Blockade of LTB$_4$/BLT$_1$ Pathway Improves CD8$^+$ T-cell-mediated Colitis

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**Background:** Leukotriene B4 (LTB$_4$) has chemotactic properties for activated T cells expressing the high-affinity receptor BLT$_1$. This study investigated whether the LTB$_4$ antagonist (CP-105,693), selective for BLT$_1$ receptor, could protect mice from colitis mediated by specific cytotoxic CD8$^+$ T lymphocytes (CTL).

**Methods:** Virus-specific colitis was induced in C57Bl/6 mice transferred with lymphoid cells from P14 TcR Tg mice which are specific to class I GP33 peptide of LCMV. Mice were immunized with GP33-pulsed dendritic cells and colitis was elicited by intrarectal administration of the peptide. Colitis was evaluated by body weight loss and macroscopic and histological analysis of colon. In vivo priming of specific CD8$^+$ CTL was determined using interferon (IFN)-γ ELISPOT and in vivo CTL assays. In some experiments mice were treated with a selective LTB$_4$ receptor antagonist.

**Results:** Immunization with GP33-pulsed dendritic cells (DCs) induced priming of specific CD8$^+$ CTL, as shown by the presence of IFN-γ-producing CD8$^+$ T cells in colon draining lymph nodes and in vivo CTL assays. Intrarectal challenge with GP33 induced severe colitis and recruitment of granzyme B$^+$ P14 CD8$^+$ cells in colon. Treatment with the specific LTB$_4$ receptor antagonist before elicitation of colitis reduced the severity of colitis and decreased the frequency of specific effectors.

**Conclusions:** Colitis can be induced by IFN-γ-producing cytotoxic CD8$^+$ CTL specific for viral antigen. Blockade of the LTB$_4$/BLT$_1$ pathway by a selective BLT$_1$ receptor antagonist attenuates colitis by inhibiting CD8$^+$ effectors recruitment in colon. These data illustrate the therapeutic potential of LTB$_4$ receptor selective antagonists in protection from CD8$^+$ T-cell-mediated intestinal inflammation.

**Key Words:** colitis, LTB$_4$, CD8$^+$ T cells, BLT$_1$ antagonists

Inflammatory bowel disease (IBD), comprised of two clinically distinct entities, Crohn’s disease (CD) and ulcerative colitis (UC), are complex chronic disorders of unknown etiology. IBD pathogenesis likely involves a combination of genetic, environmental, and immunologic factors. Studies in animal models of colitis have emphasized that IBD is mediated by T cells, including CD4$^+$ T cells producing Th1 or Th2 type cytokines. Recent reports in experimental models of intestinal inflammation using immunocompetent mice indicate that self antigen-specific CD8$^+$ cytotoxic T lymphocytes (CTL) are pathogenic effector cells that are rapidly recruited into the lamina propria (LP) and epithelium and initiate the inflammatory process by performing cytolysis of epithelial cells.

Thus, while bystander activation of proinflammatory CD4$^+$ T cells could amplify the inflammatory process, CD8$^+$ effector T cells releasing granzyme B come first and could therefore represent a therapeutic target to prevent relapses.

Among the various proinflammatory soluble mediators that are active on the recruitment of effector and effector memory CD8$^+$ T cells from blood into tissues, leukotriene B$_4$ (LTB$_4$) seems to be of major interest. Indeed, active IBD is characterized by the coordinated release of a wide spectrum of soluble inflammatory mediators with an early production of lipid mediators (eicosanoids, leukotrienes, prostaglandins) and at later timepoints secretion of cytokines and chemokines, which require biosynthesis. Although colonic mucosa from IBD patients show increased concentrations of cysteinyl-leukotrienes (LTC$_4$, LTD$_4$, LTE$_4$) compared to those from normal subjects, the few studies investigating the effects of cysteinyl-leukotriene antagonists in experimental animal models of IBD have provided conflicting results on their efficacy. Interestingly, LTB$_4$ production is enhanced in colonic mucosa of IBD patients and is chiefly involved in chemotaxis.
of leukocytes.\textsuperscript{15–21} Originally described as a potent lipid myeloid cell chemoattractant, rapidly generated from innate immune cells, including monocytes, granulocytes, and mastocytes,\textsuperscript{22} which activates leukocytes through the G-protein-coupled receptor BLT\textsubscript{1},\textsuperscript{22–25} LTB\textsubscript{4} has the unique capacity to induce chemotaxis of effector and effector/memory CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells (but not naive T cells), which express the high-affinity receptor BLT\textsubscript{1}.\textsuperscript{7,26} Thus, LTB\textsubscript{4}/BLT\textsubscript{1} interaction has been recently reported to contribute to chemotaxis of Th1- and Th2-type T cells both in vitro and in vivo.\textsuperscript{7,26,27} Studies using BLT\textsubscript{1}-deficient mice (BLT\textsubscript{1}/−/) and administration of an LTB\textsubscript{4} receptor antagonist have documented that LTB\textsubscript{4} binding to BLT\textsubscript{1} on T cells induced rapid integrin-mediated arrest of rolling effector and central memory cells in postcapillary venules and promotes their migration to the inflamed peritoneal cavity.\textsuperscript{7} In addition, animal models of asthma have emphasized that LTB\textsubscript{4}/BLT\textsubscript{1} dictates the recruitment of CD8\textsuperscript{+} effector T cells into the lung, leading to development of asthma.\textsuperscript{28–31}

Since we and others have recently documented that colitis is initiated by rapid recruitment and expansion of specific CD8\textsuperscript{+} effector T cells into the intestine, we postulated that selective blockade of LTB\textsubscript{4}/BLT\textsubscript{1} interaction might limit CD8\textsuperscript{+} T cell chemotaxis in the colon LP and exert a protective effect on colitis. In the present study we developed a murine model of colitis, induced in C57Bl/6 mice by transfer of class I-restricted GP33\textsubscript{33-41}-specific CD8\textsuperscript{+} T cells expressing a transgenic (Tg) TcRV\textsubscript{8,1} TcR transgenic mice onto a B6 background expressing a V\textsubscript{a} TcR transgenic mice (L’Arbresle, France). P14 CD8\textsuperscript{+} T cells (but not naive T cells), which express the high-affinity receptor BLT\textsubscript{1}. To study the effect of a selective LTB\textsubscript{4} receptor antagonist (CP-693,105) on the outcome of CD8\textsuperscript{+} T-cell-mediated colitis. We showed that colitis is associated with recruitment of Tg CD8\textsuperscript{+} T-cell-producing granzyme B into the LP, and that treatment with the LTB\textsubscript{4} antagonist reduces the severity of colitis by reducing effector T-cell recruitment into the colon. These data indicate that the LTB\textsubscript{4}/BLT\textsubscript{1} pathway is involved in the migration of CD8\textsuperscript{+} effectors into the colon and that the LTB\textsubscript{4} antagonist may have important implications in the treatment of colitis.

**MATERIALS AND METHODS**

**Mice**

Male C57BL/6 (8–10 weeks old) mice were purchased from Charles River Laboratories (L’Arbresle, France). P14 CD8\textsuperscript{+} TcR transgenic mice onto a B6 background expressing a V\textsubscript{a}2/V\textsubscript{8} TcR-specific GP33\textsubscript{33-41} class-I H-2D\textsuperscript{b}-restricted TcR peptide of lymphocytic choriomeningitis virus (LCMV),\textsuperscript{32} were kindly provided by H. Pircher (Institute for Microbiology and Hygiene, University of Freiburg, Germany). All experiments previously approved by the Animal care and Use Committee of the Plateau de Biologie Expérimentale de la Souris (PBES) were performed in an accredited establishment according to governmental guidelines. All mice were fed with standard mice chow pellets ad libitum.

**Bone Marrow-derived Dendritic Cells (BM-DC)**

BM-DC were generated from bone marrow cells, as previously described.\textsuperscript{33} Briefly, bone marrow was flushed from the tibias and femurs of mice prior to erythrocyte lysis. After repeated washes, a single-cell suspension was plated at 2.10\textsuperscript{5}/mL per well in 24-well plates and was cultured at 37°C in the presence of complete RPMI 1640 medium supplemented with 40 ng/mL of recombinant mouse GM-CSF (Peprotech, Neuilly-sur-Seine, France). Half of the medium was renewed every other 2 days by fresh medium and GM-CSF. Cells were collected after 7 days and the quality of the BM-DC preparation was controlled by a double-color cytometry using staining with CD11c-PE and MHC class II-FITC. Generated cells were routinely composed of >80% MHC class II\textsuperscript{+} CD11c\textsuperscript{+} DC.

**Experimental Model of CD8\textsuperscript{+} TcR Transgenic P14-induced Colitis**

Pooled leukocytes from spleen, axillary, and inguinal LN from a Tg P14 mice donor (1.5.10\textsuperscript{6} cells) were intravenously (i.v.) adoptively transferred into C57Bl/6 hosts. Twenty-four hours later, recipient mice were immunized with BM-DC pulsed with 1 μM of the H-2D\textsuperscript{b} class-I GP33\textsubscript{33-41} (KAVYNFATM) peptide of LCMV (NeoMPS, Strasbourg, France) and lipopolysaccharides (LPS) (0.3 ng/mL) (Sigma-Aldrich, Saint-Quentin Fallavier, France), as described.\textsuperscript{34} After repeated washes, 1.5.10\textsuperscript{6} GP33\textsubscript{33-41}-loaded BM-DC (BM-DC/GP33\textsubscript{33-41}) in 200 μL of phosphate-buffered saline (PBS) were injected subcutaneously in the dorsal site of C57BL/6 mice. Unsensitized mice (controls) were subcutaneously (s.c.) injected with unpulsed BM-DC (BM-DC/0).

**Colonic Elicitation with the Peptide GP33\textsubscript{33-41}**

Mice anesthetized with ketamine/xylazine received an enema of 100 μL of GP33\textsubscript{33-41} (0.8 mg/mL) diluted in a solution containing 2 mg of 2,4-dinitrobenzene sulfonate (DNBS) in ethanol 50% (v/v). DNBS, a hapten with intrinsic irritant properties, was used to favor cell recruitment into colon.

**Assessment of Colitis**

Mice were examined daily with respect to their general condition, body weight, and consistency of stools. Forty-eight or 72 hours after elicitation of the delayed type hypersensitivity (DTH) the colon was removed, opened by longitudinal incision, and macroscopic damages were immediately assessed using a dissecting microscope. Macroscopic colonic lesions were analyzed in a double-blinded fashion using the well-validated Wallace score,\textsuperscript{35} taking
into account hyperemia, thickening of the bowel wall, extent of colonic inflammation, and presence of mucosal ulcerations. Microscopic damages were assessed on a segment of 1 cm of colon located 3 cm above the anal canal, after fixation in 4% paraformaldehyde and hematoxylin and eosin staining of paraffin-embedded sections (4 μm). Histological grading of colonic lesions, using the validated Ameho score,36 including thickness of the bowel wall, extent, and severity of the inflammatory cell infiltration and gland distortion, was performed by two investigators in a blind fashion.

In Vitro Differentiation of CD8+ Effector T Cells

CD8+ T cells from naïve C57Bl/6 mice were differentiated into T effector (T_{eff}) cells as described previously.37 Briefly, pooled cells from spleen, axillary, and inguinal LN were stimulated with coated anti-CD3 monoclonal antibodies (mAbs) (1 μg/mL) for 48 hours in RPMI 1640 medium. Cells were then washed and incubated in medium supplemented with 20 ng/mL of recombinant interleukin-2 (IL-2) (Roche Biomedical, Burlington, VT). Cells harvested at days 8–10 of culture contained >80% of CD8+ T cells, as determined by flow cytometry.

In Vitro Chemotaxis Assay

In vitro generated CD8+ effector T cells (10^6 cells) were added to upper wells of Corning Transwell microchemotaxis chambers (24 wells, 5-μm pore-size; Sigma-Aldrich, Lyon, France). Medium with or without LTB4 (10 mM) (TEBU-BIO, Le Perray en Yvelines, France) or CCL5 (RANTES) used as positive control (R&D Systems, Minneapolis, MN) was added to lower wells. In some experimental conditions, the high-affinity BLT1 receptor antagonist CP-105,69338 (kindly provided by H. Showell, Pfizer, UK) and the low-affinity BLT2 receptor antagonist LY-255283939 (Interchim Labs, Montluçon, France) were added to both upper and lower wells to determine chemotaxis in response to LTB4. After a 2-hour incubation at 37°C, cells that migrated to the lower wells were collected and then numbered. Chemotactic index was calculated as the ratio of the number of migrated cells in LTB4 containing wells divided by the number of cells that migrated to medium alone.

Interferon (IFN)-γ ELISPOT Assay

The frequency of GP33 33-41-specific IFN-γ-producing cells was determined by ELISPOT assay, as previously described,40 with minor modifications. Briefly, 96-well nitrocellulose microplate (MAHA; Millipore, Molsheim, France) wells were coated with anti-IFN-γ mAb (clone R46-A2, BD Pharmingen, Le Pont de Claix, France). Lymphoid cell suspension from spleen and colon draining lymph nodes, MLN, and caudal lymph node (CLN) were restimulated in vitro with 1 μM of GP33 33-41 or medium. After overnight incubation at 37°C, plates were washed three times with PBS-containing 0.05% Tween 20 and incubated for 2 hours at room temperature with a biotinylated anti-IFN-γ antibody (XMG 1.2, BD Pharmingen). IFN-γ-spot-forming cells (SFC) were revealed with a streptavidin peroxidase kit (Dako, Glostrup, Denmark) and AEC+H2O2. The number of IFN-γ SFC in each well was counted using a microscope and the results expressed as IFN-γ SFC / 10^6 cells.

In Vivo CTL Assay

In vivo analysis of GP33 33-41 specific CTL was assessed on day 7 after immunization using the previously described in vivo CTL assay41 with modifications. Immunized mice were injected intraperitoneally (i.p.) with a mixture of peptide GP33-pulsed and unpulsed naïve syngeneic spleen cells as targets, previously labeled with a low and high concentration of carboxy-fluorescein succinimidyl ester (CFSE), respectively. GP33 33-41-pulsed APC were prepared by 45 min incubation at 37°C with 1 μM of GP33 33-41 followed by labeling with 0.5 μM of CFSE (Molecular Probes, PooetGebouw, The Netherlands). Unpulsed targets were incubated with medium alone and labeled with 5 μM of CFSE (control targets). After extensive washing, a similar number (10.10^6) of Ag-experienced and unpulsed target cells was transferred i.v. into immunized or naïve recipient mice. MLN were harvested 24 hours after target cell transfer and 10,000 CFSE high cells were acquired using a FACSCalibur (BD Biosciences, San Jose, CA). Specific in vivo cytotoxicity was assessed by calculating the difference between the ratio (unpulsed/pulsed targets in sensitized BM-DC/GP33 versus naïve mice) – (unpulsed/pulsed targets in unsensitized BM-DC/0 versus naïve mice).

Selective LTB4 Receptor Antagonist

Mice were treated daily from day 5 to day 8 post-sensitization, with s.c. injections of the selective LTB4 receptor antagonist (CP-105,693). CP-105,693 is a potent and selective antagonist of LTB4 IC_{50} = 3.7 nM in an [3H] LTB4 human neutrophil binding assay.42 It has a long plasma half-life in humans and animals (≈400 hours).38 This compound was used at doses of 300 or 500 mg/kg diluted in PBS containing 0.25% carboxy-methylcellulose (CMC) and 0.6% Tween 80 (vehicle). Control mice were injected with vehicle alone.

Isolation of Cells from Colon LP

Lamina propria mononuclear cells (LPMC) were isolated from freshly colon specimens as described previously.4 Briefly, colon specimens were incubated for 40 minutes at 37°C in RPMI 1640 containing 1% FCS, 0.2 M
DTT, and 0.5 mM EDTA. To remove epithelial cells, the remaining tissue was further digested in RPMI 1640 supplemented with liberase (7 mg/mL) and DNase I (10 mg/mL) at 37°C in a shaking incubator. The LPMC released from the tissues was resuspended in complete medium.

**FACS Analysis**

Cells were stained using anti-CD45-PerCP-Cy5, anti-CD8-PE, CD44-FITC, Vα2-FITC, CD3-Cy5 mAbs (all from BD Pharmingen). Cells were then permeabilized with cytotoxix-cytopertin (BD Pharmingen) and stained with the mouse antihuman granzyme B-PE mAbs (Caltag, Buckingham, UK) or with appropriate mouse isotype control antibodies. Staining was analyzed by flow cytometry on a FACScan Becton Dickinson analyzer and CellQuest software (BD Biosciences).

**Statistical Analysis**

All analyses were performed with the statistical package GraphPad Prism (San Diego, CA). Values are given as mean and standard error of the mean (SEM) per treatment group. Differences between groups were analyzed using the nonparametric Mann–Whitney U-test or one-way analysis of variance (ANOVA) as appropriate. The Bonferroni multiple comparison test was used to generate P-values for selected pairwise comparisons and P < 0.05 were considered significant.

**RESULTS**

**P14 Model of Colitis Induced by GP33<sub>33-41</sub> Peptide-specific CD8<sup>+</sup> CTL**

We previously reported that CD8 T cells that recognize hapten-modified self peptides induced a colonic DTH response elicited by intrarectal challenge with DNBS in previously DNBS-sensitized mice. To determine whether class I-restricted CD8 CTL against a viral peptide could induce acute colitis, we set up a new model of colitis in C57Bl/6 mice transferred with lymphoid cells from P14 Tg mice, in which CD8<sup>+</sup><sup>T</sup> cells expressed a TcR<sub>ab</sub> specific for the GP33<sub>33-41</sub> class I peptide of LCMV both in coeliac (MLN) and caudal LN (5.10<sup>3</sup> SFC/10<sup>6</sup> cells) as well as in spleen (2.10<sup>3</sup> SFC/10<sup>6</sup> cells) (Fig. 1A). Direct in vivo CTL assay revealed around 30% of GP33<sub>33-41</sub>-specific CD8<sup>+</sup> CTL activity in MLN (Fig. 1B). Thus, DC immunization generates in vivo priming of specific CD8 CTL specific for the GP33<sub>33-41</sub> class I peptide of LCMV both in colon draining LN and spleen.

To determine whether GP33<sub>33-41</sub>-specific CD8<sup>+</sup> CTL were colitogenic, mice immunized with unpulsed or GP33<sub>33-41</sub>-pulsed DC were challenged intrarectally with GP33<sub>33-41</sub> peptide alone or together with DNBS (to recruit CD8 effectors to the colon, as previously documented<sup>14</sup>) or DNBS alone. No sign of colitis was evidenced in BM-DC/0-injected (unsensitized) recipients upon challenge with either DNBS or GP33 alone or a combination of GP33<sub>33-41</sub>/DNBS (Fig. 1C,D, left panel). In contrast, mice immunized with BM-DC/GP33<sub>33-41</sub> developed a severe colitis only upon challenge with GP33<sub>33-41</sub>/DNBS (Fig. 1C,D, right panel), but not after challenge with peptide or DNBS alone (Fig. 1C). Colitis manifested within 24–48 hours after challenge as a wasting disease with body weight loss (Fig. 1C) and colon inflammation with glandular hyperplasia, leukocyte infiltration in the mucosa, and submucosa and severe lesions with disruption of the mucosal wall, and sometimes erosions or ulcerations (Fig. 1D).

**BLT<sub>1</sub> Receptor Antagonist CP-105,693 Reduces the Severity of P14 Colitis**

Since the LTB<sub>4</sub>/BLT<sub>1</sub> pathway is critically involved in effector T-cell recruitment into inflamed tissues,<sup>7</sup> we investigated whether the selective BLT<sub>1</sub> receptor antagonist CP-105,693<sup>38</sup> could reduce the outcome and/or severity of colitis in the P14 Tg mouse model. C57Bl/6 mice injected with Tg P14 cells were treated daily from day 3 postimmunization until sacrifice with CP-105,693 or vehicle alone. Vehicle-treated mice developed a severe colitis with body weight loss (17.0 ± 9.4%) (Fig. 2A) and intense colon inflammation as revealed by macroscopic (Fig. 2B) and histological scores (Fig. 2C,D) at 48 hours postchallenge. In contrast, treatment with CP-105,693 resulted in a dose-dependent improvement of colitis, with a reduction of weight loss of 6.1 ± 3.3% and 3.4 ± 4.7% after treatment with 300 and 500 mg/kg of CP-105,693, respectively (Fig. 2A). The macroscopic score of colitis was not significantly affected by treatment with CP-105,693, although it tended to be reduced in mice receiving the highest dose of 500 mg/kg of the antagonist (Fig. 2B). In contrast, both doses of the antagonist significantly reduced the Ameho score (Fig. 2C,D). Indeed, histological analysis revealed that treatment with CP-105,693 reduced the severity of colon inflammation and reduced mononuclear cell infiltrate and...
In Vivo Blockade of the LTB4/BLT1 Pathway Reduces Recruitment of CD8\(^{+}\) Effectors in Colon LP

Since we previously documented that colitis is induced by early recruitment of CD8\(^{+}\) effectors into colon LP,\(^4\) we examined whether the protective effect of the antagonist on colitis resulted from reduction in CD8\(^{+}\) effector T-cell migration in colon. FACS analysis performed in leukocyte suspensions of colon LP revealed that V\(_{\alpha2}\) CD8\(^{+}\) cells represented less than 1% of CD45\(^{+}\) leukocytes in naïve C57Bl/6 mice injected 1 day before with more than one million of P14 transgenic lymphoid cells (not shown), indicating that Tg cells were not able to reconstitute the mucosal compartment in this experimental setting. Likewise, Tg CD8\(^{+}\) T cells were barely detectable after GP33\(_{33-41}\)-DNBS challenge in the colon LP of control mice injected with BM-DC/0 (not shown). In contrast,
CD45<sup>+</sup> leukocytes from colon LP of colitic mice harvested at 48 hours after GP33<sub>33-41</sub>-DNBS challenge contained around 10% of CD8<sup>+</sup> cells, among which 18.6 ± 9.5% expressed V<sub>a<sup>2</sup></sub>. Around half of these CD8<sup>+</sup>V<sub>a<sup>2</sup></sub><sup>+</sup> cells expressed granzyme B. Similar findings were obtained in vehicle-treated mice (Fig. 4C). These data indicated that colitis was associated with migration of P14 Tg CD8<sup>+</sup> CTL effectors into the colon LP. Treatment with CP-105,693 resulted in a dose-dependent decrease of CD8<sup>+</sup> CTL recruited into the colon. Indeed, the proportion of granzyme B-producing V<sub>a<sup>2</sup></sub><sup>+</sup> transgenic cells among CD8<sup>+</sup> T cells of colon LP decreased from 11.8 ± 9.0% in vehicle-treated mice to 4.7 ± 4.2% and 1.0 ± 1.0% in mice treated at doses of 300 and 500 mg/kg of CP-105,693, respectively (Fig. 3A,B). As shown in Figure 3C, 43 ± 12% of transgenic V<sub>a<sup>2</sup></sub> CD8<sup>+</sup> T cells in vehicle-injected mice expressed granzyme B as compared to 6.2 ± 5% in mice with the high dose of antagonist treatment. These data demonstrate that LTB<sub>4</sub>/BLT<sub>1</sub> blockade by treatment with the antagonist induced a dose-dependent decrease in effector CTL chemotaxis into the inflamed colon.

Blockade of the LTB<sub>4</sub>/BLT<sub>1</sub> Pathway Reduces CD8<sup>+</sup> T Cell Effector Chemotaxis In Vitro

We next examined the relative efficacy of LTB<sub>4</sub> antagonists, selectively binding to the low- (BLT<sub>2</sub>) and high-affinity (BLT<sub>1</sub>) receptors, to impair CD8<sup>+</sup> effector T-cell chemotaxis in response to LTB<sub>4</sub> in vitro. CD8<sup>+</sup> T-cell effectors were generated by culture of naive CD8<sup>+</sup> T cells with soluble anti-CD3 mAbs þ IL-2, as described. After 7–9 days of culture, 90% of the cells expressed a CD25<sup>+</sup>CD44<sup>+</sup>L-selectin<sup>−</sup>C<sub>0</sub> phenotype characteristic of effector/memory cells (not shown). CD8<sup>+</sup> effectors migrated in response to LTB<sub>4</sub> (10 mM) as efficiently as in response to the chemokine RANTES (2.5 μg/mL) used as positive control (Fig. 4). CD8<sup>+</sup> effector migration in response to LTB<sub>4</sub> was unaffected by addition of the BLT<sub>2</sub> receptor antagonist LY-255283, whereas it was almost completely inhibited by the BLT<sub>1</sub> receptor antagonist CP-105,693. This indicates that CD8<sup>+</sup> effector chemotaxis could be blocked by antagonist of the high- (BLT<sub>1</sub>) but not the low-affinity (BLT<sub>2</sub>) receptor for LTB<sub>4</sub>, both expressed on CD8<sup>+</sup> effectors.

DISCUSSION

We have documented in earlier studies that DNBS-specific colitis in Balb/C mice can be initiated by IFN-γ-producing CD8<sup>+</sup> T cells, which are recruited into the colon as granzyme B-producing effectors. In this study we describe a novel murine model of severe acute colitis which results from a colonic DTH response against an exogenous viral protein, and is mediated by MHC class I-restricted CD8<sup>+</sup> CTL. Our data show that C57Bl/6 mice can rapidly develop an acute and severe colitis mediated by
FIGURE 3. The BLT₁ receptor antagonist CP-105,693 reduces the recruitment of CD8 CTL effectors into colon LP. BM-DC/GP33₃₃-₄₁ immunized mice were either untreated (0 mg/kg) or treated daily from day 3 until sacrifice with 300 mg/kg or 500 mg/kg of CP-105,696 and challenged on day 7 with GP33₃₃-₄₁ and DNBS. The proportion of P14 Tg CD8⁻ effector cells recruited into the colon LP at 48 hours after challenge was analyzed by FACS analysis. (A) Representative dotplot analysis show the proportion of granzyme B⁻Va₂⁺ cells among gated CD8⁺ T cells in untreated mice (left panel) and mice treated with 300 mg/kg (medium panel) or 500 mg/kg (right panel) of CP-105,696. (B) Proportion of granzyme B⁻-producing TcRVa₂⁺ Tg cells among CD8⁻ T cells of colon LP at 48 hours after challenge in individual mice (5–7 mice/group) untreated (square) or treated with 300 mg/kg (triangle) or 500 mg/kg (circle) of CP-105,696. (C) Proportion of granzyme B⁻-producing TcRVa₂⁺ Tg cells among total Va₂⁻ Tg CD8⁻ T cells from the colon LP at 48 hours postchallenge in mice treated with 0 mg/kg (white bar), 300 mg/kg (gray bar), and 500 mg/kg (black bar). The results are representative of three experiments using each five to seven mice per group. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
MHC class I-restricted CD8⁺ CTL in the absence of specific CD4 T-cell help. Cytolytic CD8⁺ effectors generated in mucosal and systemic lymphoid organs by s.c. immunization can be recruited as granzyme B⁺ T cells in colon upon challenge with the specific peptide. Interestingly, contrary to other models of colitis generated by toxic effect of chemicals on the gut epithelium (such as sodium dextran sulfate or high dose of the hapten TNBS) or by increased gut permeability and mucus layer disruption by ethanol alone, colitis in our model is elicited by attraction of specific CD8⁺ T cells in colon upon challenge with a low dose of the hapten DNBS. In this model, IFN-γ-producing CD8⁺ CTL specific for hapten-modified self peptides migrated into colon upon challenge and rapidly induced colitis. These studies emphasized that in an immunocompetent host, CD8⁺ T cells may come first and that danger signals induced by apoptosis of epithelial cells may promote subsequent migration of inflammatory cells, including bystander Th1-type CD4⁺ T cells. Our present model of colitis confirms the prime role of CD8⁺ T cells in colitis and offers several advantages over the existing models of CD8⁺ T-cell-mediated colitis including 1) analyses of the effector phase of the pathogenic immune response leading to colon lesion; 2) dissection of innate and acquired immune mechanisms that take place entirely in an immunocompetent host; and 3) screening of drugs and biotherapies for their efficacy to prevent or treat relapses of IBD.

One important issue is whether CD8⁺ T cells may be targeted by selective drugs to prevent their rapid mobilization at sites of inflammation. Identification of a novel LTB₄ antagonist, which can prevent effector T cell and memory T-cell chemotaxis in response to LTB₄ rapidly secreted in inflamed tissues, suggests that blockade of the LTB₄/BLT₁ pathway may be of invaluable importance for the treatment of IBD relapses. Previous studies have confirmed that BLT₁ is induced on CD8⁺ effector T cells, and that the antagonist CP-105,693 inhibits activated CD8⁺ T-cell chemotaxis in inflamed tissue, by blocking the LTB₄/BLT₁ pathway. We show here that this LTB₄ antagonist,

FIGURE 4. In vitro blockade of CD8 effector T cell chemotaxis by the BLT₁ receptor antagonist CP-105,693. Effector CD8⁺ T cells (1.10⁶) were added to the upper wells of chemotaxis chambers in the presence of 10 mM LTB₄ or RANTES (as a positive control). Addition of BLT₁ receptor antagonist CP-105,693 or of the BLT₂ receptor antagonist LY-255,283 on CD8⁺ T cells migration in response to LTB₄ was compared. The chemotactic index was calculated by dividing the number of migrated cells exposed with LTB₄ by the number of cells that migrated spontaneously towards media alone. Results expressed as mean (±SD) are representative of two independent experiments. *P < 0.05.
CP-105,693, can protect mice against colitis and wasting disease by inhibiting CD8 effector migration into the colon mucosa. We also found a similar protective effect of CP-105,693 in our previously described model of CD8+ T-cell-mediated DNBS-specific colitis in Balb/C mice, in which treatment of sensitized mice prevented body weight loss, colon infiltration with mononuclear cells and histopathological lesions of colitis, and recruitment of CD8+ granzyeme B+ cells in colon (data not shown).

Several observations in IBD patients support that LTB4 represents a relevant therapeutic target. LTB4 production is enhanced in inflammatory colonic mucosa from IBD patients. Jupp et al11 found an increased number of cells expressing 5-lipoxygenase and leukotriene A4 (LTA4) hydrolase, an enzyme responsible for the LTA4 conversion into LTB4 in intestinal mucosal biopsies from IBD patients with active, but not quiescent disease. However, little is known regarding the contribution of the LTB4/BLT1 pathways in IBD.11,55,56

Our data are consistent with studies reporting significant effects of other LTB4 receptor antagonists, using different animal models of colitis generated by chemically induced disruption of epithelial permeability and gut damage. Guo et al56 showed in TNBS-induced colitis in rats that oral administration of ONO-4057 significantly ameliorated the drop in body weight and the elevation of colonic MPO activity. In spontaneous colitis occurring in cotton-top tamarins, long-term oral treatment with SC-53228 was safe and efficient to attenuate intestinal inflammation (assessed by weight change, stool consistency, and histologic lesions). Fretland et al57 investigated in several animal species (rat, guinea pig, and rabbit) the influence of locally administration of the BLT1 antagonist SC-411930 on acetic acid-induced colitis. They found that LTB4 receptor antagonist substantially ameliorates colonic inflammation. However, it is not likely that protection in these models was due to blockade of the LTB4/BLT1 pathway mediating chemotaxis of neutrophils and possibly monocytes, rather than T cells.

Although additional studies are required to investigate the efficacy of LTB4 receptor antagonists on memory T-cell responses during chronic colitis, our study highlights the importance of LTB4/BLT1 interactions in the early stage of progression towards IBD and supports that selective antagonists of the LTB4 receptor BLT1, which inhibits the migration of effectors and effector/memory T cells from peripheral blood into the colon, might represent a novel approach for the treatment of IBD.

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REFERENCES


