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Intraepithelial Lymphocytes Traffic to the Intestine and Enhance Resistance to Toxoplasma gondii Oral Infection

Dominique Buzoni-Gatel,†* Hajar Debbabi,‡ Magali Moretto,‡ Isabelle H. Dimier-Poisson,* Anne C. Lepage,† Daniel T. Bout,* and Lloyd H. Kasper†

Toxoplasma gondii Ag-primed intraepithelial lymphocytes (IEL) from the mouse intestine have been shown to be protective against a lethal parasite challenge when adoptively transferred into recipient mice. In the present study, we observed that Ag-primed IEL traffic to the intestine of naive mice following i.v. administration. Primed and CD8β+ IEL were the most efficient cells at homing to the host organ. In congenic mice, IEL migrated from intestine within several hours posttransfer. On Ag reexposure, the primed IEL return to the intestine where they enhance resistance as determined by reduction in the number of brain cysts. Treatment of recipient mice with anti-α4 and anti-αaa Abs partially inhibited IEL intestinal homing. The Ab treatment dramatically impaired resistance to a subsequent oral infection. These finding indicate that lymphocyte homing is an important parameter in establishing long term immunity to recurrent infection with this parasite. The Journal of Immunology, 1999, 162: 5846–5852.

The mucosal epithelial layer provides the interface between the external and the internal environments of the gastrointestinal tract. The intestine-associated lymphoid tissue serves as an immunological barrier against a wide range of infectious agents, including orally acquired parasites such as Toxoplasma gondii. The most conspicuous population of T cells associated with the mucosa of the small and large intestine are the intestinal intraepithelial lymphocytes (IEL). Most of the IEL express the CD8αβ phenotype that can be either CD8 heterodimeric αβ-chains or homodimeric α-chains. Of the CD8αβ population, ~40% are γδ TCR+ and 20% are αβ TCR. IEL provide a number of important immunological functions including cytokotoxic activity (1, 2); secretion of cytokines including IL-2, IL-3, IL5, TNF-α, TGF-β, and IFN-γ (3, 4); as well as modulation of epithelial cell death and regeneration.

Infection with T. gondii in humans and other mammals is acquired via oral ingestion of tissue cysts containing bradyzoites from infected meat or oocytes containing sporozoites from contaminated soil. Previous observations from our laboratories have demonstrated an essential role for intestine-derived mucosal immunity against this parasite. IEL isolated from orally infected mice exhibit Ag-specific CTL activity in vitro (5). Moreover, adoptive transfer of these Ag-primed IEL into the naive host protects against a lethal parasite challenge (6). Recently, we demonstrated that Ag-primed IEL provide long term protection following lethal parasite challenge as determined by reduced mortality and decreased number of brain cysts in the recipient. The protective IEL are CD8αβ, αβ TCR. Protection is partially dependent on the presence of intact γδ TCR as well as endogenous production of IFN-γ (6, 7) in the host. Increased expression of the activated memory T cell phenotype, in particular Ly-6C, was noted in the protective IEL cell population.

Activated T cells traffic to the intestine although the molecular mechanisms that allow for this migration are not fully appreciated. Several integrins and chemokines including crg-2 and MuMig may enhance the mobilization of CD8 T cells into intestinal mucosa (8–11). The αβγ integrin is strongly expressed by IEL (9, 12, 13). This ligand is involved in the binding of IEL to epithelial cells via interaction with E-cadherin (14, 15). The IEL deficiency associated with a lack of β7 expression would suggest that αββγ is required for entry and or retention of T cells in the intestinal epithelium (16, 17). T cell activation results in the accumulation of αβγ high cells in the mesenteric lymph node, lamina propria, and IEL compartment suggesting a role for this molecule in lymphocyte homing. Another integrin, αβγ, expressed in low frequency on IEL is evident on lamina propria lymphocytes and on ~50% of T cells. Activated lymphocytes expressing αβγ can bind to several receptors, the most prominent of which is MadCAM-1, a protein expressed by high endothelial venule in Peyers’ patches and mesenteric lymph nodes and the flat endothelium in the lamina propria (18, 19). Recent studies indicate that the interactions of αβγ and MadCAM-1 play a major role in lymphocyte homing to Peyers’ patches, lamina propria, and mesenteric lymph nodes (20).

In this study, we report that Ag-primed IEL traffic to a wide range of host organs following adoptive transfer by i.v. administration. Protection against lethal challenge is dependent on the ability of these lymphocytes to traffic to the intestine and other host organs.

Materials and Methods

Mice and parasites

Female 8–10-wk-old inbred CBA/J (H-2d) mice were obtained from Janvier Breeding Center (Le Genest St. Isle, France) and congenic Thy-1.1 and Thy-1.2 C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Strain 76K of T. gondii was used in this study. This strain produces large numbers of cysts containing bradyzoites in the brains of infected mice. Mice were infected perorally by intragastric gavage of cysts collected from the brains of infected mice. Cysts are maintained by passage every 2 mo into naïve mice. Brain tissue containing strain 76K cysts was
suspended in saline buffer, and the suspension was adjusted to contain 100 or 40 cysts in each 0.5-ml dose to infect the CBA/J or Thy-1.2 C57BL/6 donor mice, respectively. The recipient mice were intragastrically challenged with 100 cysts for CBA/J and 40 for Thy-1.1 C57BL/6 mice, 3 days following the IEL passive transfer.

**Isolation of IEL and subset purification**

IEL were isolated as previously described with modification (21). The small intestine was flushed with PBS and cut into 2-mm sections. After removal of the Peyer’s patches and fat, the intestine was divided longitudinally. The mucosa were scraped and dissociated by mechanical disruption on a stirring platform for 15 min in RPMI 1640 containing 4% FCS and 1 mM dithioerythritol. Tissue debris and cell aggregates were removed by passage over a glass wool column in RPMI 1640–4% FCS. The lymphocytes were obtained by centrifugation on a Ficoll layer (d = 1.077) and the cells were suspended in complete medium. Primed IEL were isolated at day 13 and day 9 after oral infection of the CBA/J and C57BL/6 donor mice, respectively. Control unprimed IEL were obtained from uninfected mice.

Splenocytes were prepared at the same time (day 13 after infection of CBA mice) from primed donors and were used as control cells in short term homing experiments.

IEL were resuspended in RPMI with 4% FCS and washed before separation. Thirty million cells were incubated with rat anti-mouse CD8β mAb (PharMingen, San Diego, CA) for 15 min on ice. The cell suspension was applied to a prewashed miniMACS (Miltenyi Biotec, Bergisch Gladbach, Germany) column in PBS plus 10% FCS. Both positive and negative IEL fractions were assayed. Cells were stained with FITC-conjugated rat anti-CD8β mAb and analyzed by FACS. This purification procedure produced a highly pure CD8β+ population (>98%).

**Immunofluorescence staining**

Cell suspensions containing 2 × 10^6 cells were added to 96-well plates and washed twice in PBS. The cells were resuspended in 50 µl of normal rabbit serum for 10 min at room temperature to prevent nonspecific Ab staining. The cells were then incubated for 1 h on ice in the presence of FITC-labeled rat mAb (1:1000 dilution) directed to Thy-1.2, CD8α, CDβ8. Cell surface phenotype of IEL were also assayed for expression of αβ and βαβ, the homing integrins. To accomplish this, αβ and βαβ molecules were tagged with the purified PS/2 from rat (American Type Culture Collection, Manassas, VA) and the 2E7 from hamster mAbs, respectively (gift from L. Lefrancois) (22, 23). PS2 and 2E7 were produced by culture of the hybridoma cells and purified by protein G affinity chromatography. After incubation with the latter, the appropriate FITC conjugate (anti-rat or anti-hamster) were used as a secondary reagent for detection. After staining, the cells were washed and fixed with 1% paraformaldehyde buffer and analyzed by FACScan (Becton Dickinson, Mountain View, CA) the following day.

**Short term homing**

Purified IEL (1 × 10^7) were labeled with 50 µCi of 35Ct per ml for 1 h at 37°C. Nonincorporated 35Ct was removed by centrifugation. After washing, 1 × 10^7 IEL were injected into the tail vein of recipient mice. The recipient mice were sacrificed 2 h after the transfer. Blood was collected, and intestine, lungs, kidneys, liver, spleen, brain, mesentric lymph nodes, and peripheral lymph nodes were removed. Peripheral lymph nodes removed comprised the superficial inguinal nodes, the brachial and popliteal nodes, the superficial cervical nodes, and the iliac lymph nodes. All the Peyer’s patches were collected from the intestine. Intestines were washed by flushing 20 ml of buffer. Organs were carefully homogenized in 3 ml of water-1% Triton X-100, and radioactivity was counted from all the organs in a gamma counter. Values were expressed as percentage of radioactivity recovered in the organ and the remaining body. Two mice were used in each experiment, and each experiment was repeated at least three times. Because of the spontaneous release, the chromium study could not last more than some hours. To study IEL trafficking after 2 h, a fluorescent assay was conducted from naive or 13-day-infected CBA/J mice were resuspended in PBS (1 × 10^7/ml) and stained with 5-(and -6-)carboxyfluorescein diacetate, succinimidy ester (CFSE) as described elsewhere (24). Briefly, aliquots of 1 × 10^7 cells were labeled with 5 mM CFSE for 15 min at 37°C. Labeling was stopped by adding cold PBS-10% FCS. Cells were washed twice with cold PBS-10% FCS and resuspended in PBS before injection. IEL (1 × 10^7) were injected i.v. into naive recipient mice. The recipient mice were sacrificed 24 h after the transfer and IEL were isolated from their intestine. Cell suspensions were also prepared from Peyer’s patches, mesentric lymph nodes, spleen, lungs, liver, kidneys, and heart.

Fluorescence from all these organs was analyzed by FACS. Six recipient mice were used in each group. Results are expressed as the percentage of tagged IEL recovered in the organs compared with the injected population.

**Long term homing**

Congenic C57BL/6 mice were also utilized to determine whether the adoptively transferred IEL persist in the recipient host mice. In this experiment, IEL from Thy-1.2 C57BL/6 mice were isolated at day 9 postinfection (pi) and transferred (2 × 10^6) into Thy-1.1 recipients. Three days after the transfer, mice were challenged. Homing was assessed by phenotypic analysis for Thy-1.2 expression in various organs including the intestine at increasing time points before or after the challenge. Control included a phenotypical analysis within the untransferred Thy-1.1 mice. Six mice were used in each group.

**Homing blocking**

IEL were collected either from infected CBA or Thy-1.2 mice. Chromium-labeled or unlabelled purified IEL were incubated simultaneously with 50 µg of anti-αβ and 50 µg of anti-αβ mAbs (30 min, 37°C). At the very moment of the tail vein injection into recipient mice (CBA or Thy-1.1), 400 µg of each purified mAb were added to the IEL suspension. Two hours after the transfer, mice that had received the 51Cr-labeled cells were killed, and intestine homing was assessed as previously described. CBA mice that were transferred with unlabeled cells were challenged and treated every 3 days until day 15 after the transfer with 200 µg of each Ab administered by i.p. injection. Thy-1.1 mice transferred with treated or untreated Thy-1.2 IEL received i.p. injection of mAbs as described above and were killed 8 days after the challenge. Their IEL and mesentric lymph nodes were isolated and analyzed for Thy-1.2 cells as previously described. Control groups included mice sham treated with irrelevant Abs (Sigma Chemical, St. Louis, MO, for rat and Pharamingen for hamster Ig), mice transferred with unprimed IEL and challenged, and mice untransferred and unchallenged but treated with the same amount of mAbs.

To study the consequence of trafficking blocking of endogenous cells, untransferred mice were challenged and injected in the same way with the two mAbs. In that case, control mice were challenged but not treated with the Abs. Whatever the group or the experiment, mice were sacrificed 1 month after the challenge and brain cysts were enumerated. Six mice were used in each group, and each experiment was performed three times.

**Results**

IEL traffic to the intestine and other organs following adoptive transfer

Our previous observations indicated that primed IEL were protective against parasite challenge when adoptively transferred into naive mice. A radioisotope trafficking study was done to determine whether Ag-primed IEL home to the intestine and other host organs. Two hours after the i.v. injection of 51Cr-labeled primed IEL (1 × 10^7), 22% of the total radioactivity was detected in the small intestine of the recipient mice (Fig. 1). Increased radioisotope activity was observed in the liver (22%), spleen (15%), lungs (24%), and kidneys (15%) but not within the central nervous system (<2%). The level of radioactivity was 2% or less in other immune compartments of the intestine including Peyer’s patches (<1%), mesenteric lymph nodes (2%), superficial lymph nodes (iliac, inguinal, brachial, and retrocervical), and blood. In comparison, Ag-primed splenocytes traffic preferentially to the spleen rather than the intestine. For example, following splenocyte transfer, most of the radioactivity was recovered in either the spleen (37%) or the lungs (35%); whereas only 5% of the splenocyte radioactivity was detected in the intestine (Fig. 1).

To explore the importance of Ag priming in relation to lymphocyte homing, IEL were isolated from the intestine of orally infected mice at day 13 pi, labeled with chromium, and adoptively transferred into naive mice. Approximately 22% of the total radioactivity was detected in the intestine of the mice that received primed IEL, whereas <10% of the total radioactivity could be recovered from the intestine of mice transferred with unprimed IEL (Fig. 2). In the spleen, ~20% of the total radioactivity could be observed in recipient mice. There was no significant difference...
Intestine-derived IEL and splenocytes were isolated from the same infected CBA mice at day 13 pi. IEL and splenocytes were incubated with \(^{51}\)Cr. Cells \((1 \times 10^7)\) were injected into recipient mice. Two hours after the transfer, radioactivity was measured in each organ. Homing to the intestine or other organs was compared between IEL and splenocytes. Results are expressed as the percentage of radioactivity recovered from the specific organ compared with the total radioactivity in the mouse. m.l.n, mesenteric lymph nodes.

These observations were confirmed by a fluorescent cell analysis. For this, IEL from naive and infected CBA mice at day 13 pi were isolated, labeled with CFSE, and administered by i.v. injection into naive recipient mice. Homing to receptor organs was assayed 1 day after adoptive transfer. Twenty percent of the primed IEL migrated to the intestine, whereas <10% of the unprimed IEL traffic to this organ (Table I). This confirmed that primed IEL displayed the more efficient intestine-selective trafficking pattern. The day after transfer, primed IEL could be recovered from the Peyer’s patches (17%) and mesenteric lymph nodes (20%) of the recipients. In comparison, <2% of the IEL could be detected in these organs at 2 h posttransfer. Unprimed IEL could be detected in the mesenteric lymph nodes 1 day after transfer (30%) but not in the Peyer’s patches (Table I).

In the radioactive counts observed in the other organs between mice receiving Ag-primed vs unprimed IEL.

To determine the preferential homing of specific CD8\(^{+}\) subpopulations to the intestine, IEL were isolated from infected mice and separated into their respective CD8\(^{+}\) and CD8\(^{-}\) subsets. We observed that both the CD8\(^{+}\) and CD8\(^{-}\) subsets home to the intestine following adoptive transfer via i.v. administration. There was, however, a preferential increase in the homing ability of Ag-primed CD8\(^{+}\) (26%) compared with primed CD8\(^{-}\) (17.5%) to the intestine (Fig. 3). Also noted was an increase in the number of CD8\(^{+}\) IEL in the spleen, liver, and lungs compared with CD8\(^{-}\).

Expression of \(\alpha_4\) and \(\alpha_e\) molecules and their role in the homing

The involvement of the integrins \(\alpha_4\beta_7\) and \(\alpha_4\beta_2\) in trafficking of the lymphocytes to the intestine was evaluated. For these studies, IEL were isolated at varying time intervals pi and assayed for ligand expression. An increase in the expression of \(\alpha_e\) molecule was observed on the purified IEL at day 3 pi. By day 13 pi, 90% of the IEL were \(\alpha_4^{1+}\) and 50% expressed \(\alpha_4^{2+}\) (Fig. 4). Our previous studies had indicated that optimal protection occurred when IEL were harvested from infected CBA mice at day 13 pi. Phenotypic analysis of the IEL subsets at day 13 revealed that \(\alpha_4^{1+}\) expression was increased in both CD8\(^{+}\) and CD8\(^{-}\) population. There was a corresponding decrease in the expression of \(\alpha_4\) in the CD8\(^{-}\). At day 13 pi, 81 and 60% of the primed CD8\(^{+}\) population were respectively expressing the \(\alpha_4\) and \(\alpha_e\) molecules and 58.5 and 84% of the primed CD8\(^{-}\) were respectively expressing the \(\alpha_4\) and \(\alpha_e\) molecules.

### Table 1. Detection of tagged IEL by fluorescent cell analysis

<table>
<thead>
<tr>
<th>% of IEL Recovered</th>
<th>Unprimed</th>
<th>Primed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestine</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Peyer’s patches</td>
<td>1.2</td>
<td>17</td>
</tr>
<tr>
<td>Mesenteric lymph nodes</td>
<td>30</td>
<td>20</td>
</tr>
</tbody>
</table>

*a IEL were isolated from infected (primed) or uninfected (unprimed) CBA mice, tagged with a CFSE fluorescent dye, and injected into recipients. Twenty-four hours after the transfer, a FACScan analysis was performed with different organs. Results are expressed as the percentage of tagged cells in the organs compared with the number of injected cells.

CD8\(\alpha\beta\) home preferentially to the intestine

In the radioactive counts observed in the other organs between mice receiving Ag-primed vs unprimed IEL.

These observations were confirmed by a fluorescent cell analysis. For this, IEL from naive and infected CBA mice at day 13 pi were isolated, labeled with CFSE, and administered by i.v. injection into naive recipient mice. Homing to receptor organs was assayed 1 day after adoptive transfer. Twenty percent of the primed IEL migrated to the intestine, whereas <10% of the unprimed IEL traffic to this organ (Table I). This confirmed that primed IEL displayed the more efficient intestine-selective trafficking pattern. The day after transfer, primed IEL could be recovered from the Peyer’s patches (17%) and mesenteric lymph nodes (20%) of the recipients. In comparison, <2% of the IEL could be detected in these organs at 2 h posttransfer. Unprimed IEL could be detected in the mesenteric lymph nodes 1 day after transfer (30%) but not in the Peyer’s patches (Table I).

In the radioactive counts observed in the other organs between mice receiving Ag-primed vs unprimed IEL.
To examine the functional role of \(\alpha_4\beta_7\) and \(\alpha_4\beta_2\) in lymphocyte trafficking, IEL were first incubated with anti-\(\alpha_4\) and anti-\(\alpha_6\) mAbs and then transferred into naive recipient mice. At 2 h posttransfer (Fig. 5), nearly 50% of the Ab-treated IEL were unable to home to the intestine as determined by radioactive count. Same results were obtained with anti-\(\alpha_4\) alone (data not shown).

Long term homing

A genetic approach was utilized to evaluate IEL homing in long term immunity to the parasite. For this, primed IEL from C57BL/6 Thy-1.2\(^+\) donor mice, infected 9 days before, were adoptively transferred into congenic C57BL/6 Thy-1.1\(^+\) mice. At that time, IEL from C57BL/6 mice displayed the maximum protective capacity (7). Cells from the isolated organs were recovered and analyzed by FACS for expression of Thy-1.2\(^+\). As shown in Fig. 6, at the time of parasite challenge (day 0) which corresponded to 3 days posttransfer, 8% of the purified IEL from the intestine of the recipient Thy-1.1 expressed Thy-1.2\(^+\). Also noted was a 10% increase of the Thy-1.2 phenotype in the mesenteric lymph nodes. At 3 days postchallenge, a decline in the expression of Thy-1.2\(^+\) type cells in all organs assayed was observed. At 8 days postchallenge, Thy-1.2\(^+\)-expressing cell populations increased in the intestine (16%) and the mesenteric lymph nodes (30%). Treatment of Ag-primed IEL with Ab to \(\alpha_4\) and \(\alpha_6\) inhibited the trafficking of these cells to the intestine at day 8 postchallenge (Fig. 7).

Inhibition of IEL trafficking increases susceptibility to parasite challenge

An inhibition assay was performed to determine whether \(\alpha_4\) and \(\alpha_6\) have a functional role in host protection against infection. For this study, anti-\(\alpha_4\) and \(\alpha_6\) Abs were administered to recipient mice 1 day before and every 3 days after adoptive transfer of Ag-primed IEL. Preliminary data indicated that exposure to anti-\(\alpha_4\) and -\(\alpha_6\) alone had a nominal effect on host susceptibility to infection. Protection was evaluated by enumeration of brain cysts in the survivors at 30 days postchallenge. As shown in Table II, Ab treatment directed at \(\alpha_4\) and \(\alpha_6\) abrogated the protection conferred by the transfer of primed IEL. In comparison, control mice that received primed IEL without blocking Ab were protected against the challenge as measured by the number of brain cysts.

To establish whether lymphocyte trafficking is important during primary infection, mice were treated with anti-\(\alpha_4\) and anti-\(\alpha_6\) Ab and challenged with an oral dose of parasites. As illustrated in Table III, treatment with the blocking Abs to these integrins significantly (\(p < 0.05\)) impaired resistance to primary infection. There was no effect of these Abs on either the CD4\(^+\) or the CD8\(^+\) population from the spleen, mesenteric lymph nodes, and intestine of uninfected mice.

Discussion

Our data suggest that lymphocyte homing is an important regulator of mucosal immunity to orally acquired \(T. gondii\). The process of lymphocyte homing can target immune effector cells to the site of microbial invasion. The integration and control of systemic immune responses depend on the regulated trafficking of lymphocytes (25). At least two integrin ligand molecules, \(\alpha_4\beta_7\) and \(\alpha_4\beta_2\), are associated with the lymphocyte trafficking response in this model, since blocking of these molecules increases host susceptibility to parasite challenge.

Once ingested \(T. gondii\) invades the intestinal epithelial cells and is disseminated to a variety of organs including muscle and the
central nervous system. Thus, an intact mucosal surface replete with Ag-primed immune cells is essential for long term protection against recurrent infection in all mammals. Previous studies in our laboratories have demonstrated that T. gondii-primed IEL, when passively transferred into naïve mice, confer complete protection against an orally administered lethal parasite challenge (6). We observed that splenocytes preferentially home to the spleen whereas adoptively transferred intestine-derived IEL traffic to the intestine, although increased numbers of these cells could also be found within the spleen and other organs posttransfer. The increased activity within the liver and lungs following i.v. administration of IEL is consistent with earlier observations (20). The increase in cell number within these organs can be explained by either the clearance of damaged cells or perhaps physiological recirculation that may occur within these organs (26).

Ag-stimulated lymphocytes display tissue-selective trafficking patterns (25, 27). Our studies indicate that primed IEL traffic efficiently to the intestine when administered by i.v. injection. Analysis with the fluorescent tagged IEL as well as the phenotype analysis within the congenic mouse model establish that adoptively transferred IEL could be recovered from the mesenteric lymph nodes and the Peyer’s patches posttransfer, although little recovery of the IEL was apparent at 2 h posttransfer. Recently, it has been shown by in vitro culture that IEL that migrate through the intestinal epithelial cell monolayer and settle among the enterocytes depart the monolayer within 24 h (28). Thus, the IEL that traffic to the intestine within 2 h may disseminate to other organs, in particular the Peyer’s patch and mesenteric lymph nodes. Similarly, IEL located within the spleen, lung, and liver may traffic to other immune compartments as well as mesenteric nodes and Peyer’s patch at a further time point, such as 24 h posttransfer. Although T. gondii-primed IEL can migrate from the intestine, our study in congenic mice illustrated that IEL traffic back to the intestine after Ag reexposure. It is this recirculation that is probably critical for the establishment of long term immunity to reinfection.

We observed that both primed CD8β⁺ and CD8β⁻ T cells traffic to the intestine although CD8β⁺ are perhaps more efficient. Previous observations indicate that it is the CD8 β⁺ T cell population that is responsible for increased survival against a lethal parasite challenge and the establishment of long term immunity. Memory T cells expressing αβ, or B220⁺ may exhibit up-regulation of αββ and are involved in intestine tropism as well as

Table II. Brain cyst load in mice treated with IELa

<table>
<thead>
<tr>
<th>IEL Treatment</th>
<th>Brain Cyst Load</th>
</tr>
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<tbody>
<tr>
<td>Unprimed</td>
<td>2181 ± 472</td>
</tr>
<tr>
<td>Primed, sham treated</td>
<td>720 ± 274</td>
</tr>
<tr>
<td>Primed, treated with anti-α4 and anti-α6</td>
<td>2142 ± 420</td>
</tr>
</tbody>
</table>

a Recipient CBA mice were treated with blocking anti-α4 and anti-α6 mAbs on the day before the transfer of 2 × 10⁷ primed IEL which were preincubated with the Abs. Along with the primed IEL, recipients received 0.4 mg of each Ab and were treated with 200 μg of the mAbs in the same way every 3 days for 15 days. Recipients were challenged 3 days after the transfer. Control groups included mice receiving primed IEL sham treated with irrelevant Abs and mice receiving unprimed IEL. The protection was assessed 1 mo after the challenge by brain cyst enumeration.

Table III. Brain cyst load in CBA micea

<table>
<thead>
<tr>
<th>IEL Treatment</th>
<th>Brain Cyst Load</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected, sham treated</td>
<td>3390 ± 900</td>
</tr>
<tr>
<td>Infected and treated with anti-α4 and anti-α6</td>
<td>5250 ± 353</td>
</tr>
</tbody>
</table>

a CBA mice were orally infected with cyst and treated with 0.2 mg of anti-α4 and anti-α6 mAbs every 3 days for 15 days. One month after the infection, brain cysts were enumerated. The control group was infected and sham treated with irrelevant Abs.

FIGURE 7. Recipient Thy-1.1⁺ C57BL/6 mice were injected the day before the transfer with the blocking anti-α4 and anti-α6 mAbs. Primed IEL were isolated from mice infected 9 days before and were incubated with anti-α4 mAb, anti-α6 mAb, and 51Cr simultaneously. Additional purified Abs were mixed to the cells just before the transfer into the tail vein. Mice were treated with mAbs every 3 days after the transfer. Mice were challenged 3 days after the transfer. The blocking efficiency was determined at day 8 after the challenge by performing a FACS analysis for Thy-1.2 expression among the purified IEL population.
immunity to enteric pathogens such as rotavirus (30–32). We observed that both CD8β − and CD8β −primed IEL express an α4 molecule, although CD8β + express this molecule in higher proportion. Our data suggest that it is the trafficking and recirculation of the CD8β + IEL that is responsible for long term protection.

It appears that at least two integrin ligands (α4 and αE) are required for the homing process. Interaction of the MadCAM-1 molecule on the mucosal surface with its integrin ligand, α4β7, on the lymphocyte allows for the selective recruitment of these cells to intestinal sites (18, 20, 33). We observed high expression of α4 on IEL isolated from the intestine of infected mice. Exposure of either the recipient mouse or Ag-primed IEL to anti-α4 mAb, in combination with anti-αE mAb partially blocked IEL intestine homing and increased susceptibility following parasite challenge. Treatment with anti-αE alone did not impair the trafficking of IEL, and as one-half of the IEL were expressing the α4 molecule at the time of the transfer, the blocking effect could not be complete. This suggested that other molecules are probably involved in the homing. Although MadCAM receptor can be identified among the sinus-lining cells closest to the lymphoid white pulp of the spleen, Ab to its ligand α4β7 was insufficient to prevent IEL trafficking to the spleen. This is consistent with the reports of others that demonstrate that blocking of α4 and other integrins (e.g. VLA-4, VCAM-1, and L-selectin) can inhibit lymphocyte trafficking into inflammatory tissue but has either no effect or causes a paradoxical increase on lymphocyte trafficking into the spleen (20, 34).

α4β7 is the predominant cell adhesion molecule on the surface of intestine-derived IEL. There is a substantial increase in the number of intestine IEL expressing α4 molecule after T. gondii infection. The predominant functional role of α4β7 is to retain lymphocytes within or closely apposed to epithelial cells. For IEL, α4β7 and E-cadherin interaction may be an important signaling event between T cells and epithelial cells. Ab cross-linking of α4β7 can trigger the TCR and provide a potent costimulation for IEL proliferation, cytokine secretion (36), and mucosal CTL activity against colorectal cancer cells (15, 37). Previous studies from our group demonstrate that it is the IEL isolated at day 13 postinfection that exhibit the greatest protective and CTL function against parasite-infected target cells. By FACs analysis, we observed that expression of the αE molecule was greatest among the primed IEL population. Synthesis of the αE subunit is induced by TGF-β cytokine (23, 35). This cytokine is abundant in the intestinal epithelial cells, especially those in the distal region of the villus, and induces αE synthesis in T cells on their arrival in the epithelial microenvironment. During this period, the αE subunit is down-regulated on IEL and almost replaced by αA as a partner for β7. Preliminary data in our laboratory indicate that the IEL obtained from orally infected mice produce substantial quantities of TGF-β.

There is potential importance for expression of these integrins in host immunity to this enterically derived pathogen. Since IEL are cytotoxic for T. gondii-infected enterocytes in vitro (5), αEβ7 may play an integral role in that interaction. Further, this ligand may be important in cell-cell signaling between the IEL and the enterocyte. The integrin could provide a means by which T cells directly influence fundamental aspects of epithelial cell function. Intestinal epithelial cells down-regulate IEL (38) and may be involved in the extensive intestinal hyperinflammatory response that we and others have observed in certain strains of mice (39, 40). IEL produce chemoattractant mediators or chemokines that may initiate or modulate the immune response against T. gondii at the mucosal level (41).

Taken together, our data suggest that Ag-primed IEL can traffic to the intestine and stimulate long term immunity to reinfection. The ability of these cells to traffic to the intestine is dependent on the expression of the appropriate integrins which if blocked increases susceptibility to parasite challenge. If α4 molecule seems involved in IEL trafficking as already described by others (20), the interaction of the α4 molecule with its receptor the E-cadherin appears necessary for the IEL to fully express their protective abilities. Further studies are currently under way to determine the mechanism by which these IEL regulate the host immune response to the parasite at the mucosal level.

References


