was achieved by ConA treatment for 48 h. The maximum proliferation of T cells was induced at a concentration of 2.5 µg/ml as determined by [³H]thymidine incorporation (Table 1). Therefore, in all subsequent experiments, ConA was employed at a concentration of 2.5 µg/ml.

The expression of various adhesion molecules (CD11a, α₄-integrin, L-selectin, and ICAM-1) on the surface of T lymphocytes was determined using specific MABs. FACS analysis revealed that the expression of L-selectin was
Adherent lymphocytes gradually increased in number in the middle-sized (25–50 µm) PCVs of Peyer’s patches during the observation period, especially during the initial 30- to 40-min period (Fig. 2). Figure 3A illustrates the time course of changes in the number of adherent lymphocytes in PCVs of Peyer’s patches and the effect of ConA activation. Lymphocytes located both inside and along (extravasated) microvessels are also illustrated. The number of adherent lymphocytes in PCVs of the control group was 165.0 ± 18.0 and 195.2 ± 14.8 cells/mm² at 20 and 30 min, respectively. ConA treatment significantly inhibited the number of adherent lymphocytes, particularly within the first 30 min (Fig. 2), with the number of these lymphocytes being 18.3 ± 4.0 and 25.1 ± 5.1 cells/mm² at 20 and 30 min, respectively (Fig. 3A). In vitro pretreatment of lymphocytes with antibody functionally blocking α4-integrin (MRα4–1) almost completely abolished the adherence of ConA-activated lymphocytes to PCVs of Peyer’s patches, but anti-L-selectin antibody (HRL3) did not affect these interactions (Fig. 3A).

Although the density of sticking lymphocytes was quite low in the villus (nonlymphoid) mucosa compared with PCVs in Peyer’s patches, we constantly observed a number of adherent lymphocytes in these tissues. In submucosal venules (third-order branches) of villi, the number of lymphocytes adherent to venular endothelium gradually increased during the observation period (Fig. 3B). In contrast to the PCVs of Peyer’s patches, ConA treatment did not significantly decrease the number of adherent lymphocytes in this region (12.8 ± 1.8 in control vs. 11.5 ± 1.0 cells/mm² in ConA treated at 40 min). In vitro pretreatment with MRα4–1 but not HRL3 significantly decreased the adherence of ConA-activated lymphocytes to submucosal venules (Fig. 3B). Figure 3C illustrates the number of adherent lymphocytes in villus tip capillaries as observed from the mucosal surface. In the control group, the adherent lymphocytes in villus tip capillaries amounted to 11.0 ± 2.0 cells/mm² at 10 min, but their number did not significantly change during the 40-min observation period (12.0 ± 1.3 cells/mm² at 40 min). ConA treat-

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<th>Table 1. Proliferative response of T lymphocytes with concanavalin A as determined by [3H]thymidine uptake</th>
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Values are means ± SE from 6 experiments. T cells from intestinal lymph were stimulated with several concentrations of concanavalin A (ConA) and incubated in 96-well flat bottom microtiter plates for 48 h. [3H]thymidine (1 µCi) was added to each well 6 h before the end of incubation. Cultures were harvested onto glass filter papers using a Skatron cell plate counter. T cells were washed extensively to remove the activating stimulus before use in the presence of 25 mM methyl α-D-mannoside. cpm, Counts per minute.

downregulated on activation with ConA (Fig. 1). ConA treatment slightly increased the intensity of CD11a and ICAM-1 on T lymphocytes, but it did not alter the expression of α4-integrin.

In vivo study of T lymphocyte migration into intestine. Characteristic lymphocyte-endothelium interaction were observed in PCVs of Peyer’s patches after infusion of T lymphocytes. At an early stage (10 min after cell administration), some lymphocytes transiently interacted with the vessel wall by rolling and then soon detached and returned to the blood stream. During the period of interaction, these lymphocytes rolled on the endothelium of PCVs at various speeds and for varying distances. The velocity of rolling lymphocytes, which slowly traveled along the venules, was calculated and compared between control and ConA-stimulated T cells. The mean flow velocity of RBCs in 30-µm-diameter venules was 3.0 ± 0.8 mm/s in the control group, and there was no significant change in RBC velocity when ConA-treated T lymphocytes were administered. A histogram summarizing lymphocyte rolling shows that 60–80 µm/s was the most frequent rolling velocity in the control group. A significant increase in rolling velocity was observed after ConA treatment, with the most frequent rolling velocity being 140–160 µm/s in the ConA-treated group.

Fig. 1. Expression of adhesion molecules L-selectin (A), α4-integrin (B), CD11a (C), and intercellular adhesion molecule (ICAM)-1 (D) on T lymphocytes determined by flow cytometric analysis. Effect of concanavalin A (ConA) treatment is shown. Control, naive T lymphocytes from intestinal lymph. ConA, T cells stimulated with ConA (2.5 µg/ml) for 48 h. Lymphocytes (1 x 10⁶) were first incubated with anti-rat monoclonal antibodies against CD11a (WT-3), α4-integrin (MRα4–1), L-selectin (HRL3), and ICAM-1 (1A-29). Cells were incubated with 1 ml FITC-labeled anti-rat IgG or anti-hamster IgG. Flow cytometry analysis was performed using FACScan (Epic Elite, Coulter). Data were obtained using CONSORT software on viable cells, as determined by forward light scatter intensity. Representative data from at least 4 individual measurements are shown.
ment did not induce a different level or time course of lymphocyte accumulation in villus tip capillaries compared with naive lymphocytes. Treatment with MR\(_{\alpha4-1}\) did not affect the lymphocyte accumulation in this area.

In vivo study of T lymphocyte migration into spleen and liver and the effect of antiadhesion molecules. The interaction of CFSE-labeled T lymphocytes with microvessels was visualized in the spleen. There was almost no significant lymphocyte rolling along the endothelium of these microvessels, and T lymphocytes appeared to adhere immediately on entering the splenic microcirculation. The time course of accumulation of adherent lymphocytes in splenic microvessels is shown in Fig. 4. In control animals, the number of adherent T lymphocytes gradually increased over the 60-min period. In contrast, treatment of T cells with ConA significantly inhibited the accumulation of adherent lymphocytes in the spleen (133.5 ± 7.3 in control vs. 37.9 ± 7.2 cells/mm\(^2\) in ConA treated at 30 min), although the total flux of appearing lymphocytes in spleen was not significantly different between the two groups. To determine which adhesion molecules were involved in T lymphocyte adherence within the splenic microcirculation, several MAbs against adhesion molecules were studied. As shown in Fig. 4, all of the adhesion molecule-specific antibodies tested (anti-L-selectin, anti-CD11a, and anti-\(\alpha4\)-integrin) did not alter the accumulation of unstimulated (Fig. 4A) as well as ConA-activated (Fig. 4B) T lymphocytes in the splenic microcirculation, suggesting that these adhesion molecules are not involved in the splenic T cell migration observed under normal physiological conditions.

In the liver, most of the infused, labeled T lymphocytes emerged (appeared) vertically from the periportal area (PVs), crossed the hepatic sinusoid, and drained into the THVs (Fig. 5). Flow velocity of these lymphocytes was 256 ± 28 µm/s in venules of the midzonal area and 320 ± 30 µm/s in venules of the perivenular area. ConA treatment did not significantly affect the flow velocity of T lymphocytes in either the midzonal or perivenular area. Some lymphocytes attached to the hepatic sinusoids especially in the portal area and perivenular area after transient rolling. The percentage of rolling flux was significantly greater in activated lymphocytes (53.3 ± 5.0%) than in unstimulated lymphocytes (37.5 ± 3.5%) in the midzonal area at 10 min after infusion. The time course of accumulation of adherent lymphocytes in hepatic sinusoids was determined in both periportal and perivenular areas, and the effects of treatment of T cells with ConA are shown in Fig. 6. The number of adherent T lymphocytes in the periportal area reached a maximum within 10-20 min (295 ± 52 and 310 ± 44 cells/mm\(^2\) at 10 and 20 min, respectively). ConA stimulation significantly enhanced the number of adherent lymphocytes in the periportal zone, with remarkable increases observed at 10 min (779 ± 129 cells/mm\(^2\)) and 20 min (960 ± 138 cells/mm\(^2\)) after cell activation (Fig. 6A). However, the number of T lymphocytes adherent to hepatic sinusoids of the perivenular area was greater for naive vs. ConA-treated lymphocytes during the first 10 min (Fig. 6B). Thereafter, activated T lymphocytes accumulated in sinusoids of the perivenular area, whereas the number of adherent naive lymphocytes diminished. Consequently, the number of adherent ConA-treated T lymphocytes exceeded that of controls at 20 min (Fig. 6B). T lymphocytes drained into the THV, with 280 ± 28 cells·mm\(^{-2}\)·10 min\(^{-1}\) observed at 10 min after the infusion of naive T lymphocytes. On the other hand, ConA stimulation remarkably inhibited the lymphocyte flux into THVs (35 ± 8 cells·mm\(^{-2}\)·10 min\(^{-1}\)) at 10 min because many activated lymphocytes were trapped in the periportal zone.

To determine which adhesion molecule is involved in the enhanced adherence of activated T lymphocytes within hepatic sinusoids, we assessed the actions of the adhesion molecule-specific MAbs on the number of adherent lymphocytes (Fig. 7A) and the lymphocyte flux into THVs (Fig. 7B) at 30 min after the T lymphocyte infusion. Values are expressed as a percentage of the adherent unstimulated or ConA-treated T lymphocytes not exposed to an antibody. Anti-ICAM-1 (1A-29) significantly decreased lymphocyte adherence to 67.7 ± 7.0% of that of unstimulated lymphocytes and 59.3 ± 11.3% of that of ConA treated (Fig. 7A). However, although not shown in Fig. 7, no further decrease in lymphocyte adherence was observed when in vitro pretreatment of lymphocytes with 1A-29 in conjunction with 1A-29 systemic injection was compared with the 1A-29 infusion alone. In vitro pretreatment of lymphocytes with anti-\(\alpha4\)-integrin (MR\(_{\alpha4-1}\)) did not significantly attenuate lymphocyte sticking (98.0 ± 9.0% and 84.8 ± 9.2% of the antibody untreated values in unstimulated and ConA-treated lymphocytes, respectively). Lymphocyte sticking was reduced to 76.0 ± 11.0% and 74.3 ± 13.0% by treatment with anti-CD11a (WT-3) in unstimulated and ConA-treated lymphocytes, respectively, although these decreases were not significantly greater than in the antibody untreated controls (84.3 ± 13.6% and 82.8 ± 11.5%, respectively).
statistically significant. Pretreatment of lymphocytes with HRL3 did not significantly affect the adherence of these lymphocytes (Fig. 7A). In accordance with the results of the effect of MAbs on lymphocyte adherence

Fig. 4. Time course changes of T lymphocyte sticking in microvessels of rat spleen. Effects of adhesion molecule antibodies on unstimulated lymphocytes (A) and ConA-activated lymphocytes (B) are shown. ConA (2.5 µg/ml) was used for stimulation of T lymphocytes. In some experiments, lymphocytes were treated with a monoclonal antibody against CD11a (WT-3), L-selectin (HRL3), and α4-integrin (MRα4-1) before infusion. Lymphocytes located in the 1-mm² observation field were determined. *P < 0.05 compared with controls (unstimulated lymphocytes). Values are means ± SE from 6 animals.

in the periporal area, treatment with 1A-29 significantly increased the flux of T lymphocytes into THVs and this increase was especially remarkable for ConA-activated lymphocytes (Fig. 7B). WT-3 treatment also

Fig. 3. Effect of ConA activation on the time course of T lymphocyte sticking in postcapillary venules of rat Peyer’s patches (A), third-order submucosal venules of villi (B), and villus tip capillaries (C) and the effect of monoclonal antibodies against α4-integrin (MRα4-1) or L-selectin (HRL3) on ConA-induced changes of T lymphocyte sticking in these area. Lymphocytes were incubated with ConA (2.5 µg/ml). Lymphocytes located both inside and along venules were counted in the 1-mm² observation field. Peyer’s patches and submucosal venules were observed from the serosal side, and villus tip capillaries were observed from the mucosal side. *P < 0.05 compared with controls. #P < 0.05 compared with ConA alone. Values are means ± SE from 6 animals.
significantly accelerated the flux of both unstimulated and ConA-treated lymphocytes into THVs, whereas MRα4-1 and HRL3 did not significantly alter lymphocyte flux of these lymphocytes (Fig. 7B).

The depletion and inhibition of Kupffer cells in rat liver by treatment with GdCl3 significantly attenuated lymphocyte adherence to 55.5 ± 5.4% of that observed with ConA treatment, but this was not the case for unstimulated lymphocytes (86.0 ± 10.0%) (Fig. 7A). Furthermore, GdCl3 drastically increased the flux of activated T lymphocytes in THVs as shown in Fig. 7B.

DISCUSSION

The migration of lymphocytes through different lymphoid organs and other tissues of the body is a carefully controlled process that enables immune cells to encounter the cognate antigen and to disseminate memory and effector cells for immunologic surveillance (7). In this study, we have demonstrated, using intravital video microscopy, that activation of T lymphocytes by ConA leads to profound alterations in the sequential migration of these cells in the microcirculation of different lymphoid and nonlymphoid organs. The activated T cells either lose or exhibit a diminished capacity to enter Peyer's patches and the spleen, whereas they exhibit a preferential distribution to the liver. A similar behavior of activated lymphocytes in lymph nodes or Peyer's patches has been reported in previous studies, although these were in vivo homing studies that used 51Cr-labeled lymphocytes or an in vitro binding assay of frozen sections (8, 13, 14). Our study also clearly demonstrates that stimulation of T lymphocytes leads to a selective suppression of lymphocyte binding to PCV of Peyer's patches in the intestinal mucosa. The altered homing of activated lymphocytes may reflect changes in the expression of cell surface adhesion molecules. Recently, we have demonstrated that α4-integrins mediate the rolling and sticking of T cells in PCVs of rat Peyer's patches (34). In this study, we were unable to detect any significant changes in the surface expression of α4-integrins on T lymphocytes after ConA treatment. Instead, we showed (using flow cytometric analysis) that the expression of L-selectin was downregulated when activated with ConA. Data suggest that the decreased L-selectin expression of ConA-treated lymphocytes is responsible for increased rolling velocity; other work shows that even a 50% reduction in L-selectin expression can dramatically reduce homing (33). Lymphocytes from L-selectin knockout mice show impaired homing to Peyer's patches, and in vivo homing studies have shown that an antibody to L-selectin partially blocks lymphocyte migration to Peyer's patches (1, 12). Our present results show that blocking of α4-integrins mediating lymphocyte adherence to PCV of Peyer's patches may be responsible for the altered homing of activated lymphocytes.
integrin of ConA-treated lymphocytes almost completely inhibited the lymphocyte-endothelial interaction in Peyer's patches, suggesting the concerted action of L-selectin and $\alpha_4$-integrin molecules in this region. It appears likely that L-selectin plays a primary role in the initiation of lymphocyte contact (rolling and adherence) with Peyer's patch HEVs in association with $\alpha_4$-integrin (6), whereas $\alpha_4\beta_7$ and LFA-1 sequentially participate in processes of firm adhesion and transendothelial migration (2).

In the present study, we observed profound differences in the magnitude and kinetics of T lymphocyte recruitment into lymphoid (Peyer's patch) and nonlymphoid (villus mucosa) regions of the intestinal microcirculation. In contrast to Peyer's patch HEVs, we were unable to demonstrate significant differences in the accumulation of stationary naive vs. ConA-activated lymphocytes in submucosal venules and villus tip capillaries of the rat intestinal mucosa. These observations are somewhat different from previously published reports of an enhanced migration of activated lymphocytes into the gut mucosa (3, 4, 7, 27, 30). Berlin et al. (4) reported an increased in situ interaction of activated murine lymph node lymphocytes with venules in the small intestinal lamina propria compared with resting lymph node cells. They also found that $\alpha_4\beta_7$ can mediate the direct L-selectin-independent interactions of activated lymph node cells with mucosal address in cell adhesion molecule-1. The mucosal immunoblast is considered to traffic efficiently to the intestine (27), and recent studies indicate that mucosal T cell blasts preferentially bind to human mucosal HEVs, primarily via $\alpha_4\beta_7$, but with contributions from CD44 and LFA-1$\alpha$ (30). This characteristic gut tropism of gut-derived activated T cells was not observed in the present study. The exact reason for the difference between previous reports and our observations is not known but may relate to the use of different cell sources (cells from intestinal lymph vs. lymph node cells or lamina propria cells), type of stimulation (ConA vs. other activators), and the species difference (rats vs. mice or humans). On the other hand, Salmi et al. (30) also showed that lamina propria lymphocytes (LPLs) stimulated in vitro with phytohemagglutinin and interleukin-2 did not...
bind any more avidity than the "not-as-activated" small LPLs. Hamann et al. (13) suggested that in vitro activation by mitogen induces only one peculiar type of migration behavior. It may be that in vitro stimulation and differentiation may not mimic the in vivo situation very closely. In our present results, the accumulation of activated T lymphocytes to microvessels of villus tips was not inhibited by anti-α4-integrin antibodies. We also recently demonstrated that T lymphocyte adhesion in periglandular and villus tip capillaries is significantly increased by endotoxin, but these interactions were independent of α1- or β3-integrins (23).

Additional work is needed to more fully characterize the homing of activated lymphocytes to different sites within the intestinal mucosa, such as lymphoid vs. nonlymphoid regions, as well as submucosal venules vs. mucosal capillaries.

The predominant role of the spleen in lymphocyte migration has been postulated because higher numbers of lymphocytes enter this regional circulation than enter the thoracic duct (25) of rats. Lymphocyte homing to the spleen does not involve HEVs, and the adhesion of these leukocytes in the splenic microcirculation may depend on the expression of different lymphocyte surface molecules. Our results confirm the view that T lymphocytes readily adhere to splenic microvascular endothelium and further demonstrate that none of the known adhesion molecules (L-selectin, α4-integrin, and CD11a/ICAM-1) is involved in this process. Activated lymphocytes, in contrast, have a strongly reduced capacity to enter the spleen compared with resting lymphocytes, which is consistent with previous findings (15). The reduced capacity of activated lymphocytes to enter the spleen is not likely a consequence of L-selectin shedding because treatment of T lymphocytes with an anti-L-selectin MAb did not induce a significant reduction in adhesion, which is comparable to the behavior of activated lymphocytes. These findings suggest that unknown alterations in the T cell phenotype occur on activation, which contribute to the reduced ability of these lymphocytes to reach the spleen.

In this study, we demonstrated a significant accumulation of activated T lymphocytes in the liver microcirculation, particularly in the periportal region, although nonactivated lymphocytes interact less effectively with hepatic sinusoids. Our results from flow cytometric analyses and blocking studies performed using MAbs against CD11a and ICAM-1 suggest the possibility that an interaction between LFA-1/ICAM-1 accounts for the lymphocyte accumulation in hepatic sinusoids. There is histochemical evidence that ICAM-1 is expressed on liver endothelium but mainly in the portal area (35). Together, these results indicate that activated T lymphocytes may preferentially adhere in hepatic sinusoids of the periportal region using an ICAM-1-dependent process. A common picture emerges that activation seems to induce a general change in the migration properties of lymphocytes, diminishing their entry into lymphoid tissue while enhancing their transit through nonlymphoid sites such as the liver (13, 15).

There is some evidence that a major portion of blasts remain in the liver and die there. Hence, the liver may constitute a temporary depository for activated lymphocytes within the body. Recently, Huang et al. (18) demonstrated that, as injected T cells expressing the transgene T cell receptor disappear from lymph nodes and spleen after administration of an antigenic peptide, a massive accumulation of these cells occurs in the liver, where they undergo apoptosis.

Our study also demonstrates that inhibition and depletion of Kupffer cells in the liver by GdCl3 treatment significantly attenuate the accumulation of activated T lymphocytes in the periportal area, suggesting that Kupffer cells contribute to lymphocyte recruitment in the liver microcirculation. Recently, we have demonstrated that Kupffer cell-mediated cytolysis against hepatoma cells occurs through cell-cell adhesion via ICAM-1/CD18, causing calcium mobilization and oxidative activation of nuclear factor-kB, which may lead to the increased production of nitric oxide in Kupffer cells (20, 29). Thus there is also a possibility that activated T cells could be retained by Kupffer cells when they enter the hepatic microcirculation through an ICAM-1-independent interaction. The dominant distribution of Kupffer cells in the portal area supports this possibility. Once activated lymphocytes interact with Kupffer cells or endothelial cells, an increased synthesis of tumor necrosis factor-α may occur, which in turn could enhance the expression of ICAM-1 expression in hepatic sinusoids. The molecular basis for the increased migration of activated lymphocytes to the liver and the significance of this process to the immunologic response warrant further attention.

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