P-Selectin and P-Selectin Glycoprotein Ligand 1 Are Major Determinants for Th1 Cell Recruitment to Nonlymphoid Effector Sites in the Intestinal Lamina Propria

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Abstract

The recruitment of activated T cell subsets to sites of effector immune responses is mediated by homing receptors induced upon activation in secondary lymphoid tissue. Using an adoptive transfer model, the intestinal recruitment of CD4+ T cells activated with intraperitoneal antigen in complete Freund’s adjuvant was examined. The data demonstrate that activated CD4+ T cells recruited to intestinal Peyer’s patches (PP) and lamina propria (LP) up-regulate functional P-selectin glycoprotein ligand 1 (PSGL-1). Blockade of IL-12 inhibited functional PSGL-1 expression and reduced PP and LP CD4+ T cell recruitment by >40%. P-Selectin blockade reduced LP recruitment of activated cells by 56% without affecting PP recruitment. Studies of mice examined 3 d after adoptive transfer of differentiated T cell subsets revealed that Th1 but not Th2 cells were recruited to small intestine PP and LP. Mucosal addressin cell adhesion molecule blockade reduced Th1 recruitment to PP by 90% and to LP by >72%, whereas P-selectin blockade reduced Th1 recruitment to PP by 18% and Th1 recruitment to LP by 84%. These data suggest that IL-12–induced functional PSGL-1 expression is a major determinant for the recruitment of Th1 effector cells to noninflamed as well as inflamed intestine.

Key words: memory T cells • lamina propria • small intestine • interleukin-12 • leukocyte migration

Introduction

The participation of effector T cells in intestinal nonlymphoid tissue is critical for mucosal host defense. CD4+ lymphocytes that populate the intestinal lamina propria (LP)* are comprised of previously activated, memory-like T cells that make high levels of effector cytokine (e.g., IFN-γ, IL-4, or IL-10) but not IL-2 (1–3). As naive T cells do not typically migrate to the LP, effector cells must be recruited after activation elsewhere. Leukocyte recruitment involves a multistep process that includes selectin-mediated rolling and integrin-mediated attachment to endothelial surfaces. Attachment of α4β7 integrin to mucosal addressin cell adhesion molecule (MAdCAM)-1 is essential for optimal recruitment of activated T cells into intestinal lymphoid Peyer’s patches (PP) structures and nonlymphoid LP (4). Papers from Lefrancois and colleagues suggest that for CD8+ T cells, an α4β7-independent pathway of LP recruitment exists (5), and that β2 integrins (LFA-1) play an important role in intestinal LP and PP T cell recruitment (6). However, there are few papers that address the molecular mechanisms of selectin binding in migration of activated CD4+ T cells to the LP.

*Abbreviations used in this paper: APC, allophycocyanin; C2GalNAcT-I, core 2 β-1,6-N-acetylgalcosaminyl transferase I; Fus-T VII, fucosyltransferase VII; MLN, mesenteric LN; PLN, peripheral LN; PP, Peyer’s patches; PSGL-1, P-selectin glycoprotein ligand 1.
Functional ligands for E- and P-selectin on Th1 cells mediate recruitment to nonlymphoid tissue in inflamed joints, skin, and peritoneum (7–11). Expression of functional P-selectin glycoprotein ligand (PSGL)-1 is controlled through posttranslational activity of the enzymes, core 2 β-1,6-N-acetylglucosaminyl transferase I (C2GlcNAcT-I) and α-3 fucosyltransferase VII (Fuc-T VII; references 12–15). Expression of Fuc-T VII and C2GlcNAcT-I are enhanced through activity of the Th1-polarizing cytokine, IL-12 (13, 14, 16). As the LP is a major site for Th1 effector responses, we postulated that T cell activation in the presence of IL-12 would promote Th1 recruitment to the LP through selective expression of functional PSGL-1.

In this paper, we analyze the role of functional PSGL-1 in CD4+ T cell recruitment to the LP using antigen-specific adoptive transfer mice. We find a substantial reduction in recruitment of in vivo–activated cells to the LP in anti–IL-12 or anti–P-selectin–treated mice, and a virtual absence of LP recruitment in anti–P-selectin–treated mice given differentiated Th1 cells. These data assign important roles for IL-12 and functional PSGL-1 in the migration of activated T cells to nonlymphoid tissue in the intestine, and suggest that P-selectin and functional PSGL-1 are major determinants of Th1 recruitment to the LP.

Materials and Methods

Mice. BALB/c mice were obtained from The National Cancer Institute. DO11.10 mice carrying a transgenic TCR specific for OVA 323–339 peptide plus I-A^d (obtained from D. Loh, Hoffman-LaRoche, Nutley, NJ) were backcrossed to RAG-1 knockout mice (RAG-1−/−) from R. Coffman (DNAX Research Institute, Palo Alto, CA). All mice were bred and maintained in the specific pathogen-free facility at the Lakeside Veteran's Administration Medical Science Building or at Northwestern University Feinberg School of Medicine in accordance with guidelines of the Northwestern University Animal Care and Use committee.

Cell Isolation. Spleen, mesenteric LN (MLN), or pooled peripheral LN (PLN; brachial, axillary, and inguinal) from individual mice were mechanically dissociated, respectively, and RBCs were lysed with ACK lysis buffer. Cell suspensions were washed and stored in DMEM containing 5% FBS on ice until used. Cells from the spleen, MLN, PLNs, LP, and PP were freshly isolated as described previously. For three or four color analysis, cells were stained with FITC-, PE-, and allophycocyanin (APC)-conjugated mAbs (all were obtained from BD Biosciences unless otherwise indicated). In some experiments, the cells were stained for 5% DMEM. The next day, the cells were incubated for 1 h at 37°C. Viable cells were obtained by suspending the cells in 0.3 μg/ml dihydroethidium (GIBCO BRL) in DMEM and centrifuging them over Nycoprep 1.077 (Accurate Chemical).

T Cell Enrichment and Generation of Th1 and Th2 Cell Lines. Freshly isolated spleen and MLN cells from DO11.10 × RAG-1−/− mice were resuspended in column buffer and passed through a T cell enrichment column (R&D Systems) according to the manufacturer’s instructions. To generate Th1 and Th2 cells, the enriched T cells were cultured with 1 μg/ml immobilized anti–CD3 and anti–CD28 (BD Biosciences) in a 24-well plate (Costar) at 2 × 10^6 cells/well for 2 d, followed by 6 d in a FACLON tissue culture flask (Becton Dickinson) at 2 × 10^6 cells/ml, in the presence of 10 ng/ml IL-12 (BD Biosciences) and 10 μg/ml anti–IL-4 (a gift from C. Reynolds, National Institutes of Health, Bethesda, MD) for Th1 cell differentiation, or IL-4 (R&D Systems) and anti–IFN-γ (XMG-1.2, a gift from S. Hurst, DNAx Corp, Palo Alto, CA) for Th2 cell differentiation. Cells were harvested on the eighth day of the culture, and prepared for adoptive cell transfer.

Adoptive Cell Transfer and Ag Challenge. The enriched naive DO11.10 T cells, Th1 cells, or Th2 cells were passed through Nitex filters to remove any debris, washed 3–4 times with PBS, and diluted with PBS in the appropriate concentration. Approximately 5 × 10^6 T cells were given i.v. tail vein injection (200 μl) to BALB/c recipients. Adoptive transferred BALB/c mice were challenged i.p. with 150 μg OVA 323–339 (University of North Carolina) in CFA (Sigma-Aldrich), or PBS/CFA as a control. In some cases, mice were treated i.p. with 150 μg OVA 323–339 and 100 μg LPS (Escherichia coli serotype 055:B5; Sigma-Aldrich) as described previously (17).

Blocking Antibodies. Anti–IL-12 mAb was purified from supernatant from clone 17.8 obtained from G. Trinchieri (Scribing-Plough Research Institute, Dardilly, France; reference 18). Hermes-1 rat isotype control hybridoma was obtained at the Developmental Studies Hybridoma Bank. MECA-367 (anti–MAC-1), 17.8, and Hermes-1 hybridomas were grown in a bioreactor (Heraeus) and as ascites and antibody purified with the Gammabind plus protein G column (Amersham Biosciences). The mouse E-selectin blocking mAb (9A9E3.F10, rat IgG2b) and the mouse P-selectin blocking mAb (RMP-1, a mouse IgG2a) were gifts from A. Issekutz (Dalhousie University, Halifax, Nova Scotia, Canada; references 19, 20). Isotype control mouse IgG2a (clone G155–178) and rat IgG1 control (R3–34) were purchased from BD Biosciences. Purified antibodies for in vivo use were checked for endotoxin by the limulus assay (Associates of Cape Cod) and found to be <1 EU/ml. For mAb treatment, 2 mg anti–IL-12 or control mAb was given i.p. 2 h before Ag challenge. 0.2 mg anti–MAC-1 or control Ab was given daily in the indicated experiments. The anti–P-selectin, anti–E-selectin, or control mAbs was given 0.3 mg i.p. 2 h before Ag challenge, followed by 0.2 mg i.p. on the third and fifth days after Ag challenge. In addition, a rat anti–mouse P-selectin mAb (RB40.34, rat IgG1) (a gift from K. Ley, University of Virginia, Charlottesville, VA) was used to confirm the result (see Fig. 2).

FACS® Analysis. Adoptive transferred BALB/c mice were killed three or seven days after Ag challenge. Cells from the spleen, MLN, PLNs, LP, and PP were freshly isolated as described previously. For three or four color analysis, cells were stained with FITC-, PE-, and allophycocyanin (APC)-conjugated mAbs for CD4, KJ1–26.1, and Thy-1, or isotype-matched mAbs (all were obtained from BD Biosciences unless otherwise indicated). In some experiments, the cells were stained for...
L-selectin, α4β7, or functional PSGL-1 expression in addition to CD4 and KJ1.26. Functional PSGL-1 expression was determined by P-selectin binding using a murine P-selectin/human IgM chimeric Ab (P-selectin-Ig; a gift from L. Stoolman, University of Michigan, Ann Arbor, MI), followed by APC-labeled anti–human IgM (Jackson ImmunoResearch Laboratories) as described previously (16). Parallel staining with control chimeric CD45-Ig or P-selectin–Ig was performed to rule out nonspecific binding. Reported staining was calculated as the percentage of positive cells. A subset of MLN DO11.10 cells (15%) expressed functional PSGL-1 and α4β7 expression. By comparison, 9% of MLN DO11.10 cells expressed functional PSGL-1 when mice were stimulated with i.p. OVA/CFA, 59% of DO11.10 cells recruited to the LP expressed functional PSGL-1, whereas 18% and 8% in the PP and spleen bound P-selectin–Ig, respectively. These results are consistent with normal BALB/c and C57BL/6 B6 mice, where 53–58% of endogenous LP CD4+ T cells were found to bind P-selectin–Ig (unpublished data). Together, these data suggest that IL-12 plays a role in the intestinal recruitment of activated CD4+ T cells.

To directly address the effect of IL-12 neutralization on surface expression of tissue homing receptors, cells were isolated from control and anti–IL-12 mAb–treated mice and analyzed by flow cytometry. Consistent with previous studies (7, 11), IL-12 neutralization reduced functional PSGL-1 expression by MLN DO11.10 cells by >50%. Data presented in Fig. 2 A indicate that IL-12 does not affect α4β7 up-regulation or L-selectin shedding. Interestingly, we detected a subset of MLN DO11.10 cells (15%) that expressed α4β7 and functional PSGL-1 when mice were stimulated with i.p. LPS and OVA323–339 peptide for 48 h. These results suggest that conditions that promote Th1 differentiation (other than CFA) also induce coexpression of functional PSGL-1 and α4β7 (Fig. 2 C). Together, the data in Fig. 1 D along with data in Fig. 2 (B and C) suggest that most cells in MLN and PLN up-regulate functional PSGL-1 upon activation with “Th1-promoting” CFA or LPS.

The Role of PSGL-1 in Recruitment of Activated CD4+ T Cells to the LP and PP. To directly test whether P–selectin and PSGL-1 were involved in activation-induced intestinal T cell migration, mice were treated with blocking mAb directed against tissue P-selectin (19). Data in Fig. 3 (A–C) suggest that P–selectin blockade reduced DO11.10 CD4+ T cell recruitment to the LP while increasing cells in the spleen and MLN. Analysis of the percent change in DO11.10 T cell yields indicates that anti–P–selectin mAb treatment increased numbers of DO11.10 T cells in the spleen and MLN by 14 ± 3% and 8 ± 3%, respectively, while decreasing DO11.10 cells in PP by 6 ± 2% (Fig. 3 C). In comparison, data show that anti–P–selectin Ab reduced LP DO11.10 cell numbers by 59 ± 3% (P < 0.001). Results in mice treated with anti–E–selectin mAb failed to show significant effects of E–selectin blockade in this model (Fig. S1 available at http://www.jem.org/cgi/content/full/jem.20020691/DC1). Together, these data suggest that a substantial number of activated T cells utilize P–selectin for intestinal LP migration.

The Role of P–Selectin in the Selective Migration of Th1 Cells to the PP and LP. To directly address the role of P–selectin in the intestinal recruitment of different differentiated T cell subsets, we studied the influence of anti–P–selectin mAb on...
localization of Th1 and Th2 cells to PP and LP. Differentiated Th1 cells were generated by activating naive cells with immobilized anti-CD3 and anti-CD28 mAbs followed by culturing for 7 d in IL-12 and anti-IL-4 or IL-4 and anti-IFN-γ as published previously (14). Data from mice examined 72 h after cell transfer suggest that differentiated Th1 DO11.10 cells were recruited to the small bowel LP in relatively high numbers compared with Th2 cells (Fig. 4, A and C). To determine whether accumulation of Th1 cells in the intestine was due to recruitment versus local expansion, mice were treated with anti-MAdCAM mAb (21). Consistent with previous findings, MAdCAM blockade reduced Th1 recruitment to PP by 82 ± 2% (P < 0.008) and to the LP by 71 ± 7% (P < 0.03) (Fig. 4, A and B). Results in anti-P-selectin mAb-treated mice indicate that P-selectin blockade reduced Th1 PP recruitment by 18 ± 4%
and Th1 LP recruitment by >83 ± 2% (P < 0.0003). Together, these data suggest that P-selectin is a major determinant of Th1 recruitment to extralymphoid sites within the intestinal LP.

Discussion

We present new information regarding the role of IL-12 and PSGL-1 in generating effector immune responses in the intestine. Administration of i.p. Ag in CFA induced migration of activated effector T cells to the intestinal PP and LP. In this model, DO11.10 cells are initially activated in the MLN and spleen, where they expand and up-regulate functional PSGL-1 (Fig. 1 D). Findings in anti–IL-12–treated mice suggest that PSGL-1 is not required for DO11.10 T cell activation or peripheral expansion. These data are consistent with findings by Xie et al. (7), who noted a similar lack of effect of anti–P-selectin and E-selectin mAbs on PP recruitment of Th1 effector cells. In contrast, our findings suggest that PSGL-1 is a major determinant of Th1 recruitment to extralymphoid sites within the intestinal LP.

The pathway described here provides a means for generating mucosal T cell responses to enteric antigen presented in draining LNs. The pathway permits the induction of immune responses to enteric Ag normally contained in mucosal tissue. Data from Huang et al. support the notion that mucosal dendritic cells carry enteric pathogen-derived antigen to MLNs, where they produce IL-12 and activate naive T cells (23). Given our findings, we suspect that IL-12 P-selectin mAb. Anti–P-selectin mAb significantly reduced intestinal LP recruitment of DO11.10 T cells activated with i.p. OVA/CFA (Fig. 3, A–C) and nearly abrogated recruitment of differentiated Th1 cells to the LP (Fig. 4, A and B). The relatively modest effect of P-selectin blockade on PP recruitment is consistent with data from Kunkel et al. (22), which suggested that P-selectin plays a minor role in recruitment of effector T cell recruitment to PP tissue. In contrast, the effect we observed for Th1 cell recruitment to the LP was pronounced in all the studies performed, suggesting that this pathway is an important means for recruiting Th1 effector cells to this compartment. Based on these data, we hypothesize that effects of IL-12 combine with TCR signaling to induce functional differentiation and expression of homing receptors needed for recruitment of effector T cells to sites of mucosal immune responses within the intestine.
released at the time of activation of naive T cells within MLN promotes Th1 functional differentiation and enhances functional PSGL-1 expression on cells that reenter the circulation. Binding of PSGL-1 to P-selectin slows the transit of activated cells through mucosal blood vessels and allows cells to sample endothelial surfaces for activating factors such as chemokines (24). These events enable the recruitment of appropriate Ag-specific populations of Th1 cells to the intestine where they deliver effector immune responses. It should be noted that data in Fig. 4 suggest that recruitment under these conditions need not require that Ag be present in the tissue. This allows memory T cells to seed distant sites within a host before reexposure to a mucosal pathogen.

The current data suggest that P-selectin rather than E-selectin mediates recruitment of activated T cells to the intestine under noninflamed conditions. Data from Granger and colleagues indicate that P-selectin, but not E-selectin, is expressed constitutively on endothelial cells throughout the intestine (23). Both may be induced with endotoxin and/or cytokine (TNF). Although enhanced P-selectin expression is long-lived (lasting several days), increased E-selectin expression tends to be short-lived (<8 h; reference 25). Blockade of P-selectin, but not E-selectin, re-
duces leukocyte rolling in states of ischemia/reperfusion (26), experimental colitis (27, 28), and radiation injury (29). Taken with our own, these data suggest that functional PSGL-1 and P-selectin play a greater role in recruitment of effector T cells to the intestine compared with E-selectin ligand and E-selectin.

A possibility not addressed in these previous works was whether PSGL-1 could bind E-selectin. Using PSGL-1−/− mice, McEvers and colleagues found that PSGL-1 may play a novel role in tethering leukocytes to E-selectin under flow (30). To address this issue, we examined the effect of E-selectin on recruitment of activated T cells to the intestinal LP. We found that blockade of P-selectin (Fig. 3), but not E-selectin (Fig. S1), reduced recruitment of activated DO11.10 T cells to the intestinal LP. Thus, these data are consistent with the notion that E-selectin does not play a major role in recruitment of activated Th1 cells to the LP in noninflamed mice and that PSGL-1 is not the major ligand for E-selectin. However, it remains possible that during states of intestinal inflammation (infectious or regional enteritis, ischemia, sepsis, etc.), E-selectin may increase in the intestine. Under these conditions, E-selectin may bind functional PSGL-1 or E-selectin ligand expressed by Th1 cells, thus, increasing intestinal recruitment of these effector T cell subsets.

The data presented suggest that T cells required a relatively prolonged period of activation and differentiation before recruitment to the intestine occurs. In previous studies by Iezzi et al. (31), it was suggested that nonpolarized cells generated by a short (24-h) antigenic stimulation are intermediate between naive and effector cells and migrate to peripheral lymphoid but not nonlymphoid tissue. In contrast, cells activated with Ag for longer periods (96 h) in the presence of differentiating cytokine become memory-like effector cells that preferentially migrate to nonlymphoid compared with lymphoid tissue. These findings suggest that the pattern of distribution of activated cells to peripheral tissue may change within relatively short periods of time. In the model presented here, DO11.10 T cells were activated with i.p. Ag in CFA. The use of CFA allows Ag to persist while inducing local IL-12 production by APC (32, 33), thus, promoting functional PSGL-1 and recruitment to the intestinal LP. Studies by Campbell et al. examined expression of homing receptors on DO11.10 T
cells after a limited, 48-h period of activation with OVA protein and LPS (17). These data suggested that short-term Ag priming with LPS and OVA protein generates distinct T cell subsets expressing either α4β7 or functional PSGL-1, but not both. In contrast, we found that a distinct subset of MLN DO11.10 T cells in adoptive transfer mice given i.p. LPS plus OVAα32-339 peptide express both α4β7 and functional PSGL-1 (Fig. 2 C). It is possible that repeated stimulation of cells with Ag and LPS (beyond 48 h) may have induced even greater numbers of cells expressing both α4β7 and functional PSGL-1. Together with data in OVA/CFA–treated mice, we suspect that prolonged periods of activation with Ag and differentiating cytokine (IL-12) generates populations of activated T cells analogous to the memory-like, effector cells described by Lanzavecchia and colleagues (31). Thus, the data presented fit with the emerging paradigm that selectin ligands mediate the recruitment of differentiated, memory-like effector cells to nonlymphoid compartments in tissue like the LP.

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