Direct Presentation of Antigen by Lymph Node Stromal Cells Protects Against CD8 T-Cell-Mediated Intestinal Autoimmunity

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See editorial on page 1249.

Background & Aims: Disruption of the enteric glial cell (EGC) network is an early pathologic feature in Crohn’s disease. To determine the contribution of antigen-specific CD8 and CD4 T cells to the breakdown of the EGC network, we studied specific autoimmune targeting of an ectopic antigen expressed by EGCs. Methods: Transgenic mice (GFAP-HA), which express the influenza hemagglutinin (HA) in EGCs, were either crossed with mice transgenic for a T-cell receptor (TCR) specific for a major histocompatibility complex (MHC) class I epitope of HA (CL4-TCR) or were adoptively transferred with conventional CL4 T cells. These were compared with GFAP-HA mice transferred with conventional T cells specific for an MHC class II epitope of HA (6.5).

Results: Both CD8 and CD4 T-cell subtypes were activated in vivo in an antigen-specific manner; however, they differed substantially in their ability to expand in the mesenteric lymph nodes, trigger proinflammatory cytokines, and induce autoimmune damage in the intestine. Direct presentation of antigen, provided by lymph node stromal cells, caused the activation and deletion of CD8 T cells. This mechanism of T-cell tolerance did not affect CD4 T cells, which produced antigen-specific lethal autoimmunity.

Conclusions: Our observations support a recently identified mechanism of peripheral T-cell tolerance that specifically protects against autoimmunity mediated by conventional CD8 T cells. Furthermore, we show that conventional CD4 T cells are not affected by this mechanism of tolerance, and their targeting of EGCs produces lethal intestinal autoimmunity.

Autoimmune diseases that affect the bowels, including inflammatory bowel diseases (IBDs), encompassing Crohn’s disease (CD) and ulcerative colitis, are characterized by an abnormal mucosal immune response that can be facilitated by microbial factors. Disruption of the enteric glial cell (EGC) network is an early pathologic feature in CD and coincides with intestinal and mesenteric T-cell infiltration, vasculitis, T helper cell 1 (Th1) cytokine production, and bowel inflammation. Selective destruction of EGCs compromises epithelial and vascular integrity within the gastrointestinal tract and results in fulminating inflammation, hemorrhage, and necrosis. EGCs constitutively express major histocompatibility complex (MHC) class I and selectively up-regulate MHC class II in CD, or upon in vitro treatment with cytokines, and can be recognized by CD4 and CD8 T cells. Thus, EGCs are candidate targets of CD8 and CD4 T-cell triggering of IBD.

There is evidence that both CD8 and CD4 T cells can trigger lethal autoimmunity when peripherally activated by antigen in a proinflammatory setting. In contrast, recent reports have suggested that, in the absence of inflammation, peripheral CD8 T cells with specificity to self are deleted following direct presentation of antigens by nonprofessional antigen-presenting cells (APCs) in the lymph node (LN) stroma. In our mouse model, the influenza virus membrane protein hemagglutinin (HA) is expressed under the control of the glial fibrillary acidic protein (GFAP) promoter (referred to as GFAP-HA hereafter). HA peptides are expressed by EGCs and recognized by CD8 T cells that express a T-cell receptor (TCR) specific for Kd:HA512–520 (CL4) or CD4 T cells that express a TCR specific for IEd:HA107–119 (6.5), respectively. We have reported that CL4 T cells express high affinity receptors and that the transfer of as few as 8000 conventional CL4 T cells from RAG-2−/− TCR transgenic mice can generate potent cytotoxicity and reject aggressive transplanted tumors that express HA. A pathology reminiscent of the one observed in CD is produced in compound mu-

Abbreviations used in this paper: BM, bone marrow; DTg, double transgenic; EGC, enteric glial cell; GFAP, glial fibrillary acidic protein; HA, hemagglutinin; LN, lymph node; MHC, major histocompatibility complex; MLN, mesenteric lymph node; STg, single transgenic; TCR, T-cell receptor.
tant progeny of GFAP-HA mice crossed to CL4-TCR mice. Here, we have examined the susceptibility of GFAP-HA mice to the adoptive transfer of limited numbers of conventional CL4 CD8 or 6.5 CD4 T cells. Our observations support a recently identified novel mechanism of CD8 T-cell tolerance through direct presentation of antigen. This mechanism affects conventional positively selected CD8 but not CD4 T cells transferred to GFAP-HA recipients from RAG-2−/− TCR transgenic mice that do not express the cognate antigen. In contrast, in hosts that express the antigen, pathogenic CD8αβ T cells that escape thymic negative selection induce lethal autoimmunity against the intestine. These observations reveal a distinct susceptibility of the intestine to autoimmunity mediated by conventional CD4 T cells. Furthermore, they are consistent with distinctly different vulnerability of conventional as compared with truly self-reactive CD8 T cells to peripheral deletion.

Materials and Methods

Mice

CL4-TCR × RAG-2−/− BALB/c mice, 6.5-TCR × RAG-2−/− mice, DPE-GFP × CL4-TCR × RAG-2−/− mice, Thy1.1 BALB/c mice, and GFAP-HA BALB/c mice have been previously described. H2b-BALB/c mice were purchased from Jackson Laboratory (Bar Harbor, ME). Mice were bred in the Harvard School of Public Health animal facility under specific pathogen-free conditions, and all experiments using these mice were approved by and performed according to the guidelines of the Animal Ethics Committee of Harvard University.

Cells

CL4 and 6.5 T cells were purified from TCR-Tg-RAG-2−/− mice and labeled with 10 μmol/L 5,6-carboxyfluorescein diacetate succinimidyl diester (CFSE) as previously described. Green fluorescent protein (GFP) expressing CL4 cells were purified from DPE-GFP × CL4-TCR × RAG-2−/− mice. T cells were injected into the lateral tail vein in 200 μL phosphate-buffered saline. Intestinal mononuclear cells and stromal cells or dendritic cells (DCs) were purified as described before to >90% purity.

Monoclonal Antibodies and Fluorescence-Activated Cell Sorter Analysis

6.5 Clonotypic monoclonal antibodies (mAb) were purified according to standard procedures. αβ mAb were purchased from eBioscience (San Diego, CA). UEA-I mAb was purchased from Vector Labs (Burlingame, CA). All other mAbs were purchased from BD Pharmingen. Fluorescence-activated cell sorter (FACS) analysis was done with FlowJo software (Tree Star, Ashland, OR).

Cytokine Analysis

A total of 10⁴ 6.5 cells purified out of mesenteric lymph nodes (MLN) and intestine were cultured with syngeneic irradiated APCs and HA₁₀⁷.₁₁₉ peptide. Supernatant was harvested after 3 days and kept frozen at −80°C until analysis. For serum preparation, blood was drawn and allowed to clot at room temperature. The levels of individual cytokines were determined in the supernatant using the Luminec xMAP technology platform (Austin, TX), with kits from Upstate Biotechnology (Charlottesville, VA). Intracellular cytokine stainings were performed using the BD Pharmingen kit according to manufacturer’s instructions after 4-hour in vitro restimulation of LN cell suspensions in the presence of HA peptide at 37°C.

Bone Marrow Reconstitution

GFAP-HA mice were irradiated at a lethal dose of 850 rad in a GammaCell 40 irradiator and adoptively transferred with 2 × 10⁵ Lin− bone marrow (BM) by negative-immunomagnetic cell enrichment for CD3, B220, DX5, Gr1, and TER-19 negative cells followed by sorting of the negative fraction on a FACSARia. Purity was greater than 95%.

Reverse-Transcription Polymerase Chain Reaction

RNA was prepared with miniprep isolation kit (Stratagene). Complementary DNA (cDNA) was synthesized using the Omniscript reverse transcription kit and random hexamers (Qiagen). Real-time polymerase chain reaction (PCR) was carried out in a final volume of 30 μl with optimal concentrations of primers (HA primers: 5′-CTCCCTTCTCCGTCAGCCATAGCAA-3′ and 5′-AGACAGCCACAACGGAATGACTG-3′ and Actin primers: 5′-TGGATTCCTGTGGCATCCATAGCAA-3′ and 5′-TAAAACGCGATGATACAGTCCG-3′) using the SybrGreen kit (Applied Biosystems). Reactions were run on a GeneAmp 7300 Sequence Detection System (Applied Biosystems) in triplicate, and expression values were normalized to Actin expression using the comparative threshold cycle (CT) method.

Statistical Analysis

Data are expressed as mean ± SEM. Differences between groups were calculated using a 2-tailed unpaired t test using the Prism4 software.

Supplemental Data

Supplemental data include 3 Figures (see Supplemental data online at www.gastrojournal.org).

Results

The Intestine of GFAP-HA Mice Is Protected Against HA-Specific CD8 T Cells

GFAP-HA × CL4-TCR double transgenic (DTg) mice succumb to Crohn’s-like disease in the first 8 days of life. We sought to determine the status of CL4 T cells in the intestine and spleen of GFAP-HA × CL4-TCR DTg newborn mice and the ability of adoptively transferred
CD8 T cells to induce lethal IBD. As previously reported,\textsuperscript{3} the intestines of GFAP-HA × CL4-TCR DTg newborn mice were severely damaged and massively infiltrated by leukocytes (Figure 1A, a and b). CD11b\textsuperscript{+}Gr1\textsuperscript{+} proinflammatory myeloid cells accounted for the largest fraction of the infiltrate (Figure 1B and see Supplementary Figure 1A online at www.gastrojournal.org). Significant numbers of

CL4 cells were observed in the intestine (Figure 1B) and the spleen (Figure 1C). Although the greater fraction of CL4 cells infiltrating the intestine expressed CD8α\textsubscript{α} (Figure 1D), consistent with these being agonist selected and regulatory in nature,\textsuperscript{20} approximately 30% of the cells expressed CD8αβ, consistent with these being pathogenic T cells that had escaped negative selection (Figure

\textbf{Figure 1.} Lethal intestinal inflammation in newborn GFAP-HA × CL4-TCR mice but not GFAP-HA mice transferred with CL4 T cells. (A) H&E-stained paraffin intestine sections 6 days postparturition. GFAP-HA × CL4-TCR (a and b), age-matched CL4-TCR (c), GFAP-HA (d and e), or WT BALB/c (f) transferred IP with 10\textsuperscript{6} (d and f) or 5 × 10\textsuperscript{4} (e) CL4 T cells 1-day postparturition. Arrows: (a) lymphatic swelling, (b) submucosal leukocytes infiltration, (c) healthy villi and submucosa, (d) submucosal edema. Original magnification, ×40, n = 4 independent experiments. (B) Numbers of intestine infiltrating CL4 and Gr1 cells in indicated mice 6 days after transfer (n ≥ 4/group). *P < .05, **P < .01. (C) Absolute numbers of CL4 cells in the spleen of age-matched GFAP-HA × CL4-TCR (DTg) and CL4-TCR (STg) mice. n = 5/group. (D) Characteristics of spleen or intestine derived CL4 T cells, from STg or DTg newborn mice, gated for Vβ8.1-8.2 and CD8α and plotted for expression of CD8β; n ≥ 3/group.
In the spleen, over 90% of the CL4 cells expressed CD8αβ and had presumably escaped negative selection (Figure 1D). Lack of gut infiltrating CD8αβ CL4 T cells in the CL4-TCR single transgenic (STg) mice was consistent with no agonist selection in the absence of HA (Figure 1D).

We had reported that, in nontransgenic hosts, conventional CL4 T cells exhibit potent cytotoxicity against HA-expressing transplanted tumors. To test the fate of CL4 T cells upon encounter of antigen, Thy1.2+ HA-specific conventional CD8 T cells isolated from CL4-TCR × RAG-2−/− mice were adoptively transferred to Thy1.1+ GFAP-HA STg newborn mice in titrating numbers. In contrast to the GFAP-HA × CL4-TCR DTg mice, GFAP-HA STg mice that received 10^6 CL4 T cells showed only a mild submucosal edema (Figure 1A, d). GFAP-HA STg mice that received 5 × 10^6 CL4 cells and WT mice that received 10^6 CL4 cells did not show any intestinal inflammation (Figure 1A, e and f). The numbers of gut infiltrating CL4 and myeloid cells both increased as more T cells were transferred (Figure 1B). Similar numbers of gut infiltrating CL4 T cells as in GFAP-HA × CL4-TCR DTg mice (5.6 × 10^3/intestine calculated as 30% of the total CL4 cell pool) were achieved in GFAP-HA STg mice (4.7 × 10^3/intestine) by transfer of 10^6 conventional CL4 cells (Figure 1B), yet the GFAP-HA STg intestine accumulated much less CD11b+Gr1+ myeloid cells than the GFAP-HA × CL4-TCR DTg intestine (Figure 1B), and the pathology was much less severe (Figure 1A, d). These observations indicate that conventional CD8 T cells can access the intestine of newborn pups but are significantly less autoaggressive than pathogenic CD8 T cells that have escaped thymic negative selection in GFAP-HA × CL4-TCR mice.

We pursued our studies in adult mice. Adult (6–10 weeks old) Thy1.1+ GFAP-HA mice received intravenous (IV) injections of up to 10^7 Thy1.2+ CL4 T cells labeled with CFSE and were examined in a time course manner. The transferred cells proliferated in all peripheral LNs (Figure 2A), in line with the expression of HA in glial cells throughout the body, displayed an activated profile as shown by down-regulation of CD62L and up-regulation of CD25 and CD44 (Figure 2B), but did not express the gut-homing integrin α4β7, failed to accumulate in the gut-associated lymphoid tissues, and significantly decreased in frequency and absolute numbers with time after adoptive transfer (Figure 2C). Finally, the mice neither manifested HA-specific CD8 T-cell nor myeloid infiltration in the intestine and exhibited no clinical sign of disease at least 3 weeks after transfer (data not shown). To probe further the pathogenic potential of CD8 T cells, CL4 T cells were transferred to Thy1.1+ GFAP-HA × RAG-2−/− mice. Lymphopenia-driven expansion of the cells in the MLNs provided for over an order of magnitude better accumulation than in immune-competent mice (compare Figures 2C and 3A), however, still not reaching the levels of control nontransgenic recipient mice (Figure 3A). None of the mice showed any histologic sign of intestinal disease (Figure 3B), and no increase in numbers of intestinal CL4 T cells or proinflammatory CD11b+Gr1+ cells was observed in GFAP-HA × RAG-2−/− as compared with RAG-2−/− mice (Figure 3C and see Supplementary Figure 1 online at www.gastrojournal.org). In GFAP-HA mice, the transferred CL4 T cells produced similar interferon (IFN) γ but less tumor necrosis factor (TNF) α than upon transfer to control RAG-2−/− recipient mice (Figure 3D). Thus, adoptively transferred CL4 T cells were poor effectors of IBD even after lymphopenia-driven expansion and activation, reflecting a profound CD8 tolerance in GFAP-HA mice. These obser-
The intestine of GFAP-HA mice is resistant to HA-specific CD8 T-cell autoimmunity. Thy1.2+ CL4 cells were isolated from mice on RAG-2−/− background, and 10⁶ cells were adoptively transferred in Thy1.1+ GFAP-HA × RAG-2−/− or RAG-2−/− controls. Intestines were analyzed on day 7. (A) Frequency (left) and accumulation (right) of transferred cells in MLN of GFAP-HA × RAG-2−/− or RAG-2−/− controls 7 days after transfer. N = 5 mice per condition and 3 independent experiments. (B) H&E staining of paraffin sections of small intestine of GFAP-HA × RAG-2−/− mice, 7 days after transfer of CL4 T cells (a) or no transfer (b). Control RAG-2−/− intestine 7 days after transfer of CL4 T cells (c). (C) Left: representative FACS dot plots of CL4 cells and neutrophils in the small intestine. Right: total numbers of infiltrating CL4 cells and neutrophils in the small intestine. N = 4 mice/group, n ≥ 3 independent experiments. *P < .05. (D) FACS dot plots of cytokine expression by CD8+ Thy1.2+ CL4 cells, derived from MLN and restimulated in vitro with HA512−520 peptide, prior to analysis. N = 3−5 mice per group.

Figure 3.

HA-Specific CD4 T Cells Trigger IBD in GFAP-HA × RAG-2−/− Mice

We have reported that GFAP-HA × TCR-6.5 DTg mice harbor HA-specific regulatory T cells (Tregs) and are profoundly tolerant to HA.22 Here, we investigated whether GFAP-HA mice were also tolerant to IV transferred 6.5 T cells purified from TCR-6.5 × RAG-2−/− transgenic mice. The cells proliferated extensively in all LNs examined (Figure 4A), were activated as shown by down-regulation of CD62L and up-regulation of CD44 and CD25 (Figure 4B), and accumulated with time in the MLNs of GFAP-HA mice (Figure 4C). However, the 6.5 T cells did not express the gut-homing integrin α4β7 (Figure 4B) and did not infiltrate the intestine (data not shown), revealing additional requirements for IBD.

Transfer of polyclonal naïve CD4 T cells to lymphocyte-deficient SCID mice readily promotes colitis, presumably because of lymphopenia-driven proliferation and activation of T cells with cross-reactivity to gut microfloral antigens. We therefore tested the ability of the HA-specific CD4 T cells to induce inflammation in the intestine in an antigen-dependent manner. To this aim, we transferred 5 × 10⁶ naïve HA-specific CD4 T cells in lymphopenic GFAP-HA × RAG-2−/− or control mice. The HA-specific CD4 T cells accumulated in the MLNs (Figure 5A) but also infiltrated the intestine, mediated destruction of crypts (Figure 5B), and caused death within 7 days in all mice analyzed. Autoimmunity was directed to HA-expressing cells because 6.5 T cells transferred to RAG-2−/− mice did not infiltrate in the MLNs (Figure 5A), but also infiltrated the intestine, mediated destruction of crypts (Figure 5B), or trigger autoimmunity (Figure 5C). Accordingly, upon transfer of 6.5 T cells, we counted in the intestine of GFAP-HA × RAG-2−/− mice 34 times more CD11b+Gr1+ myeloid cells as compared with RAG-2−/− mice and observed a massive infiltration by 6.5 T cells (Figure 5C). The number of CD11b+F4/80+ macrophages did not increase (data not shown). Proinflammatory cytokines were elevated locally (Figure 5D, a) as well as systemically (Figure 5D, b), with dominance of IFN-γ in the intestine and gut-associated lymphoid tissues and of IFN-γ, TNF-α, interleukin (IL)-2, and IL-6 in the serum. Thus, expression of HA in the intestine rendered...
GFAP-HA × RAG-2-/- mice susceptible to antigen-specific CD4 T-cell autoimmunity involving proinflammatory cells and cytokines. The Th1 profile of the disease is in line with earlier models based on polyclonal CD4 T cells of unknown specificity.25,26

LN Stroma Promotes Preferential Deletion of HA-Specific CD8 T Cells

We investigated a recently reported mechanism for CD8 T-cell tolerance caused by the direct presentation of self-antigens.14,27 To eliminate cross presentation of antigen by hematopoietic cells, we generated GFAP-HA mice that harbored MHC incompatible BM from H2b-BALB/c mice. Expression of H-2Kb on lymphocytes and DCs of the reconstituted mice revealed successful BM chimerism (see Supplementary Figure 2 online at www.gastrojournal.org). For control, H-2Kd BM Lin- cells from WT BALB/c mice were used to repopulate irradiated GFAP-HA mice. The mice were then adoptively transferred with 10⁶ CFSE-labeled naive CL4 or 6.5 T cells, and the CFSE dilution of the transferred cells was analyzed on day 3. As a further control, T cells were adoptively transferred to BALB/c mice.

CL4 T cells proliferated (Figure 6A) and up-regulated activation markers CD44, CD69, and the integrin αβ7, and down-regulated the LN-homing receptor CD62L equally well in H2d and H2b BM-chimeric GFAP-HA mice (Figure 6B), but few cells secreted IFN-γ (Figure 6B) or accumulated in the MLNs (Figure 6C). They remained quiescent after transfer to control BABL/c mice. The absolute numbers of CL4 T cells after transfer were significantly higher in WT BALB/c mice than in H2d or H2b chimeric GFAP-HA mice, suggesting deletion of CL4 T cells in the latter. Deletion of CL4 T cells was more pronounced in H2d chimeric mice (Figure 6C), possibly reflecting a contribution of cross presentation to CD8 tolerance.

The response of 6.5 T cells was distinct from that of CL4 T cells. In control H-2d chimeric GFAP-HA recipients, 6.5 T cells proliferated vigorously (Figure 6A), up-regulated activation markers and produced IFN-γ (Figure 6B), and accumulated (Figure 6C). In contrast, 6.5 T cells remained quiescent after transfer to H-2b chimeric GFAP-HA recipients or BALB/c mice. Our observations agree with a recent report that implicates direct presentation of antigens by LN stromal cells in maintaining CD8 T-cell tolerance to self.14 Although this study had demonstrated expression of both MHC class I and class II antigens by the LN stromal cells, it had not directly addressed antigen presentation to CD4 T cells. In our model, conventional CD4 T cells did not seem to be affected by direct presentation of antigen.

To relate our in vivo observations to LN stromal cells, we examined the expression and presentation of HA antigens by LN-derived cells. LN stromal cell populations were purified from collagenase-treated LNs of GFAP-HA mice, based on the cell surface expression of UEA-I and the absence of the hematopoietic marker CD45.14 For comparison, DCs were purified, based on the expression of CD11c together with or without CD8α.28 HA expression was detected by quantitative RT-PCR in the LN stromal cell populations of GFAP-HA mice but not in the corresponding DC population (Figure 7A). We then tested for presentation of HA antigens to CD8 and CD4 T cells by performing ex vivo proliferation assays. Stromal cells or CD8α+CD11c+ or CD8α-CD11c- subsets of DCs were purified and cocultured separately with naïve CFSE-labeled 6.5 or CL4 T cells. Stromal cells from GFAP-HA but not control WT mice promoted vigorous proliferation of the CL4 T cells without the need for addition of the HA512–520 peptide (Figure 6B). Proliferation was also observed in cocultures with the CD8α+ subset of CD11c+ cells from GFAP-HA mice but not the CD8α- subset (Figure 7B), without the need to add exogenous peptide. These results confirm direct antigen...
presentation by stromal cells and cross presentation by CD8αCD11c+ DCs. The lack of cross presentation by CD8αCD11c− subpopulation of DC is in agreement with a recent report on the specialization of this DC subset for presentation of MHC class II specific antigens.28 The 6.5 T cells proliferated when placed in culture with GFAP-HA-derived CD8αCD11c+ cells but not CD8αCD11c− cells (Figure 7B), again confirming recent findings.28 However, they failed to proliferate in cocultures with GFAP-HA stromal cells (Figure 7B). Addition of HA107–119 to stromal cell cultures promoted little proliferation of the cells (see Supplementary Figure 3 online at www.gastrojournal.org), compatible with low expression of MHC class II (Figure 7C).

These observations highlight preferential activation of conventional CD8 T cells by nonprofessional APCs in the LN stroma. In an otherwise healthy mouse, CD4 T cells were not susceptible to this mode of activation and were resistant to activation-induced deletion in the LN s of GFAP-HA mice.

Discussion

We have developed a new model of antigen-specific acute and fatal intestinal inflammation triggered by CD4 T-cell targeting of EGCs. In contrast to CD4 T cells purified from RAG-2−/− TCR transgenic donors, similarly prepared specific CD8 T cells transferred to GFAP-HA failed to cause serious pathology. This model revealed a very different impact on CD8 T cells compared with CD4 T cells, mainly because, in the recipient mice, LN stroma constitutively expressed MHC class I and HA but not MHC class II. As a result, endogenous HA antigen was preferentially presented to HA-specific CD8 T cells in the periphery, leading to their depletion and peripheral tolerance. Glial cells are not known to be present in LNs21 and can therefore be eliminated as potential APC. Our observations confirm recent reports of a novel mechanism of CD8 peripheral tolerance, which relies on peripheral antigen display by LN stroma.14,27 We have also demonstrated that cross presentation of HA antigen by DC contributes to the elimination of CD8 T
cells that responded to antigen. In both models, the elimination process impacted conventional CD8 T cells, positively selected in mice lacking the cognate antigen. CL4 T cells transferred to adult GFAP-HA mice did not access the intestine and were rapidly eliminated from the periphery. This is similar to the peripheral deletion of OT-1 T cells transferred to iFABP-OVA mice that expressed OVA in gut enterocytes. In contrast, Westendorf et al reported that transfer of conventional CL4 T cells to Villin-HA transgenic mice induces severe pathology in the gut. The difference may be the spatial pattern of expression of HA. In GFAP-HA mice, HA was expressed by LN stromal cells, EGCs, and glial and astrocytic cells throughout the body, as well as by Ito cells in the liver. The Villin promoter may be strictly active in enterocytes, limiting antigen presentation to mucosal DC. Imprinted T cells would then migrate to the gut, encounter nonphysiologic levels of HA, and trigger autoimmunity. Another interesting difference is the fate of GFAP-HA × CL4-TCR DTg mice. The intestine of GFAP-HA × CL4-TCR newborn mice harbored mostly CD8αα CL4 T cells. These were presumably agonist selected suppressive cells. We also detected a small but significant subpopulation of CD8αβ T cells, which may have escaped negative selection and caused the lethal autoimmune pathology. In contrast Villin-HA × CL4-TCR DTg mice were healthy. Their MLN had an 8-fold increase in the frequency of FoxP3+ CD8+ T cells compared with Villin-HA STg mice.
may be better expressed in LN stromal cells than the Villin promoter. These possibilities remain to be tested.

Efficient negative selection of pathogenic autoreactive cells and agonist selection of functional regulatory T cells require the same conditions in the thymus. Conversely, defective negative selection (equals escape of negative selection) implies also defective agonist selection and defective regulatory T cells. With this in mind, it is likely that, in the double transgenic mice where CD8αβ T cells “escape” negative selection, the agonist selection of CD8αα T cells is also defective. This could imply that the CD8αα T cells present in these animals are functionally defective and cannot control the pathogenic self-reactive CD8αβ T cells.

This is the first time that targeting of EGCs by CD4 T cells has been shown to induce lethal intestinal inflammation. Lymphopenia-mediated proliferation and encounter of cross-presented HA antigen by the 6.5 T cells were both required. Because of the broad expression of HA, both mucosal and nonmucosal DCs were likely to be involved in antigen presentation, but only the former would have imprinted (induction of α,β) the primed CD4 T cells to migrate to the gut.32 Although, in immune-competent mice, the mucosal DCs are mostly regulatory and prime CD4 T cells to differentiate to regulatory T cells, it is not clear whether this is also the case in RAG-2−/− mice, in which the DCs may be defective in promoting Treg differentiation. Absence of direct presentation of HA to CD4 T cells in healthy GFAP-HA mice does not rule out the possibility that, under proinflammatory conditions, the LN stromal cells could up-regulate MHC II and directly present to CD4 T cells. However, the outcome of direct presentation to CD4 cells by LN stromal cells remains to be determined. Our model shows that, under noninflammatory conditions, DC-mediated presentation of EGC antigens to CD4 T cells can result in lethal intestinal autoimmunity and illustrates a distinct susceptibility of the intestine to autoimmunity by conventional CD4 T cells.

In conclusion, this study brings together important new insights related to T-cell-mediated intestinal autoimmunity. Peripheral elimination by LN stromal cells can prevent autodestruction by conventional CD8 T cells, responding to endogenous antigens in the periphery (as shown by the adoptive transfer of CL4 T cells). It is possible that this peripheral elimination process does not prevent IBD induced by pathogenic self-reactive CD8 T cells that have escaped negative selection (as shown with the GFAP-HA × CL4-TCR mice). These cells do not need to go to peripheral LNs to become activated and to migrate to the gut but can go directly to the intestine, similar to other unconventional T cells.33,34 Self-reactive T cells can cause lethal autoimmunity in the presence of excess CD8αα T cells of identical specificity, suggesting functional defects of the latter. Finally, at least in noninflammatory conditions, CD4 T cells are insensitive to this mechanism of tolerance, as seen by autoimmunity caused by the transfer of 6.5 CD4 T cells to GFAP-HA × RAG-2−/− recipient mice. At the same time, the stark differences in susceptibility to CD8 T-cell-mediated autoimmunity between our EGC and the reported IEC

**Figure 7.** Lymph node stromal cells express HA and efficiently present HA peptide-MHC complexes to CD8 but not CD4 T cells. (A) HA mRNA determined by real-time PCR in purified LN stromal cells (UEA-I+), DCs (CD11c+), or whole MLN from GFAP-HA mice. HA expression value in MLN was defined as 1. N = 3 independent experiments conducted on 1 pool of 5 mice. (B) In vitro proliferation of CL4 (top) or 6.5 (bottom) cells cocultured with 5 × 10⁴ MLN-derived UEA-I+ stromal cells (left) or CD8ααCD11c− and CD8ααCD11c+ DC subsets (right) from GFAP-HA or WT control mice. Cells were cultured at a 1:1 ratio for 86 hours with CFSE-labeled naive CL4 or 6.5 cells. Histograms show dilution of CFSE. N = 2 independent experiments. (C) MHC class I and II expression on stromal cells or DCs. Stromal cells and DCs from LNs were purified by collagenase digestion; stained with CD11c, CD45, UEA-I, MHC class I (H-2Kb), MHC class II (I-Aq), or the respective isotype mAbs; and analyzed by flow cytometry. Histograms are gated on either CD11c+ cells or CD45+ UEA-I+ cells. N = 5 mice.
models of IBD,\textsuperscript{29} even when the same antigen and same T cells are involved, invite caution in generalizing mechanisms of T-cell-mediated autoimmunity and indicate that the pattern of expression of antigen and cellular targets involved might be of critical importance.

**Supplementary Data**

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi:10.1053/j.gastro.2008.01.070.

**References**