In situ demonstration of intraepithelial lymphocyte adhesion to villus microvessels of the small intestine

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Abstract

The recirculation of lymphocytes through the intestinal mucosa is important for specific immune defense, but the origin and differentiation of intraepithelial lymphocytes (IEL) are not fully understood. The present study therefore used intravital microscopy to investigate the migration of IEL to the villus mucosa and Peyer’s patches of the small intestine. IEL were separated from inverted murine small intestine and mesenteric lymph node (MLN) T cells were also isolated. The adhesion of fluorescence-labeled lymphocytes to postcapillary venules (PCV) of Peyer’s patches and arcade microvessels of small intestinal villi was observed after injection. In some experiments, the effect of antibodies against adhesion molecules on cell kinetics were investigated. IEL time-dependently accumulated in villus microvessels of the small intestine, whereas few MLN cells did. Few IEL adhered to the PCV of Peyer’s patches. IEL were shown to express αEβ7-integrin but not L-selectin. The accumulation of IEL in villus arcade was significantly inhibited by antibody against β7-integrin or mucosal addressin cell adhesion molecules (MAdCAM)-1, but not by αE-integrin. The combined blocking of β7-integrin and MAdCAM-1 further attenuated the sticking of IEL in this area, although it did not entirely block the IEL adherence. The adherence of CD4+ or TCRγδ IEL to villus microvessels was significantly greater than that of CD4+ or TCRγδ IEL. It was demonstrated in situ for the first time that IEL adhered selectively to the villus microvessels of the small intestine partly via β7 and MAdCAM-1.

Introduction

Intestinal intraepithelial lymphocytes (IEL) are a very large cell population of cells in the epithelial layer of the small intestine. They are αβ and γδ TCR-bearing cells with phenotypic and functional features distinct from those of cells in peripheral lymphoid tissues. In vitro these lymphocytes, mainly CD8+ T cells, proliferate poorly in response to T cell mitogens and to stimuli of the CD3 pathway (1,2). Although IEL have intimate cellular and molecular cross-talk with intestinal epithelial cells, their physiological significance remains to be determined (3,4).

These cells have diverse origins: some develop in the thymus; some are derived from extraintestinal, extrathymic sources; and some may arise in the gut epithelium (5). The normal IEL in the blood probably gain access to the intestinal mucosa (6), and injected peripheral T cells can fill the epithelial compartments and acquire the characteristics of IEL in adequate conditions (5,7). αEβ7-Integrin is expressed on 90% of the IEL and it has therefore been said that αEβ7 mediates the binding of IEL to epithelial cells (8,9). Its epithelial ligand has been identified as E-cadherin, a membrane protein previously known for its role in the homophilic interactions of adherent junctions (10).
Surveillance for foreign antigens is a crucial function of the immune system, and the trafficking of lymphocytes from the blood stream into and through lymphoid organs is a receptor-regulated process essential for normal immune surveillance in mammals (11,12). Recent in situ microscopy experiments with Peyer’s patches have demonstrated that lymphocyte homing involves organ-specific multistep cascades of adhesion and signaling events in specialized blood vessels called high endothelial venules (HEV). Bargatzke et al. found that the initial attachment of naïve cells in Peyer’s patches is L-selectin-dependent, and that established rolling involves both L-selectin and α4β1-integrins that interact with mucosal addressin cell adhesion molecules (MAdCAM)-1 (13). Lymphocyte homing to peripheral lymph nodes may be mediated in a similar multistep adhesion cascade in which the first step, initiated by L-selectin, is followed by a G-protein-coupled activation event and activation-induced engagement of LFA-1 (14).

Tissue-specific homing is thought to control the extent and scope of immune responses, and to account for the regional compartmentalization of immune system function. Most memory and effector lymphocytes can also access and recirculate through extralymphoid immune effector sites, such as the intestinal lamina propria, the pulmonary interstitium, and the mesenchymal compartment of the skin. However, their exact migration pattern in lymphoid organs and non-lymphoid tissues in different organs has not been fully understood. Although the final stage in the migration of the normal IEL to the intestinal epithelial compartment might be mediated by their characteristic interaction of αEβ7-integrin with E-cadherin, how they reach the microvessels of the intestinal villi is not known, and there have been few direct in vivo observations of normal IEL migrate into the intestinal lymphoid area (Peyer’s patches) and villus mucosa.

We therefore used the intravital microscopic procedure for monitoring lymphocyte migration so that we could determine the migration of IEL into different compartments of the intestine (Peyer’s patch and villus mucosa), assess the differences between the ways different populations of IEL (CD4+ and CD4– TCRβ+ and TCRγδ) interact with the endothelium of the intestinal mucosa and investigate the possible contribution of various adhesion molecules to these adhesive interactions.

Methods

Collection and separation of lymphocytes
Female BALB/c mice and male C57BL/6 mice at 6–10 weeks old were used. They were purchased from Charles River Japan (Tokyo, Japan) and maintained on a standard laboratory chow diet, and their care and use were in accordance with the National Institute of Health guidelines for the care and use of laboratory animals. Mesenteric lymph node (MLN) lymphocytes (MLNL) were isolated from mesenteric lymph nodes by mechanical dissociation and red blood cells were lysed in an ammonium phosphate/chloride lysis buffer. The T cell-rich fraction of MLNL was obtained by using a nylon-wool column. After 2×107 cells in 3 ml of RPMI 1640 medium (Gibco, Grand Island, NY) with 1% FCS were incubated in 1 g of nylon wool (Wako Pure Chemical, Osaka, Japan) in a column for 1 h at 37°C, the passed fraction was designated the T cell fraction.

IEL were isolated using procedures slightly modified from those described previously (17). Briefly, inverted intestine was cut into four segments and the segments were transferred to a 50 ml conical tube containing 45 ml of 5% FCS in Ca2+, Mg2+-free HBSS (Gibco). After the tube was shaken at the horizontal position in an orbital shaker for 45 min at 37°C, cell suspensions were collected and passed through a glass-wool column to remove cell debris and adherent cells. The cells were then resuspended in 30% (w/v) Percoll (Pharmacia Biotech, Uppsala, Sweden) and centrifuged for 20 min at 1800 r.p.m. After this centrifugation, the cells at the bottom of the solution were subjected to Percoll discontinuous-gradient centrifugation and IEL were recovered at the interface of 44% and 70% Percoll. Cell suspensions (MLNL and IEL) were washed and until used were stored on ice in RPMI (pH 7.4) containing 5% FCS.

In some experiments, IEL were separated into subpopulations of CD4+ and CD4– cells by using magnetic cell sorting (MACS). Briefly, IEL (1×107 cells) were suspended in 90 µl of PBS containing 0.5% BSA and 5 mM EDTA and were incubated with 10 µl of anti-mouse CD4 (L3T4)-labeled MACS microbeads (Miltenyi Biotech, Bergish Gladbach, Germany) for 15 min at 6°C. The suspension was then passed through a separation column (type MS; Miltenyi Biotech), which was placed in the magnetic field of a MACS separator. The magnetically labeled CD4+ cells are retained in the column, while the unlabeled CD4– cells passed through it. After the column was removed from the magnetic field, the retained CD4+ cells were eluted and the purity of each subpopulation was confirmed by flow cytometry. The purity of CD4+ cells was shown to be at least 96%. These cells were washed and resuspended in RPMI with 5% FCS on ice until used.

TCR β mutant (β–/–) mice and TCR δ mutant (δ–/–) mice have been described elsewhere (4,18). The β–/– and δ–/– mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and had the genetic background of (129/ola×C57BL/ 6)F1 mice. The protocol described above was also used to isolate IEL from these β–/– and δ–/– mice.

Lymphocyte labeling with fluorochrome
Carboxyfluorescein diacetate succinimidyl ester (CFDSE; Molecular Probes, Eugene, OR) was dissolved to 15.6 mM in DMSO and until the experiments. A small aliquot (300 µl) was stored under argon in a sealed cuvette at –80°C. Lymphocytes (1×107) were incubated in a CFSE solution (20 µl of the 15.6 mM stock solution diluted with 20 ml of RPMI) for 30 min at 37°C. The labeled lymphocytes were immediately centrifuged through a cushion of heat-inactivated FBS and washed twice with cold suspension medium. The cells were resuspended in 0.2 ml of the medium and used within 30 min.

Intravital observation of lymphocyte migration in murine villus mucosa and Peyer’s patches
After an i.p. injection of sodium pentobarbital (50 mg/kg), the abdomen was opened via a midline incision. The microcirculation of intestinal villi was observed from the mucosal surface and lymphocyte migration was observed. A 7-cm ileal segment was gently extended onto the plate and a longitudinal
incision along its anti-mesenteric border was made by using a microcautery. The intestine was kept warm and moist by continuous superfusion with physiological saline warmed to 37°C while the adjacent intestinal segment and mesentery were covered with absorbent cotton soaked with Krebs–Ringer solution. The behavior of fluorescently labeled lymphocytes in villus tips was visualized, as previously described (19), on the television monitor of a fluorescence microscope equipped with a silicon intensified target image tube camera with a contrast-enhancing unit (C-2400-08; Hamamatsu Photonics, Shizuoka, Japan). Epillumination was achieved with filters of excitation at 470–490 nm and emission at 520 nm. In this setting, the tip of each villus was observed as an oblique circle and arcade microvessels in villi were observed.

For the observation of Peyer’s patches, a 5-cm ileal segment ending at the cecal valve was chosen for observation and placed gently on a plastic plate. Two small incisions in the bowel wall were made with a microcautery and 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 10 cmH₂O with warm Krebs–Ringer solution to minimize intestinal motility and obtain appropriate resolution. Suitable areas of the microcirculation in Peyer’s patch were observed through the serosa by using an inverted type fluorescence microscopy (Diaphot TMD-2S; Nikon, Tokyo, Japan) and were recorded by a television videotape recording system. Lymphocytes (1×10⁷ resuspended in 1 ml) were injected into a jugular vein for 3 min. The cell kinetics of infused lymphocytes and their interaction with the microvascular beds of intestinal villi or Peyer’s patches were monitored and recorded on S-VHS videotapes continuously for the first 20 min and then at 10-min intervals for 60 min. The lymphocytes adhering to but moving along the venular walls were defined as rolling lymphocytes. The adhering lymphocytes that remained motionless for >30 s were defined as sticking lymphocytes and the number of them in a 1-mm² area was observed on the video image. The distribution of lymphocytes at different depths was determined by adjusting the focusing plane, at 5-µm intervals, from the surface to a depth of ~50 µm.

Histological examination
The localization of infused IEL to intestinal mucosa was assessed immunohistochemically by using the LSAB (labeled streptavidin–biotin) method. After i.v. infusion of CFSE-labeled IEL, the small intestine was removed and fixed in a PLP (periodate, lysine–paraformaldehyde) solution. The intestine samples were then embedded in OCT compound (Miles, Elkhart, IN) before being frozen in dry ice and acetone. Six-micrometer cryostat sections were transferred to PLL-coated slides and air-dried for 1 h at 20°C. After they were washed in PBS (pH 7.4) containing 1% Triton X for 5 min, they were incubated in 5% normal goat serum in PBS (pH 7.4) containing 1% BSA and after the last one a cover slip was applied using glycerol jelly. Fluorescent preparations were examined using a laser-scan microscope (Carl Zeiss, Jena, Germany) equipped with an argon laser and an emission filter system providing sequential scannings at 488 nm for CFSE and 543 nm for rhodamine.

Administration of antibodies and pertussis toxin (PTX)
In some experiments, lymphocytes were reincubated with mAb that functionally block adhesion molecules. Antibodies against L-selectin (MEL-14), α₄-integrin (M290), β₇-integrin (FIB27) and CD11a (M17/4) were purchased from PharMingen (San Diego, CA), and antibody against α₂-integrin (PS/2) was obtained from ATCC (Manassas, VA). As a control, rat IgGa (R35-95; PharMingen) was used under the same conditions. Cells (1×10⁷) were incubated at 100 µg/ml of mAb for 30 min before the infusion of T lymphocytes. In some experiments, 2 mg/kg anti-MAdCAM-1 mAb (MECA-367; PharMingen) dissolved in 0.2 ml saline was infused into the jugular vein 30 min before the injection of T lymphocytes.

In another experiments, lymphocytes were incubated at 37°C with PTX (200 ng/ml; List Biological, Campbell, CA) for 2 h before injection in order to find out whether a PTX-sensitive signal transduction pathway might play an essential role in lymphocyte–microvessel interaction in vivo.

FACS analysis
The cell suspensions were washed in HBSS containing 0.2% BSA and 0.1% Na₂. This medium was used throughout the staining procedure and all cells were kept at 4°C during experimental procedures. For immunofluorescence staining, 2×10⁶ lymphocytes in 25 µl medium were first incubated with 1 µg of anti-mouse mAb to characterize and quantify adhesion molecules. Antibodies against mouse L-selectin (MEL-14, rat IgG2a), α₄-integrin (R1-2, rat IgG2b), α₂-integrin (M290, rat IgG2a), β₇-integrin (FIB27, rat IgG2a), CD11a (M17/4), CD3 (145-2C11, hamster IgG), CD69 (H1.2F3, hamster IgG) and CD45RB (16A, rat IgG2a) (PharMingen) were used for this study. After incubation for 30 min, the cells were washed in 400 µl HBSS and centrifuged 3 times for 5 min at 1500 g. They were then washed twice and resuspended for analysis. For controls, lymphocytes were reincubated with isotype-matched irrelevant antibodies. Positively stained cells were detected with FITC-conjugated anti-rat/hamster IgG. Flow cytometric analysis was performed using FACSsort (Becton Dickinson, Mountain View, CA) and dead cells were excluded from analysis according to the results of a test for iodide dye exclusion.

Statistics
All results were expressed as means ± SEM. Differences among groups were evaluated by one-way analysis of variance (ANOVA) and Fisher’s post hoc test. Statistical significance was set at P < 0.05.

Results
Migration of IEL and MLNL in villus mucosa and Peyer’s patches
We investigated the migration of IEL and MLNL to the villus mucosa by the observation from the mucosal side. The IEL
Intraepithelial lymphocyte migration

Fig. 1. Representative image of the distribution of CFSE-labeled MLNL (A) and IEL (B) adhered to the arcade microvessels of villus tip of the ileal mucosa 20 min after infusion. (C) Higher-magnification image of IEL adhered to arcade microvessels (arrow) of villus tips (arrowhead) (×20). (D) The time-course of changes in T lymphocyte sticking in microvessels of villus mucosa between IEL and MLNL. The lymphocytes located both inside and along venules were counted in the 1-mm² observation field. *P < 0.05 as compared with MLNL. Values are means ± SEM for six animals. Bar represents 100 µm.

used in the migration studies comprised four subpopulations (65% CD4⁺CD8⁺ cells, 8% CD4⁺CD8⁻ cells, 22% double-negative cells and 5% double-positive cells), and the MLNL were 70% CD4⁺CD8⁻ cells and 16% CD4⁺CD8⁺ cells. The IEL expressed both CD45RBhigh (68%) and CD69 (83%) on their surface, whereas in MLNL there was little expression of CD69 (4%) in spite of expression of CD45RBhigh (38%). The numbers of lymphocytes which had entered villus microvessels within 10 min after the infusion did not differ significantly between IEL (19.8 ± 3.2/min) and MLNL (17.8 ± 1.5/min). Figure 1(A and B) show the microscopic picture of T lymphocytes adhered to the arcade microvessels of villus tip in the ileal mucosa. As shown on the left (Fig. 1A), 20 min after injection some MLNL had accumulated in the microvessels of villus tip. As shown in the Fig. 1(B and C), on the other hand, the number of IEL accumulated in this area was significantly greater than that of MLNL accumulated there at that time. The IEL adhered to the capillaries of villus mucosa without rolling. Some were also observed at the base of crypt, but there were few IEL inside the submucosal venules (data not shown). Figure 1(D) illustrates the time-course change of the number of sticking T lymphocytes in villus mucosa. The number of IEL accumulated in the microvessels of villus tips was increased especially rapidly within 10 min, whereas the adherence of MLNL to microvessels of villus tips was often transient and these cells were frequently detached. The number of sticking IEL in villus mucosa was 22.5 ± 2.1 cells/mm² at 20 min, while at the same time the number of sticking MLNL was only 6.4 ± 1.4 cells/mm². The surgical preparation necessary for intravital observation of villus microvessels could affect lymphocyte accumulation, but in our preliminary study when we injected labeled IEL into intact animals and evaluated their accumulation after 60 min, their accumulation in villus mucosa was not significantly different from that of continuously observed animals (28.5 ± 3.6 cells/mm² in observation of intact animals 1 h later versus 27.3 ± 4.2 cells/mm² in continuous observation).

Figure 2(A and B) shows microscopic image of the distribution of sticking cells in postcapillary venules (PCV) of Peyer’s patches 20 min after infusion of IEL and MLNL. The number of lymphocytes which enter PCV of Peyer’s patches was not significantly different between IEL (16.5 ± 3.3/min) and MLNL (17.8 ± 2.2/min) at 10 min after infusion. More than half of the MLNL (57.0 ± 8.0% at 10 min) showed ‘rolling’ behavior in PCV, before they selectively adhered to the PCV of Peyer’s patches (Fig. 2A). The percentage of rolling lymphocytes of IEL, in contrast, was only 5.2 ± 2.0% and, as shown in Fig. 2(B), few sticking IEL were observed in this area. Figure 2(C) compares the time-course of the change in the number of cells sticking to PCV of Peyer’s patches between MLNL
and IEL. Lymphocytes both inside and along microvascular walls are included. The number of adhered MLNL gradually increased and reached 31.8 ± 6.2 cells/mm² at 40 min. On the other hand, few IEL adhered to PCV (2.4 ± 2.0 cells/mm² at 40 min) and their number did not significantly increase during the observation period.

Figure 3 illustrates the inhibitory effect of the function blocking of adhesion molecules on the sticking of IEL to arcade microvessels of villus mucosa at 20 min. The number of sticking lymphocytes in the control group was 24.0 ± 2.0 cells/mm², while the number was significantly less after pretreatment with a mAb that blocks β7-integrins. Preinfusion of anti-MAdCAM-1 antibody to mice also significantly inhibited these IEL interactions. Moreover, the combined blocking of β7-integrin with MAdCAM-1 further attenuated the sticking of IEL in this area, although it did not completely block the IEL adherence. On the other hand, antibodies either against L-selectin, α4-integrin, αE-integrin or CD11a did not significantly inhibited the IEL accumulation. Although the adhesion of IEL was not completely eliminated by the PTX treatment, it was decreased significantly (~42%) to 14.0 ± 2.0 cells/mm² (data not shown in Fig. 3). The number of lymphocytes that entered villus microvessels did not differ significantly between different treatment groups at 10 min after infusion (control, 21.2 ± 2.9/min; anti-β7-treatment, 19.8 ± 3.5/min; anti-MAdCAM-1 treatment, 20.4 ± 4.2/min; and anti-β7 with anti-MAdCAM-1 treatment, 18.8 ± 3.7/min).

To examine adherence in microvessels of intestinal villi, we compared the adhesion site of infused FITC-labeled IEL and the location of MAdCAM-1 molecules. MAdCAM-1 was found in the endothelium of venules in the lamina propria of intestinal villi as shown in Fig. 4 by rhodamine fluorescence. However, MAdCAM-1 was not uniformly found in the microvessels of villi: only some were MAdCAM-1⁺. After infusion of CFSE-labeled IEL, these cells were also found in the lamina propria of villi. It has been demonstrated that the adhesion site of some infused IEL (~38%) coincided with the presence of MAdCAM-1 in the lamina propria microvessels (Fig. 4A–C), but other cells did not correspond to the MAdCAM-1 expression sites as determined by a laser-scan microscope (Fig. 4B–D).

Migration of IEL subpopulations in villus mucosa
We also examined whether different subpopulations of IEL showed different migratory properties in villus mucosa. When IEL were separated into CD4⁺ and CD4⁻ cells using MACS, 90% of CD4⁺ IEL were found to be CD3⁺ cells and 2% to be slg⁺ cells. About 34% of this population were CD4⁺CD8⁻
Intraepithelial lymphocyte migration

Fig. 3. The inhibitory effect of function blocking of adhesion molecules on the sticking of IEL to arcade microvessels of villus mucosa at 20 min. The effect of mAb against L-selectin (MEL-14), α4-integrin (PS/2), αE-integrin (M290), CD11a (M17/4), β7-integrin (FIB27) and MAECAM-1 (MECA367) on the sticking of IEL was investigated. The IEL were treated with mAb (100 µg/ml) against L-selectin, α4-integrin, αE-integrin, CD11a or β7-integrin before infusion. In some experiments, the animals were pretreated (30 min before lymphocyte infusion) with a mAb against MAECAM-1 (2 mg/kg). The effect of functionally blocking both β7-integrin and MAECAM-1 molecules was also examined. *P < 0.05 as compared with controls. **P < 0.05 as compared with β7-integrin- or MAECAM-1-blocking alone. Values are means ± SEM for six animals.

Fig. 4. Representative pictures of simultaneous observation of CFSE-labeled IEL (green fluorescence) and MAECAM-1 expression (red fluorescence) in small intestinal villi as determined by immunohistochemistry. The PLP-fixed sections 60 min after lymphocyte infusion were observed by using a Carl Zeiss laser-scan microscope equipped with an argon laser (488 nm excitation for CFSE and 543 nm excitation for rhodamine). The primary antibodies used in the immunostaining were mAb to MAECAM-1 (MECA367). These sections were treated with rhodamine-conjugated anti-rat IgG antibody. The adhesion sites of some infused IEL coincided with the MAECAM-1 expression in the lamina propria vessels. The coincidental localization of IEL with MAECAM-1 expression was shown by yellow fluorescence (as indicated arrows) in (A)–(C). However, the other cells did not correspond to the MAECAM-1 expression sites as shown by green fluorescence in (B)–(D).
always showed a strong expression of αβ-integrin and a weak expression of γδ-integrin in IEL and MLNL was determined by fluorescence microscopy. As shown in Fig. 6, the isolated IEL always showed a strong expression of αβ-integrin, but no expression of L-selectin molecules and different state of activation in these lymphocytes. Phenotypes of MLNL (L-selectin\(^{+}\), α4β\(_7\)med and LFA-1\(^{+}\)) appear to be favorable to enable molecular cooperation with high endothelial venules of Peyer’s patches. The lack of L-selectin and low expression of α\(_{4}\)-integrin molecules on IEL may be responsible for the very few IEL interacting with Peyer’s patch HEV. Lymphocytes from L-selectin knock-out mice show impaired homing to Peyer’s patches (21) and it appears likely that L-selectin plays a primary role in the initiation of lymphocyte contact (rolling and adherence) with Peyer’s patch HEV in association with αα-integrin (22). Bargatze et al. (13) have demonstrated that MadCAM-1 is involved in both α4β\(_7\)-dependent and L-selectin-dependent interactions. The significant inhibition of adhesion of IEL, which lack L-selectin, to Peyer’s patches strongly supports the requirement of the multimolecular adhesion cascade in this site.

On the other hand, we demonstrated that significantly more IEL than MLNL adhere to the microvessels of intestinal villi. The reason for this difference remains unknown, but the lesser adhesion of MLNL may be due to their larger percentages of naive cells. Moreover, the significant adhesion of IEL to villus microvessels may be due to the strong expression of β\(_7\)-integrin on their surface. Lamina propria vessels in the intestine express MadCAM-1, yet are thought to lack L-selectin-binding carbohydrates (23). The direct binding of α\(_{4}\)β\(_7\) to MadCAM-1 seems to help explain the distinctive gut-homing properties of mucosal immunoblasts (24,25), but the contamination of α\(_{4}\)-cells in IEL was negligible and the involvement of α\(_{4}\)-integrin in IEL migration was also excluded in this study. Our intravital observation showed that antibodies against MadCAM-1 and β\(_7\) significantly abrogated the IEL migration, suggesting the involvement of these molecules in this process, but it does not indicate direct binding of β\(_{7}\)-MadCAM-1 molecules. Since there was an additive inhibitory effect of anti-β\(_{7}\)-integrin and anti-MadCAM-1 antibody on IEL adhesion, there is a possibility that these antibodies target different pathways. Several studies have shown that α\(_{4}\)β\(_{7}\) is not a ligand for MadCAM-1 and α\(_{4}\)β\(_{7}\) is not thought to participate in lymphocyte–endothelial cell interaction (26,27). Indeed, histologically most of the adhesion sites of infused IEL did not coincide with the MadCAM-1 expression in the lamina propria as determined by fluorescence microscopy. The counterligands for β\(_{7}\)-integrin or MadCAM-1 in IEL interaction with the villus mucosa remain to be identified. Moreover, it should be also noted that even the combination of both anti-β\(_{7}\)-integrin and anti-MadCAM-1 antibodies reduced the IEL adherence to microvessels of villi only to ~67% of what it

Discussion

The present study has demonstrated that the characteristics of the homing of IEL to small intestinal mucosa differed significantly from that of MLNL. The lymphocytes from different cell sources are postulated to show different migratory routes (20), and the significant difference between IEL and MLNL with regard to the lymphocyte trafficking in intestinal mucosa might be related to the differential expression of adhesion molecules and different state of activation in these lymphocytes. Intraepithelial lymphocyte migration in CD4\(^{+}\) cells was slightly greater than in CD4\(^{-}\) cells (data not shown).

Analysis by FACS

The expression of various adhesion molecules (L-selectin, α\(_{4}\)-integrin, αE-integrin, β\(_{7}\)-integrin and CD11a) on the surface of IEL and MLNL was determined by flow cytometric analysis using specific mAb. As shown in Fig. 6, the isolated IEL always showed a strong expression of α\(_{4}\)- and β\(_{7}\)-integrin molecules on their surface. There was also an expression of CD11a and a weak expression of α\(_{4}\)-integrin, but no expression of L-selectin molecules. On the other hand, MLNL significantly expressed α\(_{4}\)-integrin, CD11a and L-selectin molecules. There was also β\(_{7}\)-integrin molecule on surface of MLNL, but their expression was not stronger than that of IEL. α\(_{4}\)-integrin molecule was not significantly expressed on MLNL.

We also compared the adhesion molecules of different subpopulations of IEL. FACscan analysis revealed that the extent of expression of α\(_{4}\)- and β\(_{7}\)-integrin molecules did not differ significantly between CD4\(^{+}\) and CD4\(^{-}\) IEL or between TCRαβ IEL and TCRγδ IEL. The expression of LFA-1 molecules in CD4\(^{+}\) cells was slightly greater than in CD4\(^{-}\) cells (data not shown).

Fig. 5. (A) The time-course of changes in IEL sticking in microvessels of villus tip and the difference between CD4\(^{+}\) and CD4\(^{-}\) cells. *P < 0.05 as compared with CD4\(^{-}\) cells. Values are means ± SEM for three animals. (B) The time-course of changes in IEL sticking in microvessels of villus tip and the difference between TCRαβ IEL obtained from TCRβ\(_{2}\) mutant (β\(_{2}\)–) mice and TCRγδ IEL obtained from TCRβ\(_{2}\) mutant (β\(_{2}\)–) mice. *P < 0.05 as compared with TCRγδ IEL. Values are means ± SEM for three animals.

20 min, while at the same time fewer TCRγδ IEL had adhered (8.1 ± 1.5 cells/mm\(^{2}\)).
was in controls, suggesting that other mechanisms account for lymphocyte migration in this site. They may include the G-protein-independent mechanisms, such as capillary plugging.

The restricted distribution of αββ7 on lymphocytes led to the speculation that this integrin may be involved in lymphocyte migration to epithelial sites (28,29). Recently, αβ-integrin has been shown both entry and retention of αββ7 cells in the inflamed mucosa are necessary for the induction and maintenance of colitis (30). Since antibody against αβ did not significantly affect the initial step of lymphocyte adherence to microvessels of intestinal villi, it may function in the following migration from the vascular space toward the epithelial cell layer. Morphological studies have revealed that the basal laminae underlying the absorptive epithelium of the intestine contain numerous pores of various sizes through which lymphocytes migrate into or out of the epithelium (31,32).

Toyoda et al. recently investigated the subepithelial connective tissue of lamina propria in the rat jejunum under a scanning electron microscope and demonstrated that this reticular sheet also contains numerous foramina ranging with diameters from 3 to 7 μm, through which lymphocytes and macrophages passed (33). From these observations we can speculate that there is a pathway by which lymphocytes gain access to the intraepithelial space of intestinal villi after they leave the lamina propria venules.

In mice, IEL are distinct from other peripheral T cell population in that a large fraction of them (30–60%) bear TCRγδ. Recent findings have indicated a qualitative difference between two classes of murine IEL (αβIEL and γδIEL) with regard to their ability to modulate intestinal epithelial growth and/or immunological responses (3,18,34). In the present study we found by using TCR β chain and δ chain mutant mice that there is a significant difference in homing ability between these two classes of IEL. We also demonstrated that the adhesive capacity of the CD4+ population to villus microvessels is greater than that of the CD4- one. Since most of the CD4+ IEL expressed TCRαβ on their surface, one can speculate that most cells migrating preferentially to the villus mucosa are TCRαβ and CD4+ cells. However, it should be noted that FACS analysis showed no significant difference in surface expression of adhesion molecules between either the TCRαβ and TCRδγ population or the CD4+ and CD4- populations. This observation also supports the possibility that the IEL migration to intestinal villi is not absolutely regulated by β7-mediated interaction.

It is not known whether bone marrow-derived precursor IEL that reach the epithelium start there to rearrange their TCR genes and differentiate into mature IEL or complete all these processes somewhere else, most likely the intestinal mucosa, and then migrate into the epithelial layer. In mice, most TCRγδ IEL and many TCRαβ IEL use the CD8αβ homodimer instead of CD8αβ and develop somewhere in the intestinal mucosa, without passing through the thymus (3,35). In this regard, Ishikawa et al. have recently revealed that there are novel lymphoid tissues in murine intestinal mucosa, called crypt patches, which fulfill the criteria for precursor IEL development (36,37). On the other hand, Reinmann et al. have demonstrated that many donor-type CD4+ T cells migrate into the epithelial layer of the small intestine of scid mice and undergo striking changes in phenotype during the transit from the lamina propria into the epithelium (5). Many CD4+ T cells within the epithelial layer co-expressed the CD8 α chain on the cell surface. That is, they switched from the single-positive CD4+CD8- to the double-positive (CD4+CD8α+) phenotype. Furthermore, double-positive cells down-regulated expression of the α chain and up-regulated expression of the α chain (5).

Fig. 6. The expression of adhesion molecules (L-selectin, α4-integrin, αE-integrin, β7-integrin and CD11a) on IEL and MLNL as determined by flow cytometric analysis. Lymphocytes (2×10^6) were first incubated with anti-mouse mAb against L-selectin (MEL-14), α4-integrin (R-1-2), αE-integrin (M290), β7-integrin (FIB27) and CD11a (M17/4). They were then incubated with 1 ml FITC-labeled anti-rat IgG or anti-hamster IgG. Flow cytometric analysis was performed using FACSort (Becton Dickinson). Data were obtained using CONSORT software on viable cells, as determined by forward light scatter intensity. Representative data from four individual measurements are shown.
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Abbreviations

CFSE carboxyfluorescein succinimidyl ester
HEV high endothelial venule
IEL intraepithelial lymphocyte
MAdCAM mucosal addressin cell adhesion molecule
MLNL mesenteric lymph node lymphocyte
PCV postcapillary venules
PTX pertussis toxin

References

Intraepithelial lymphocyte migration

