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LIGHT Is Constitutively Expressed on T and NK Cells in the Human Gut and Can Be Induced by CD2-Mediated Signaling

Offer Cohavy,* Jaclyn Zhou,* Carl F. Ware, † and Stephan R. Targan2•

The TNF superfamily cytokine, lymphotoxin-like inducible protein that competes with glycoprotein D for binding herpesvirus entry mediator on T cells (LIGHT; TNFSF14), can augment T cell responses inducing IFN-γ production and can drive pathological gut inflammation when expressed as a transgene in mouse T cells. LIGHT expression by human intestinal T cells suggests the possibility that LIGHT may play a key role in regulation of the mucosal immune system. A nonenzymatic method was developed for the isolation of T cells from the human lamina propria, permitting analysis of native cell surface protein expression. Cell surface LIGHT was constitutively expressed on mucosal T and NK cells and a subpopulation of gut-homing CD4+ T cells in the periphery. In addition, CD2-mediated stimulation induced efficient LIGHT expression on intestinal CD4+ T cells, but not on peripheral blood T cells, suggesting a gut-specific, Ag-independent mechanism for LIGHT induction. By contrast, herpesvirus entry mediator expression on gut T cells was unperturbed, implicating the transcriptional regulation of LIGHT as a mechanism modulating signaling activity in the gut. Quantitative analysis of LIGHT mRNA in a cohort of inflammatory bowel disease patients indicated elevated expression in biopsies from small bowel and from inflamed sites, implicating LIGHT as a mediator of mucosal inflammation. The Journal of Immunology, 2005, 174: 646–653.

Both Crohn’s disease (CD) and ulcerative colitis (UC), collectively, are recognized as inflammatory bowel diseases (IBD) (1), are the result of a dysregulated mucosal inflammatory response (2). The intestinal immune compartment is differentially regulated, and its antigenic repertoire is independently shaped to accommodate the heavy antigenic load characteristic of the gut environment (3). In IBD, tolerance to intestinal Ags is perturbed (4, 5), and strong evidence implicates a skewed T cell-mediated Th1 response in CD (6), as well as mouse models of IBD (7, 8). TNF is implicated as an important contributor to intestinal inflammation with a subset of patients with CD responding to anti-TNF therapy (9). However, the partial success of blocking TNF emphasizes the complexity of mucosal immune regulatory mechanisms, prompting an investigation of other TNF-related ligands, such as lymphotoxin (LT) αβ and lymphotoxin-like inducible protein that competes with glycoprotein D for binding herpesvirus entry mediator on T cells (LIGHT), in human IBD pathology (2, 10, 11).

TNF, LTαβ, and LIGHT form a network of signaling systems involved in many aspects of immune function and inflammation reviewed in Refs. 12 and 13). LIGHT signaling is transduced via two members of the TNFR family, herpesvirus entry mediator (HVEM, TNFRSF14) and LTβR (TNFRSF13B) (14, 15), which also binds the LTαβ heterotrimer involved in the development and organization of peripheral lymphoid tissue (16). In addition, LIGHT is bound and putatively regulated by DecR3 (TNFRSF6B), a soluble decoy receptor (17). LTβR is broadly expressed, including stromal and myeloid cells, but absent on lymphocytes, whereas HVEM is expressed prominently on lymphocytes. LIGHT is primarily expressed in the lymphoid compartment by activated T cells, but also by monocytes (14). LIGHT-HVEM engagement is likely to play an important immunomodulatory role mediating stimulatory T-T interactions, because HVEM can deliver a costimulatory signal augmenting proinflammatory cytokine production and T cell proliferation (18). Disruption of the LIGHT gene in the mouse (19–21) revealed no abnormalities in lymphoid organ development, but CD8+ T cell differentiation in response to Ag was compromised.

Aberrant regulation of some TNF-related cytokines, including TNF (22), Fas ligand (23), or B cell-activating factor of the TNF family (24), can precipitate autoimmune diseases (25). Transgenic expression of LIGHT (TNFSF14) by T cells induced severe intestinal inflammation with autoimmune-like pathology in mice, specifically linking LIGHT-mediated signaling to the intestinal immune compartment (26, 27). In the gut, inhibition of the LTβR signaling pathway with a LTβR-Fc chimera decoy receptor alleviated inflammatory symptoms in the CD4+CD45Rαhigh T cell transfer model of colitis, suggesting a role for LIGHT or LTαβ in this CD4+ T cell-mediated pathology (10, 28). Moreover, the human LIGHT locus is closely linked to the TNF family members, CD27 ligand (CD70, TNFSF7) and 4-1BB ligand (TNFSF9), within the MHC paralogous region on chromosome 19p13.3 (29), which contains a candidate susceptibility locus for CD (30). These reports suggest that regulation of LIGHT expression or signaling may play a key role in modulating mucosal immunity, possibly contributing to the pathological inflammation of human IBD. We recently reported an enhanced potential for LIGHT expression by intestinal T cells in vitro, suggesting that mucosal location of T

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cells might promote LIGHT induction. In the present study, we provide direct evidence that LIGHT is constitutively expressed on human intestinal T and NK cells, but can also be activated through a gut-specific CD2-dependent mechanism. Expression of LIGHT mRNA was elevated in small bowel and inflamed intestinal tissue biopsy specimens from a cohort of IBD patients, further implicating LIGHT as a mediator of mucosal inflammation.

Materials and Methods

Cohort and specimen procurement

Blood leukocytes were obtained by venipuncture from healthy adult volunteers. Intestinal specimens were obtained from patients undergoing intestinal resection for clinical reasons, and biopsy specimens from patients undergoing diagnostic endoscopy to evaluate the location and severity of intestinal inflammation, or to characterize an intestinal tumor (non-IBD patients). Patient diagnosis was defined as CD, UC, or non-IBD, using standard clinical, radiographic, and endoscopic criteria (31), and gross tissue involvement was validated microscopically. Patients treated with cyclosporin A and patients with indeterminate colitis were omitted from the study. Procedures for subject recruitment, informed consent, and specimen procurement were in accordance with protocols approved by the Institutional Review Board for Human Subject Protection of the Cedars-Sinai Medical Center.

Cell isolation and culture

PBMC were isolated from uncoagulated blood by standard Ficoll-Hypaque density gradient centrifugation. Mononuclear cells from lamina propria (LPMC) were isolated, as described previously (32). Briefly, epithelial cells were removed by washing in EDTA, followed by enzymatic disruption of the lamina propria (LP) matrix, mincing, and density gradient purification of LPMC. For analysis of native surface protein expression, mucosal specimens were cut perpendicularly to the mucosal surface (1102), following the EDTA wash step, and lightly smashed to mechanically disrupt the tissue and facilitate lymphocyte leakage. Mechanically disrupted mucosa was incubated in RPMI 1640 for 2–6 h, and lymphocyte accumulation in the medium was monitored periodically for optimal yield, followed with exclusion of tissue debris through a macroporous (105-μm opening) polypropylene mesh (Spectrum Laboratories) and centrifugation at 28 x g to exclude remaining epithelial cells. Intraepithelial lymphocytes were excluded from these preparations by means of the initial EDTA wash similar to the standard enzymatic isolation protocol. Lymphocytes were cultured at 0.25–1 x 106 cells/ml in RPMI 1640 containing 2 mM L-glutamine and 25 mM HEPES buffer (Mediatech), supplemented with 10% heat-inactivated FBS (Atlanta Biologicals), 50 μg/ml gentamicin (Omega Scientific), and LPMCs with additional 0.25 μg/ml amphotericin B (Gemini Bio-Products). Where indicated, lymphocytes were stimulated by 40 ng/ml PMA and 1 μg/ml ionomycin (Sigma-Aldrich); or by Ab cross-linking of cell surface CD2 and CD28 used at 0.4 and 1 μg/ml, respectively.

T cell subset purification

Adherent cells were partially depleted from LPMC and PBMC preparations by adherence to plastic for 16–20 h before further purification or experimentation. CD4+ , CD8+ , CD4+CCR9+ , and CD4+CCR9- cells were purified from PBMC by flow cytometry (FACStar; BD Biosciences) using CD3+ staining for the CD4+ subsets or on CD4+ staining for the CD45RA/RO subsets. Purity was consistently greater than 99% for the gated markers when reanalyzed by flow cytometry (FACScan; BD Biosciences).

Ab reagents

Gem1A.1 is an anti-human LIGHT combinatorial Ab containing Vh and Vs chains generated from a BALB/c mouse immunized with soluble rLIGHT (29). Gem1A.1 is recognized by anti-mouse Ig κ. Mouse anti-methamphetamine was used as an isotype control and was provided by G. Valkirs (Biosite Diagnostics). An anti-LIGHT polyclonal antisera was produced in rats immunized with soluble human rLIGHT protein (33). A polyclonal goat anti-HVEM was generated by immunization with purified human HVEM-Fc. The serum was absorbed with immobilized human IgG, and purified IgG was prepared by protein G affinity chromatography. Anti-LIGHT Ab was used at 20 μg/ml in blocking experiments, and anti-HVEM was used at 0.2 μg/ml for T cell stimulation. The anti-CD2 Ab pair, clones GD10 and CB6, was a gift of C. Benjamin (Biogen Idec). Anti-CD28 Ab ascites, clone 9.3, was obtained from Bristol-Meyers Squibb Pharmaceutical Research Institute. The ascites was purified over a protein G column and quantified by ELISA. Anti-CCR9 was the 3C3 clone (34) from R&D Systems, anti-human integrin β2 from BD Pharmingen, and PC5-conjugated anti-CD56 from Beckman-Coulter. Additional chimeromorph-conjugated Abs specific for human CD3, CD4, and CD8 were from Caltag Laboratories.

Cell staining for flow cytometry

Cells were blocked with goat IgG for 20 min on ice, then indirectly stained for membrane-associated LIGHT using a mouse anti-human rLIGHT Fab, or isotype control Fab (Jackson ImmunoResearch Laboratories), and detected after washing by a FITC-conjugated goat anti-mouse (H and L) Fab (Jackson ImmunoResearch Laboratories) for 30 min per step. To avoid secondary Ab detection of mouse anti-CD2 Abs used for T cell stimulation, a rat anti-LIGHT serum was used and detected with a mouse-adsorbed, anti-rat secondary (Jackson ImmunoResearch Laboratories). After washing and blocking with mouse IgG or normal rat serum for 20 min, cells were stained for additional surface markers, for 20 min on ice. Flow cytometric analysis included at least 2 x 104 events on a FACScan (BD Biosciences) and analyzed with CellQuest software. Nonspecific staining by control isotypes or staining of unstained cells was subtracted from percentage staining for each cell subset to determine specific mean fluorescence.

Real-time PCR

Isolated cells or whole biopsy specimens were lysed in guanidium thiocyanate buffer, and total RNA was isolated using the RNeasy kit (Qiagen). For micro-purification of RNA from <106 cells, lysates were supplemented with 12 μg of RNA as a carrier (Sigma-Aldrich). LIGHT mRNA levels were quantified by real-time RT-PCR (iCycler; Bio-Rad) using One-Step RT-PCR mixture (Qiagen) with a LIGHT-specific dual-labeled probe and intron-spanning primers, normalized to a primer limiting 18S ribosomal RNA amplification measured in duplex. The following primer (Integrated DNA Technologies) and probe (Qiagen) sets were designed using Primer 3 software (35): LIGHT; forward primer, 5′-TCGGCTCTGAGAGATGTG-3′ and reverse primer, 5′-GGTTGACCTCGTAGACCTT-3′; Hyb probe, 5′-6-FAM-AGCTCAGGACGGCTGC-BHQ1-3′; 18S, forward primer, 5′-AAACGCTACCATCCATCCAAG-3′ and reverse primer, 5′-CCCTCAAATGCATTCTGTTA-3′; Hyb probe, 5′-TixRed-AGCAGGGGCAATATATCCC-BHQ3-3′.

Results

LIGHT is constitutively expressed on mucosal T and NK cells

LIGHT expression has only been detected on in vitro activated intestinal or peripheral blood (PB) T cells (11), but no direct evidence exists for LIGHT expression by gut lymphoid cells in vivo. We developed a mechanical disruption technique for the isolation of lymphocytes from the LP, because traditional enzymatic release of lymphocytes from intestinal tissue may remove cell surface proteins. Cells isolated using the mechanical disruption approach showed significant constitutive LIGHT expression primarily on CD8+ and CD4+ T cells (CD3+, CD4/8+) and NK cells (CD56+, CD3+) (Fig. 1A), contrary to cells isolated from enzymatically disrupted tissue, which do not stain for surface LIGHT protein. Cells from the inflamed or uninvolved colon (shown), small bowel, or rectum consistently expressed membrane LIGHT, although expression was not detected in cells from the PB or draining mesenteric lymph nodes whether inflamed or not (11). LIGHT expression diminished over 24–48 h in culture, but could be rapidly induced to maximal levels by PMA and ionomycin (P/I) activation, in accordance with the induction profile characteristic of LP T cells (Fig. 1A) (11).

LIGHT expression was further validated by quantitative real-time RT-PCR analysis of LIGHT mRNA levels in LP lymphocyte preparations isolated by mechanical disruption of the mucosa and staining positive for membrane LIGHT protein in comparison with enzymatically isolated LP lymphocytes or nonactivated PBLs that do not stain for surface LIGHT (Fig. 1B). LIGHT mRNA was detected in freshly isolated, some rested, and activated LPMC, although only activated lymphocytes from PB expressed LIGHT mRNA transcript. Although this approach does not permit further
LIGHT transcript expression was detected by quantitative real-time RT-PCR in highly purified (>99% by flow cytometry) CD4⁺/CCR9⁺ cells, but not in unstimulated CD4⁺/CCR9⁻ or unseparated PBLs (Fig. 2C).

HVEM is expressed and regulated normally on mucosal T cells

HVEM is the primary receptor for LIGHT constitutively expressed on PB T cells (18, 37). T cells from PB constitutively express HVEM, but can down-regulate expression following activation (38). Interestingly, while fresh mechanically isolated mucosal CD4⁺ and CD8⁺ T cells uniformly express HVEM (Fig. 3A), expression levels are relatively low, similar to levels expressed by activated PB T cells (Fig. 3B) (38). Surface expression of HVEM was regained by resting cells in culture, but following cell activation, HVEM was partially down-regulated, as occurs on peripheral T cells. Therefore, regulatory mechanisms governing HVEM expression in intestinal T cells do not differ from PB cells (Fig. 3).

The relatively reduced levels of HVEM in fresh, mechanically isolated gut T cells suggest in vivo activation before isolation from the mucosa. This finding agrees with the reported activated state of intestinal mucosa and suggests that gut-homing T cells in PB cells (Fig. 3).

FIGURE 1. LIGHT is constitutively expressed on mucosal T and NK cells. A, LPMC were isolated from the mucosa using a modified nonenzymatic method and surface stained with anti-LIGHT Ab (shaded), or isotype control Ab (unshaded), anti-CD3, and anti-CD4/8 or anti-CD56. Where indicated, cells were rested in culture for 24 h and then stimulated for an additional 6 h with P/I. Gated CD3⁺/CD4⁺ or CD3⁺/CD8⁺ T cell and CD3⁺/CD56⁺ NK cell histograms are shown. B, LIGHT mRNA levels were quantified by real-time RT-PCR in duplex with primer limiting 18S rRNA amplification in fresh mechanically isolated LPMC, or rested/activated LPMC in comparison with a PBL control. The 18S-adjusted threshold cycle for LIGHT signal is presented. Data are representative of three experiments. ND = not detected.

FIGURE 2. LIGHT is primarily expressed on PB T cells expressing gut-homing markers. PBMC were surface stained with anti-LIGHT Ab (shaded), or isotype control Ab (unshaded), anti-CD3, and anti-integrin β7 or anti-CCR9 following 16-h incubation in the absence (top panels) or presence (lower panels) of P/I. A, Lymphocyte-gated CD3⁺ T cell histograms are shown resolving LIGHT expression based on β7 integrin expression; B, resolving LIGHT expression based on CCR9 expression. C, LIGHT mRNA levels were quantified by real-time RT-PCR in duplex with primer limiting 18S rRNA amplification in flow-sorted CD3⁺/CCR9⁺ (>99% purity) cells, vs control unseparated PBMC activated for 16 h, where indicated. The 18S-adjusted threshold cycle for LIGHT signal is presented. Data are representative of a minimum of three experiments. ND = not detected.

separation of highly pure cell subsets, it is likely that detected LIGHT mRNA is produced by T and NK cells, which are the predominant cell type in these preparations and the exclusive cell type expressing membrane LIGHT protein by flow cytometry.

LIGHT is expressed on circulating mucosa-associated T cell subsets

The constitutive expression of LIGHT on T cells directly isolated from the intestinal mucosa suggested that gut-homing T cells in PB may express LIGHT. Gut-homing T cells were identified by surface expression of β7 integrin and CCR9 (36). In support of our hypothesis, LIGHT was identified on a small subset of integrin β7⁺ T cells (Fig. 2A) and on a major subset (>40%) of unstimulated CD4⁺/CCR9⁺ T cells (Fig. 2B). In addition, LIGHT was preferentially induced on the integrin β7⁺ subset of T cells (β7⁺ > 6% vs β7⁻ < 0.5%) following P/I stimulation (Fig. 2A), but expression was diminished on the CD4⁺/CCR9⁻ subset when compared with the CD4⁺CCR9⁺ population (Fig. 2B), suggesting maximal expression levels in the unstimulated state. Interestingly, while nonspecific stimulation induces a broad range of LIGHT expression levels on T cells, constitutive LIGHT expression, or lack of, clearly defines two major unique subsets of CCR9⁺ T cells (Fig. 2B). In support of LIGHT protein expression, constitutive LIGHT transcript expression was detected by quantitative real-time RT-PCR in highly purified (>99% by flow cytometry) CD4⁺/CCR9⁺ cells, but not in unstimulated CD4⁺/CCR9⁻ or unseparated PBLs (Fig. 2C).
regulated reciprocally to HVEM (38). HVEM expression is unlikely to be compromised considering the rapid nonenzymatic cell isolation technique and unperturbed expression of other cell surface markers such as CD3, CD4, CD8, or CD56 (data not shown).

**LIGHT can be induced on mucosal T cells by the CD2 signaling pathway**

CD2 can act as an Ag-independent activation pathway for intestinal T cells (40), which could account for constitutive expression of LIGHT. Cell surface staining and flow cytometry indicated efficient LIGHT induction by CD2 cross-linking, which was further augmented by costimulation with CD28 on LP and mesenteric T cell populations (Fig. 4A). Interestingly, CD2 cross-linking induced higher levels of LIGHT expression in the CD4<sup>+</sup> T cell subset, when compared with P/I stimulation (Fig. 4A, top and middle panels). Hence, although peripheral CD8<sup>+</sup> T cells constitute the predominant LIGHT-expressing T cell subset (11, 41), CD2-mediated activation provides a novel mechanism for efficient LIGHT induction on CD4<sup>+</sup> T cells in the intestinal compartment.

In addition, LIGHT induction via CD2 cross-linking followed similar kinetics to P/I stimulation (Fig. 4B), in accordance with the rapid LIGHT induction profile characteristic of LP T cells (11). LIGHT transcription was induced to similar peak levels and kinetics following CD2, CD2<sup>+</sup>/CD28, or P/I stimulation, indicating LIGHT up-regulation at the transcriptional level (Fig. 4C).

Conversely, PB T cells rendered responsive to CD2 by treatment with PHA (42) expressed LIGHT primarily on the CD8<sup>+</sup> T cell subset.

**FIGURE 3.** The LIGHT receptor, HVEM, is expressed and normally regulated by gut T cells. A, LPMC were isolated from the mucosa using a modified nonenzymatic method and surface stained with anti-HVEM Ab (shaded), or isotype control Ab (unshaded), anti-CD3, and anti-CD4/8. Where indicated, cells were rested in culture for 24 h and then stimulated for an additional 6 h with P/I. Gated CD3<sup>+</sup>/CD4<sup>+</sup>/CD8<sup>+</sup> T cell and CD3<sup>+</sup>/CD56<sup>+</sup> NK cell histograms are shown. B, Mean MFI changes vs isotype controls in three separate experiments are shown for CD8<sup>+</sup> (■) or CD4<sup>+</sup> (□) T cells.

**FIGURE 4.** LIGHT is induced on mucosal T cells via CD2 signaling. Lymphocytes were isolated from the LP or mesenteric lymph nodes, as described in Materials and Methods, and activated with P/I CD2 or CD2 and CD28. Cells were then surface stained with anti-LIGHT Ab and anti-CD3/8. A, Lymphocyte-gated, CD4/8<sup>+</sup> T cells following activation are plotted (shaded), in reference to the unstimulated profile (unshaded). B, Mean fluorescence changes from the isotype control are plotted for CD4<sup>+</sup> and CD8<sup>+</sup> T cells for the activation time indicated. C, LIGHT mRNA levels were quantified by real-time RT-PCR in duplex with primer limiting 18S rRNA amplification. The 18S-adjusted threshold cycle for LIGHT signal is presented for the activation time indicated. Data are representative of a minimum of three experiments.
population, with only minimal levels on the CD4\(^+\) PB T cell subset (Fig. 5A). Furthermore, up-regulation on both CD8\(^+\) and CD4\(^+\) PB T cells was consistent with the slower induction kinetics profile typical of PB T cells regardless of PHA pretreatment, with LIGHT protein levels increasing through a 24-h time point (Fig. 5B). LIGHT mRNA was effectively induced by CD2 in PHA-treated cells, peaking later than in LP T cells (by 2 h vs 1 h in LP T cells), and detected through 24 h (Fig. 5C). Interestingly, induction was transient in untreated cells, falling below detection by 6 h following P/I or CD2 stimulation, yet stabilized by CD2 and CD28 through 24 h (Fig. 5C). These data indicate that although PHA treatment can potentiate PB T cell response to CD2 signaling, gut-specific regulatory mechanisms in addition to basic CD2 responsiveness must account for the rapid induction kinetics and enhanced CD4\(^+\) T cell responsiveness unique to the LP T cell population (11).

LIGHT synthesis can possibly be regulated at the level of transcription, as LIGHT mRNA levels were significantly increased following T cell stimulation (Figs. 5C and 6C). Alternatively, intracellular stores of LIGHT protein have been reported (43) that could facilitate up-regulation of membrane LIGHT. Both CD2- and PMA-ionomycin-mediated up-regulation of LIGHT protein was effectively abrogated by cycloheximide (CHX) (>90% inhibition) (Fig. 6, A and B), thus demonstrating that de novo protein synthesis is essential to membrane LIGHT up-regulation via CD2 or P/I stimulation. In addition, effective inhibition of membrane LIGHT up-regulation was attained by transcription blocker, actinomycin D (Fig. 6, A and B, lower panels), indicating a requirement for transcriptional activation. Finally, analysis of LIGHT mRNA levels revealed unperturbed induction following stimulation in the presence of CHX, indicating that the LIGHT transcriptional machinery is performed in the resting cell (Fig. 6C). Taken together, these data suggest transcriptional regulation as a primary mechanism governing LIGHT expression in T cells.

**LIGHT mRNA expression is elevated in the inflamed and small bowel mucosa**

Gut-related LIGHT expression on T and NK cells (Figs. 1 and 2) (11) and the capacity of T cell-expressed LIGHT to induce a pathological gut-specific inflammation (26, 27) suggest that intestinal T cell activation induces LIGHT expression and a potential self-propagating proinflammatory signal driven by this elevated LIGHT expression. Accordingly, we examined whether LIGHT expression levels correlated with inflammation in the human gut mucosa. RNA samples were extracted from intestinal biopsy specimens representing a cohort of IBD patients, and LIGHT mRNA levels were measured by real-time RT-PCR. When LIGHT mRNA levels were compared between biopsies from inflamed and uninvolved intestinal regions from each patient, a significant trend for higher LIGHT mRNA levels was detected (p < 0.019, n = 11 pairs) in biopsies from inflamed tissue, regardless of diagnosis or intestinal location (Fig. 7A). However, no significant difference was detected (less than one cycle) in 3 of the 11 biopsy pairs analyzed (Fig. 7A, dotted lines). Interestingly, significantly higher levels of LIGHT mRNA (p < 0.036) were detected in small bowel biopsy specimens when compared with colonic biopsy specimens (Fig. 7B), in agreement with a previous report indicating a higher

**FIGURE 5.** CD2 mediates induction of LIGHT on PHA-activated PB-T cells, but not following LP-T kinetics of induction. PBMC were treated with PHA for 72 h, and cells were then surface stained with anti-LIGHT Ab and anti-CD3/8. A, Lymphocyte-gated, CD4/8\(^+\) T cells following activation are plotted (shaded), in reference to the unstimulated profile (unshaded), comparing LIGHT induction on PHA-treated vs untreated cells. B, Mean fluorescence changes from the isotype control are plotted for CD8\(^+\) (top) and CD4\(^+\) (bottom) T cells for the activation time indicated for PHA treated (shaded) or untreated (unshaded). C, LIGHT mRNA levels were quantified by real-time RT-PCR in duplex with primer limiting 18S rRNA amplification. The 18S-adjusted threshold cycle for LIGHT signal is presented for the activation time indicated comparing PHA treated (shaded top) vs untreated (unshaded bottom). Data are representative of a minimum of three experiments.

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potential for LIGHT expression on small bowel T cells (11). However, variable levels of LIGHT transcript were detected in the majority of mucosal specimens analyzed not significantly correlating with IBD pathology in this 16-patient cohort (3 non-IBD, 5 UC, 6 CD, and 2 indeterminant colitis).

Discussion

CD4⁺ T cells are key to maintaining immune homeostasis in the gut and can be pathogenic when reactive to bacterial Ags (44). More specifically, CD4⁺ CD45RB⁺ Th1, and a deficiency in T regulatory type 1 cells have been implicated in IBD pathology (6, 7, 28, 45). However, although excessive immune reactivity to bacterial Ags has been associated with human IBD (4), the mechanism by which breakdown of tolerance occurs remains largely unknown.

The present study provides evidence for LIGHT expression in the human intestinal mucosa, thus proposing a role for this TNF family cytokine in IBD pathology. A nonenzymatic protocol for gentle extraction of lymphocytes from the intestinal mucosa permitted the analysis of cell surface proteins that may more closely reflect in vivo expression. Using this approach, we detected constitutive LIGHT expression on both CD8⁺ and CD4⁺ T cells, as well as NK cells (Fig. 1). These observations correlate with the semiactivated state of LP T cell population (39), and denote the applicability of previous in vitro analysis indicating enhanced potential for LIGHT expression on LP T cells (11). From studies of mice, constitutive expression of LTαβ and TNF is a crucial mechanism serving as a homeostatic regulatory pathway between lymphocytes and the stroma to control tissue microenvironments within lymphoid organs. The work in this study demonstrating constitutive LIGHT expression in T cells resident in mucosal lymphoid tissue and a unique subset of mucosal trafficking T cells in blood suggests the involvement of LIGHT in homeostatic immune regulation.

Intensive immune interaction with intestinal Ags at the mucosal interface plays a key role in IBD pathology (5, 44, 46). However, T cell maturation and repertoire shaping via Ag exposure are not sufficient in themselves for the induction of an inflammatory response, given that Ag-specific T cells can be regulatory as well as effector cells (47). Constitutive LIGHT expression on NK cells that are effective IFN-γ producers (Fig. 1), and an almost exclusive LIGHT expression by IFN-γ-producing CD4⁺ T cells (11), further link LIGHT to a skewed Th1 response associated with CD pathology (6, 7). Moreover, LIGHT expression on NK cells could potentially play an additional role mediating NK regulation of DC cells, thus modulating Ag-presenting functions in the gut mucosa and perturbing the mucosal immune repertoire (4, 48).

We recently reported an enhanced potential for LIGHT expression on LP and mature peripheral CD4⁺ T cells (CD45RO), presumed to have encountered Ag (11). Because the gut is the primary

FIGURE 6. LIGHT is induced de novo by CD2 via a pre-existing transcriptional mechanism. PHA-treated PBMC were activated with P/I, CD2, or CD2 following 15-min incubation with CHX. Cells were then surface stained with anti-LIGHT Ab and anti-CD3/8. Lymphocyte-gated, CD4/8⁺ T cells following activation are plotted (shaded), in reference to the unstimulated profile (unshaded) for: A, P/I-activated cells, or B, CD2-activated cells. C, LIGHT mRNA levels were quantified by real-time RT-PCR in duplex with primer limiting 18S rRNA amplification. The 18S-adjusted threshold cycle for LIGHT signal is presented for the activation time indicated comparing mRNA levels in the presence of CHX treated (shaded) vs its absence (unshaded). Data are representative of a minimum of three experiments.

FIGURE 7. LIGHT mRNA expression is elevated in the inflamed and small bowel mucosa. RNA was extracted from biopsy specimens of an IBD patient cohort, and LIGHT mRNA levels were quantified by real-time RT-PCR in duplex with primer limiting 18S rRNA amplification. The 18S-adjusted threshold cycle for LIGHT signal is presented. A, Paired analysis comparing LIGHT mRNA levels from inflamed vs uninvolved sites for each patient. Solid lines indicate biopsy pairs with significantly elevated mRNA levels in the inflamed sample; dotted lines indicate pairs not varying significantly. B, Comparing LIGHT mRNA levels in small bowel vs colon (two-tailed p < 0.019).
sampling site for environmental Ags, we hypothesized that exposure to intestinal Ags may promote LIGHT expression preferentially in circulating gut T cells. Indeed, enhanced LIGHT expression was detected on T cells expressing integrin β7, as a gut-specific marker (Fig. 2A). Moreover, constitutive LIGHT expression was maintained in the periphery by small bowel-homing CD4+ T cells identified by β7 integrin/CCR9 expression (Fig. 2B), which represents the first PB T cell subset expressing LIGHT in the absence of cell-activating stimulation. Recently, Papadakis et al. (49) determined that the CD4+/CCR9+ T cell subset contains IFN-γ-producing cells and expresses several activation markers in the periphery. Constitutive LIGHT expression may define a unique subpopulation of CCR9+ T cells putatively producing IFN-γ, and consistent with predominant LIGHT expression on IFN-γ-producing CD4+ T cells (11).

Direct evidence for LIGHT expression in the intestinal mucosa was provided by the analysis of mRNA in intestinal biopsies indicating the presence of LIGHT transcript in the majority of tested specimens (Fig. 7). Higher LIGHT mRNA levels were detected in inflamed sites directly correlating with elevated potential for LIGHT protein expression in T cells from these sites (Fig. 7A) (11). However, expression in healthy as well as inflamed mucosa suggests that LIGHT immunoregulatory function may not be limited to the pathologically inflamed mucosa. In addition, higher LIGHT mRNA levels in small bowel biopsies (Fig. 7B) correlate with constitutive LIGHT expression on peripheral small bowel-specific (CD4+/CCR9+) T cells (Fig. 2B), as well as higher induced LIGHT levels unique to the small bowel compartment (11). These observations more specifically link LIGHT to the small bowel compartment and further support a linkage between LIGHT- and IFN-γ-mediated pathology because the small bowel is the primary activated organ in Th1-mediated CD (6). Elevated LIGHT mRNA levels in inflamed tissue as well as small bowel localization prompt for more extensive analysis of LIGHT expression levels in a larger cohort to examine correlation with disease state, localization, duration, and responsiveness to therapeutics.

HVEM is the primary receptor for LIGHT capable of transducing signals in T cells, modulating transcription via NF-kB as well as NF-kB-independent mechanisms (50). For example, LIGHT has been reported to provide T cell costimulation augmenting proliferation and cytokine production (18), and we recently demonstrated that HVEM signaling directly induces IFN-γ production in LP T cells (11). Consequently, perturbations in the LIGHT-HVEM signaling axis could directly induce chronic inflammation in the intestine, or putatively create a niche for opportunistic microorganisms exacerbating a mucosal immune response. Our data indicate intact expression and regulation of HVEM on intestinal T cells similar to peripheral T cells (Fig. 3), thus pointing to the regulation of LIGHT expression on activated T cells as a determining factor modulating signaling in the mucosa.

T cell activation by TCR-mediated stimulation is muted in the gut, to avoid harmful reactivity to the heavy antigenic load characteristic of the gut environment. However, CD2 provides an analogous Ag-independent activation pathway unique to gut T cells (32). CD2 cross-linking efficiently induced LIGHT expression on LP T cells following the rapid kinetic profile characteristic of the intestinal compartment (Fig. 4) (11). Interestingly, while LIGHT is preferentially induced on CD8 T cells (11, 38), a CD2-mediated signal was particularly effective in inducing LIGHT expression on CD4+ LP T cells, thus revealing a novel mechanism for LIGHT induction on this T cell subset (Fig. 4, A and B). Consequently, Ag-independent stimulation via CD2 could permit pathological immune activation mediated by LIGHT, and circumvent safeguards ensuring intestinal T cell tolerance. Nonetheless, additional complex gut-specific regulatory mechanisms must be in place, because CD2 responsiveness by itself could not confer the rapid LIGHT induction profile to PB T cells (Fig. 5).

LIGHT is transcribed following activation (Figs. 4C and 5C), and LIGHT mRNA was detected in gut cells (Figs. 1C, 2C, and 7), suggesting that LIGHT expression is regulated at the transcriptional level. Alternatively, intracellular stores of LIGHT have been reported (43), proposing a pre.synthesized protein pool that can be rapidly transported to the cell surface. Our data indicate that LIGHT induction is actinomycin D and CHX sensitive (Fig. 6), suggesting a requirement for transcription and de novo protein synthesis, and supporting LIGHT regulation primarily at the transcriptional level. Rapid LIGHT mRNA induction resistant to protein synthesis inhibitor, CHX, indicates the presence of preformed LIGHT transcriptional machinery in resting T cells (Fig. 6C), providing a rapid mechanism for LIGHT up-regulation. Furthermore, full-length LIGHT mRNA was not detected in unstimulated T cells (Figs. 5C and 6C), but a variant form of LIGHT has been described that lacks the transmembrane domain and cannot be displayed on the cell surface (29), most likely accounting for intracellular LIGHT detected in resting PB T cells (43). However, a more precise real-time protein-trafficking analysis will be required to exclude intracellular stores of LIGHT as a mechanism for rapid induction and to establish whether signaling activity may be terminated by shedding LIGHT (29).

In summary, our findings demonstrate for the first time that LIGHT is constitutively expressed on human mucosal T and NK cells. Furthermore, constitutive LIGHT expression on a subset of circulating small bowel-homing T cells and elevated LIGHT transcript levels in small bowel biopsies localize LIGHT expression to the small bowel compartment, suggesting a link to Th1-driven CD pathogenesis. In addition, activation of the CD2 pathway is proposed as a novel gut-specific, yet Ag-independent mechanism for LIGHT induction on CD4+ T cells. Finally, LIGHT regulation on T cells is proposed to occur primarily at the transcriptional level via a preformed transcriptional apparatus. In conclusion, gut-specific regulation of LIGHT could have significant pathological implications in human IBD, and thus, further investigation of LIGHT-associated immune regulatory mechanisms unique to the intestinal immune compartment is merited.

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References


