Gut Homing Receptors on CD8 T Cells Are Retinoic Acid Dependent and Not Maintained by Liver Dendritic or Stellate Cells

BERTUS EKSTEEN,* J. RODRIGO MORA,‡ EMMA L. HAUGHTON,* NEIL C. HENDERSON,§ LAURA LEE–TURNER,* EDUARDO J. VILLABLANCA,‡ STUART M. CURBISHLEY,* ALEX I. ASPINALL,‖ ULRICH H. VON ANDRIAN,¶ and DAVID H. ADAMS*

*Centre for Liver Research, MRC Centre for Immune Regulation, Institute for Biomedical Research, Medical School, University of Birmingham, Birmingham, United Kingdom; ‡Gastrointestinal Unit, Massachusetts General Hospital, Boston, Massachusetts; §Centre for Inflammation Research, Queens Medical Research Institute, University of Edinburgh, Edinburgh, Scotland; ‖Foothills Hospital, University of Calgary, Calgary, Alberta, Canada; and ¶Department of Pathology and the Immune Disease Institute, Harvard Medical School, Boston, Massachusetts

BACKGROUND & AIMS: Lymphocytes primed by intestinal dendritic cells (DC) express the gut-homing receptors CCR9 and $\alpha4\beta7$, which recognize CCL25 and mucosal addressin cell-adhesion molecule-1 in the intestine promoting the development of regional immunity. In mice, imprinting of CCR9 and $\alpha4\beta7$ is dependent on retinoic acid during T-cell activation. Tissue specificity is lost in primary sclerosing cholangitis (PSC), an extraintestinal manifestation of inflammatory bowel disease, when ectopic expression of mucosal addressin cell-adhesion molecule-1 and CCL25 in the liver promotes recruitment of CCR9+$\alpha4\beta7$+ T cells to the liver. We investigated the processes that control enterohepatic T-cell migration and whether the ability to imprint CCR9 and $\alpha4\beta7$ is restricted to intestinal DCs or can under some circumstances be acquired by hepatic DCs in diseases such as PSC. **METHODS:** Human and murine DCs from gut, liver, or portal lymph nodes and hepatic stellate cells were used to activate CD8 T cells. Imprinting of CCR9 and $\alpha4\beta7$ and functional migration responses were determined. Crossover activation protocols assessed plasticity of gut homing. **RESULTS:** Activation by gut DCs imprinted high levels of functional CCR9 and $\alpha4\beta7$ on naïve CD8 T cells, whereas hepatic DCs and stellate cells proved inferior. Imprinting was RA dependent and demonstrated plasticity. **CONCLUSIONS: Imprinting and plasticity of gut-homing human CD8 T cells requires primary activation or reactivation by gut DCs and is retinoic acid dependent. The inability of liver DCs to imprint gut tropism implies that $\alpha4\beta7$+CCR9+ T cell that infiltrate the liver in PSC are primed in the gut.**

Selective imprinting of the adhesion molecules CCR9 and $\alpha4\beta7$ on primed lymphocytes facilitates their tissue-specific homing to the lamina propria and intraepithelial compartments in the gut. Expression of the chemokine ligand for CCR9, CCL25, is largely restricted to epithelial cells in the thymus[1] and small bowel[2] and in combination with $\alpha4\beta7$ ligand mucosal addressin cell-adhesion molecule-1 (MAdCAM-1) provides a gut-specific "address code" to recruit gut-tropic lymphocytes.[3] More than 90% of lymphocytes in the small bowel express $\alpha4\beta7$ and CCR9,[4] and mice deficient in either have disrupted mucosal lymphocyte compartments and impaired lymphocyte trafficking to the gut.[5,6] MAdCAM-1 is up-regulated during inflammatory bowel disease (IBD) and promotes recruitment of $\alpha4\beta7$+lymphocytes[7]; and CCL25 and CCR9 expression have been linked to small intestinal Crohn's disease.[8,9] As a consequence, these receptors are therapeutic targets in IBD resulting in promising phase II/III studies using inhibitors of CCR9 or $\alpha4\beta7$.[10] Although CCL25 and CCR9 are associated with small bowel homing in mice, CCR9 is associated with human colitis, which is ameliorated by treatment with the CCR9 inhibitor Traficet EN (personal communication T. Schall, Chemocentryx).

Stagg et al first showed that human gut-derived dendritic cells (DCs) imprint $\alpha4\beta7$ on responding T cells.[11] Subsequent murine studies demonstrated that gut-derived DCs imprint CCR9 as well as $\alpha4\beta7$ expression on T and B lymphocytes.[12,13] CD103+ antigen-containing gut DCs[14] migrate from the lamina propria to mesenteric lymph nodes (MLN) via lymphatics in a CCR7-dependent process[15] where they activate naive lymphocytes to become gut-homing effector cells.[12] Murine studies suggest that the ability of gut DCs to imprint $\alpha4\beta7$ and CCR9 on lymphocytes is retinoic acid (RA) dependent.[13,16] Gut DCs express retinal dehydrogenase isoenzymes, allowing them to convert retinol to all-trans-retinoic acid (ATRA), which complexes with intracellular retinoid receptors to activate transcription of CCR9 and $\alpha4\beta7$.[16] In contrast, DCs from spleen or peripheral lymph nodes lack retinal

Abbreviations used in this paper: ATRA, All-trans-retinoic acid; GALT, gut-associated lymphoid tissue; MLN, mesenteric lymph nodes; MAdCAM-1, mucosal addressin cell-adhesion molecule-1; DC, dendritic cells; RA, retinoic acid; RAR, retinoic acid receptor; MLR, mixed lymphocyte reaction; TCR, T-cell receptor.

0016-5085/09/$36.00
doi:10.1053/j.gastro.2009.02.046

dehydrogenases and fail to generate ATRA. Vitamin A-deficient mice generate low numbers of CCR9+$\alpha4\beta7$+ lymphocytes and have reduced numbers of lymphocytes in the lamina propria.[13,16] RA is stored at several extraintestinal sites including hepatic stellate cells (HSC),[17] yet, except in the context of PSC, lymphocyte homing to these sites is CCR9/$\alpha4\beta7$ independent.[18] Recent work demonstrates that nongut DCs can generate some RA-dependent responses but that CCR9 and $\alpha4\beta7$ induction may depend on antigen dose and local RA levels.[19] The pleiotropic effects of RA are highlighted by its role in generating regulatory T cells when combined with transforming growth factor (TGF)-β.[20] Thus, microenvironmental cofactors and signals combine with RA to determine lymphocyte gut tropism.

The maintenance of gut tropism requires reencounter with gut DCs to sustain high levels of CCR9 and $\alpha4\beta7$.[21] If a gut tropic lymphocyte is reactivated by skin DCs, CCR9 and $\alpha4\beta7$ are down-regulated, and expression of skin homing receptors is increased.[21] Such plasticity enables memory lymphocytes not only to be targeted to tissue in which antigen was originally encountered but also to be redirected to other sites in which antigen is subsequently encountered. RA appears to be critical for imprinting gut homing in mice, although its role in humans is less clear. We implicated mucosal lymphocytes in the pathogenesis of inflammatory liver diseases complicating IBD by demonstrating aberrant expression of CCL25[22] and MAdCAM-1[23] in the liver of patients with primary sclerosing cholangitis (PSC)[18] associated with hepatic infiltration by CCR9+$\alpha4\beta7$+ T cells. Although this phenotype is consistent with T cells that have been primed by DCs in gut-associated lymphoid tissue (GALT),[22] an alternative explanation is that DCs in portal lymph nodes or the inflamed liver in PSC[24] acquire the ability to generate gut tropic lymphocytes. This has implications for the pathogenesis of PSC; if CCR9+$\alpha4\beta7$+ T cells can only be imprinted by DCs in GALT, this confirms that T-cell activation in PSC originates in the gut, whereas if liver DCs can acquire the ability to imprint gut homing, the defect may lie in the hepatic microenvironment. To clarify these issues, we investigated the ability of DCs isolated from the human gut, liver, and spleen to imprint $\alpha4\beta7$ and CCR9 and the extent to which this is RA dependent.

Materials and Methods

Tissue

Human liver, gut, and associated lymph nodes were obtained with consent from patients undergoing liver transplantation or intestinal resection (local ethical approval 04/Q2708/41 and REC 2003/242). Surplus splenic tissue was obtained from organ donors (donor details in Supplementary Table 1).

Mice

C57BL/6 and C57BL/6 Thy1.1 mice (Jackson Laboratories, Bar Harbour, ME) housed in an SPF/VAF facility were used in accordance with CBR/Harvard Medical School animal committees' guidelines or with approved license from the Animal Scientific Procedures Division of the Home Office.

Reagents

ATRA, Am580, carboxyfluorescein succinimidyl ester (CFSE) (Sigma, Dorset, UK) and LE540 (Wako Chemicals, Osaka, Japan) were dissolved in dimethyl sulfoxide (DMSO) in dark and anoxic conditions. Anti-mCCR9 (clone 5F2) and anti-h$\alpha4\beta7$ (ACT-1; 15 μg/mL) (M. Briskin Millennium Pharm, Inc, Cambridge, MA), anti-hCD3-RPE/Cy7 (UCHT1; 1:10; Coulter, London, UK), anti-hCD11a-FITC (MEM-25; 8 μg/mL; Caltag, Buckingham, UK), anti-hCD45RA-PE/Cy5 (HI30; 1:20 [vol/vol]; Serotec, Oxford, UK), anti-h$\beta7$-PE/Cy5 (FIB504; 1:20 [vol/vol]; BD, Oxford, UK), anti-hCD8-APC/Cy7 (SK7; 1:10 [vol/vol]; BD, Oxford, UK), anti-hCCR9-APC (FAB1791A; 2.5 μg/mL; R&D Systems, Abingdon, UK), anti-m$\alpha4\beta7$ (DATK32; BD), and anti-mCD8α (BD).

Human Peripheral Blood Lymphocyte Isolation

Blood was centrifuged over LymphoLyte-H (Cedarlane Laboratories, Burlington, Ontario, Canada) for 20 minutes at 650*g*, and peripheral blood lymphocytes were harvested and maintained in culture at 37°C in RPMI with 10% fetal calf serum (FCS). Naïve CD8 T cells were FACS sorted based on coexpression of CD8, CD45RA, and CD11a^{low} to 95% purity (Supplementary Figure 1*B*).

Isolation of DCs

Primary human myeloid DCs were isolated from MLN, spleen, liver, gut, and portal lymph nodes as described before using mechanical disaggregation in a Stomacher 400 circulator (Seward, UK) followed by with Optiprep (AxisShield, Kimbolton, UK) density gradient centrifugation.[25] Lymphocytes were depleted by anti-CD3 dynabeads and DCs isolated to >85% purity by immunomagnetic selection on CD11c (EasySep Stemcell Technologies, Vancouver, British Columbia, Canada) (Supplementary Figure 1*A*).

Isolation of Murine DC and Naive CD8 T Cells

DCs were obtained from spleens of C57BL/6 mice 12 days after injection of Flt3l-secreting B16 cells as described.[21] Spleens were enzymatically digested before positive immunomagnetic selection with anti-CD11c microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). DC (>90% CD11c$^+$) resuspended to 1×10^7/mL in Isove's modified Dulbecco's media + 10% FCS + 50