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*J Immunol* 2001; 167:2479-2486; 
doi: 10.4049/jimmunol.167.5.2479
http://www.jimmunol.org/content/167/5/2479

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The TNF Superfamily Members LIGHT and CD154 (CD40 Ligand) Costimulate Induction of Dendritic Cell Maturation and Elicit Specific CTL Activity

Yannis Morel,* Alemseged Truneh,§ Raymond W. Sweet,§ Daniel Olive,2*‡ and Régis T. Costello*†

LIGHT is a recently identified member of the TNF superfamily that is up-regulated upon activation of T cells. Herpesvirus entry mediator, one of its receptors, is constitutively expressed on immature dendritic cells (DCs). In this report, we demonstrate that LIGHT induces partial DC maturation as demonstrated by Ag presentation and up-regulation of adhesion and costimulatory molecules. LIGHT-stimulated DCs show reduced macrophagocytosis and enhanced allogeneic stimulatory capacity but fail to produce significant amounts of IL-12, IL-6, IL-1β, or TNF-α compared with unstimulated DCs. However, LIGHT cooperates with CD154 (CD40 ligand) in DC maturation, with particular potentiation of allogeneic T cell proliferation and cytokine secretion of IL-12, IL-6, and TNF-α. Moreover, LIGHT costimulation allows DCs to prime in vitro-enhanced specific CTL responses. Our results suggest that LIGHT plays an important role in DC-mediated immune responses by regulating CD154 signals and represents a potential tool for DC-based cancer immunotherapy. The Journal of Immunology, 2001, 167: 2479–2486.

Dendritic cells (DCs)3 are professional APCs that play a crucial role for the initiation of the immune response of both helper and cytotoxic T lymphocytes (1). DCs reside in many tissues in an immature state characterized by the ability to capture and present Ags. Following Ag uptake, DCs undergo a maturation process and migrate to the T cell areas of lymph nodes. During maturation, DCs decrease their Ag-processing capacity, increase cell surface expression of MHC and costimulatory molecules, and acquire the ability to produce IL-12. These phenotypic and functional changes correlate with their capacity to induce primary T cell responses.

CD40 is member of TNFR family that plays a pivotal role in both cell-mediated as well as humoral responses. CD40 has wide tissue and cell distribution including B lymphocytes, monocytes, hematopoietic progenitors, DCs, endothelial cells, and epithelial cells. In contrast, CD154, the ligand for CD40, is mainly expressed on activated CD4+ T lymphocytes. CD40 triggering on DCs induces phenotypic and functional maturation of DCs (i.e., up-regulation of costimulatory molecules such as CD54, CD58, CD80, and CD86) and enhanced capacity to induce T cell proliferation and cytokine secretion including IL-1, IL-6, IL-8, IL-10, IL-12, TNF-α, and macrophage-inhibitory protein 1α (2). In vivo activation of APCs, presumably DCs, through CD40 cross-linking can replace the requirement for CD4+ T cell help for the induction of CTL responses (3–5).

LIGHT is a recently identified member of the TNF superfamily (6) that, like CD154, is expressed on activated T lymphocytes (7), but also on monocytes, granulocytes, and immature DCs (iDCs) (8, 9). LIGHT recognizes three different members of the TNFR family, herpesvirus entry mediator (HVEM), lymphotoxin β receptor (LTβR), and decoy receptor 3 (DcR3, TR6) (6, 10). HVEM was initially characterized as mediator of HSV-1 infection (11), and this process is inhibited by LIGHT (6). HVEM is broadly expressed on cells of the immune system, such as T and B lymphocytes, NK cells (12, 13), and DCs (14), but it is also expressed on endothelial cells. LTβR plays a key role in the development and organization of lymphoid tissue, but it is absent on mature T and B lymphocytes, primary monocytes, and peripheral DCs (15). DcR3, a TNFR lacking a transmembrane region, is predominantly expressed in lung tissue and the colon carcinoma cell line SW480 and may serve to modulate LIGHT function in vivo (10).

Functionally, LIGHT can mediate apoptosis of some tumor cells in vitro and in vivo (9, 16). Although this effect appeared to require coexpression at the cell surface of both HVEM and LTβR, recent studies demonstrated that LTβR expression is necessary and sufficient (17). LIGHT-mediated apoptosis activates death signals through selective recruitment of TNFR-associated factor 3 by LTβR, implicated by their colocalization. Through its interaction with HVEM, LIGHT is also an important costimulatory molecule for T cell activation. LIGHT stimulated T cell proliferation in a three-way MLR (16), which was inhibited by a neutralizing Ab to HVEM. Moreover, blockade of LIGHT inhibited DC-mediated allogeneic T cell responses (8). LIGHT stimulation of T cells activated NF-κB (8), induced production of IFN-γ (8), and led to down-modulation of HVEM (7). In vivo, LIGHT is implicated in the development of the T cell immune response in tumor and graft-vs-host disease models in the mouse, but the molecular mechanism of LIGHT function in these models remains to be elucidated (18).

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Received for publication February 14, 2001. Accepted for publication June 11, 2001.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was funded in part by the Groupement Entreprise Français Lutte Cancer, the Association pour la Recherche contre le Cancer, the Ligue Nationale contre le Cancer, and the Institut SmithKline Beecham, Paris.

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3 Abbreviations used in this paper: DC, dendritic cell; HVEM, herpesvirus entry mediator; LIGHT, homologous to lymphotoxins, shows inductible expression, and competes with herpes simplex virus glycoprotein D for HVEM, a receptor expressed by T lymphocytes, LTβR, lymphotoxin β receptor; DcR3, decoy receptor 3; iDC, immature DC; TRANCE, TNF-related activation-induced cytokine.
Because CD40-independent pathways are implicated in the T cell help for priming of CD8+ cytotoxic T lymphocytes by DCs (19), we examined the effect of LIGHT on DC maturation and their capacity to prime a CTL response.

Here we demonstrate that LIGHT, a new member of the TNF superfamily, costimulates CD154-induced DC maturation. Moreover, this costimulation increases DC conditioning to elicit, in vitro, an enhanced primary specific CTL activity against a tumor Ag.

Materials and Methods

Blood samples and cell separation

PBMC from healthy donors were isolated on Ficoll-Hypaque gradients (20). T lymphocytes were isolated as the CD2+ PBMC population, corresponding to cells that adhere to sheep erythrocytes (21) in the E rosetting technique but fail to adhere to plastic dishes after overnight incubation in medium plus 30% FCS.

CD154-, LIGHT-, and LIGHT plus CD154-transfected cell lines

Full-length cDNA of human CD154 and human LIGHT were cloned in pcDNA3.1Neo and pcDNA3.1Hygro (Invitrogen, Groningen, The Netherlands), respectively, and transfected alone or sequentially by electroporation (960 µF, 220V) into Ltk- murine fibroblasts. Stably transfected cells, selected by resistance to geneticin (Life Technologies, Rockville, MD), hygromycin B (L�vivitrogen), or both were then selected for ligand expression by three rounds of FACS sorting. CD32-transfected fibroblasts were a kind gift from Schering-Plough (Dardilly, France).

Culture conditions and DC generation

Culture experiments were performed in RPMI 1640 with 10% FBS (Bio-Whittaker, Walkersville, MD). For DC generation, T cell-depleted PBMC were depleted of nonadherent cells by a 4-h adhesion on plastic dishes. Adherent cells were extensively washed and then cultured in RPMI 1640 (Bio-Whittaker) and 10% FCS with GM-CSF (Novartis, Bern, Switzerland) at 100 ng/ml and IL-4 (a kind gift from Schering-Plough) at 10 ng/ml for 5 days. The medium was replenished with cytokines every 2–3 days. At day 5, final maturation was induced by the addition of irradiated (75 Gy) L cells at a ratio of 1:10 for an additional 72 h.

Flow cytometry studies

For cell surface staining, cells were processed following standard procedures, and analysis was performed on a FACScalibur flow cytometer (BD Biosciences, San Jose, CA). The mAbs directed against HVEM (12C5 and 20D4, both murine IgG1) and LIGHT (2C8, murine IgG2b) were generated at SmithKline Beecham Pharmaceuticals (King of Prussia, PA) by conventional hybridoma methodology from mice immunized with the respective recombinant proteins and screening the hybridomas by ELISAs. The mAbs to CD1a, CD3, CD4, CD8, CD14, CD19, CD25, CD40, CD54, CD56, CD69, CD83, and HLA-DR were purchased from Beckman Coulter (San Diego, CA). The mAb to CD80 was obtained from BD Biosciences, and the mAbs to CD86 and CD154 from BD Pharmingen (San Diego, CA). Cell surface CD40 and HVEM were quantified on iDCs by indirect immunofluorescence staining using QFIKIT (DAKO, Glostrup, Denmark).

Primary MLR

Serial dilutions (3 × 10^3–14 cells/well) of irradiated (25 Gy) stimulator cells were cultured in triplicate with 10^5 allogeneic naive CD4+ T cells in 96-well round-bottom plates (Costar, Corning Glass, Corning NY). Naive CD4+ T cells were prepared from purified T cells by three rounds of negative depletion using magnetic beads (Beckman Coulter) incubated with mAbs to CD8 and CD45RO (Beckman Coulter). Proliferation of T cells was monitored by measuring [methyl-3H]thymidine (1 µCi/well; Amersham, Little Chalfont, U.K.) incorporation during the last 16 h of a 6-day culture. Thymidine uptake was counted on a gas-phase beta counter (Mark 960; Packard Instruments, Meriden, CT). The iDCs or DCs matured by coincubation with L cells expressing CD154, LIGHT, or LIGHT plus CD154 were used as stimulators.

Macropinocytosis assay

Mannose receptor-mediated fluid phase macropinocytosis, measured by the cellular uptake of FITC-dextran, was used as a surrogate marker for Ag capture. On day 8, 10^5 of the iDCs and matured DCs were incubated in medium containing 0.5 mg/ml FITC-dextran (molecular mass of 40,000 kDa; Sigma-Aldrich, St. Louis, MO) for 1 h at 37°C or 4°C (negative control). After four washes with cold PBS, cells were analyzed by flow cytometry for uptake of the FITC-dextran.

Cytokine determination

When h 72 h of final maturation, DC cultures were harvested and cell-free supernatants were frozen. After thawing, cytokine concentrations were quantified by ELISA (IL-12p75, IL-1β (R&D Systems, Minneapolis, MN), IL-6, and TNF-α (Beckman Coulter)).

Induction of specific anti Melan-A CTLs

The iDCs and matured DCs were pulsed for 2 h at 37°C in serum free X-Vivo 15 (BioWhittaker, Walkersville, MD) with a 10-µg/ml Melan-A26–35 peptide analog (ELAGILGILTV; the kind gift of P. Coulie, Ludwig Institute for Cancer Research, Brussels, Belgium) (22) together with β2-microglobulin (3 µg/ml). After two washes, 2 × 10^5 peptide-pulsed DCs were cultured with 10^6 CD8+ purified T cells (95% by flow cytometry), obtained by two rounds of negative selection from purified T cells using anti-CD4 mAb (13B8.2), in 2 ml CTL medium in the presence of IL-2 (10 U/ml) and IL-7 (5 ng/ml). The CTL medium used was IMDM (BioWhittaker) supplemented with l-arginine (550 µM), l-asparagine (240 µM), l-glutamine (1.5 mM), 1% penicillin-streptomycin, and 10% pooled human serum. On day 7, CTLs were harvested and restimulated at the initial ratio with the corresponding peptide-pulsed DCs. Cytotoxic activity was measured on days 13–14 in the 51Cr-release assay described below.

Cytotoxicity assay

The T2 cell line was labeled by incubating 10^6 cells in 100 µCi sodium 51Cr-labeled chromate for 2 h at 37°C and washing three times. Labeled target cells (10^5) and serial dilutions of effector cells in triplicate were incubated in RPMI 1640 with 10% FCS in 96-well V-bottom plates at 37°C for 4 h in the presence or absence of Melan-A26–35 (1 µg/ml) and a 30-fold excess of unlabeled K562 cells. Supernatants were then analyzed in a microplate scintillation counter (TopCount; Packard Instruments). The percentage of lysis was determined for each triplicate experiment as ([experimental 51Cr release – spontaneous 51Cr release]/(maximal 51Cr release – spontaneous 51Cr release)) × 100. Results are expressed as the percentage of specific lysis (percentage of lysis in the presence of peptide – percentage of lysis in the absence of peptide).

IFN-γ-secretion assay

On days 13–14, 10^5 CTLs, obtained as previously described, were stimulated with 2 × 10^5 peptide-pulsed, autologous irradiated (25 Gy) PBMC. After 48 h, the levels of IFN-γ in the culture supernatants were measured by ELISA (OptEIA; BD Pharmingen).

Results

HVEM is constitutively expressed on iDCs, whereas LIGHT is induced upon T cell activation

HVEM, like CD40, is expressed on peripheral blood monocyte-derived iDCs but at a lower level. LIGHT and CD154 are not expressed on resting T lymphocytes, but both are up-regulated at the cell surface following activation (Ref. 7 and Fig. 1A). These cellular distributions suggest that, similar to CD154 and CD40, the interaction of LIGHT with HVEM is important in T cell communication with DCs. Thus, we investigated the effects of CD154 and LIGHT, alone or in combination, on the maturation of DCs.

LIGHT induces phenotypically mature DCs

The iDCs were generated from T cell-depleted adherent PBMC by a 5-day culture in the presence of GM-CSF and IL-4. They were then incubated for 72 h with irradiated (25 Gy) PBMC. After 48 h, the levels of IFN-γ in the culture supernatants were measured by ELISA (OptEIA; BD Pharmingen).

Results

HVEM is constitutively expressed on iDCs, whereas LIGHT is induced upon T cell activation

HVEM, like CD40, is expressed on peripheral blood monocyte-derived iDCs but at a lower level. LIGHT and CD154 are not expressed on resting T lymphocytes, but both are up-regulated at the cell surface following activation (Ref. 7 and Fig. 1A). These cellular distributions suggest that, similar to CD154 and CD40, the interaction of LIGHT with HVEM is important in T cell communication with DCs. Thus, we investigated the effects of CD154 and LIGHT, alone or in combination, on the maturation of DCs.
up-regulation of the adhesion molecule CD54. Moreover, LIGHT stimulation induced DC cluster formation, there was little effect on CD80. Consistent with the costimulatory molecules CD86 and CD40, but unlike CD154-stimulated DCs, there was little effect on CD80. Consistent with the observation of DC cluster formation, LIGHT stimulation induced up-regulation of the adhesion molecule CD54. Moreover, LIGHT down-regulated its own receptor, HVEM, as previously observed on T lymphocytes (7). The iDCs cocultured with L cells expressing both LIGHT and CD154 acquired a mature phenotype distinct from that obtained by CD154 or LIGHT stimulation alone. In comparison to stimulation with CD154 alone, these DCs expressed higher levels of HLA-DR, CD54, and costimulatory molecules (particularly CD86, with the mean fluorescence intensity rising from 1014 ± 235 for CD154 stimulation alone to 1646 ± 310 for LIGHT plus CD154 stimulation; n = 5 and p < 0.01) and showed down-modulation of HVEM. These phenotypic changes were specific for CD154 and LIGHT stimulation because addition of mAbs to CD154 and/or LIGHT (10 μg/ml) completely inhibited the elaboration of these markers. Similarly, iDCs cultured with CD32-transfected L cells maintained the immature phenotype of CD83+/HLA-DR++CD80++CD86+++. Moreover the effect of LIGHT on DC maturation is inhibited by blocking anti-HVEM mAb (18D4) (Fig. 2B).

LIGHT down-regulates pinocytic activity and cooperates with CD154 to enhance DC-mediated allogeneic T cell responses

During the maturation process, DCs lose their ability to capture exogenous Ag and, in turn, acquire potent Ag-presenting capacity. We examined the effect of LIGHT stimulation on these functional parameters of DC maturation. On day 8, iDCs, CD154−, LIGHT−, and LIGHT plus CD154-matured DCs, generated as described above, were harvested and evaluated for their macrophagic activity. FITC-dextran uptake was measured by flow cytometry and the results expressed as percentage of positive cells (Fig. 3A). Under the control condition of incubation with CD32 L cells, most of the iDCs (79 ± 4% of positive cells) captured FITC-dextran, whereas few of the CD154-matured DCs (12 ± 7% positive cells) showed uptake. LIGHT stimulation resulted in the loss of the capacity to take up FITC-dextran for a subpopulation of the DCs (47 ± 4% positive cells). This subpopulation corresponds to the CD83− DCs (Fig. 3B), consistent with the expected phenotype of mature DCs. As expected, LIGHT plus CD154-matured DCs, like CD154-matured DCs, did not capture FITC-dextran (9 ± 1% positive cells).

The Ag-presenting capacity of these differentially matured DCs was then investigated in a primary allogeneic MLR (Fig. 3C). CD154-matured DCs show a stronger capacity to activate T cells compared with iDCs. LIGHT-matured DCs showed only modest enhancement of T cell proliferation and only at DC:T cell ratios of 1:10.

LIGHT synergizes with CD154 for some cytokine secretion

The DC maturation process is associated with cytokine synthesis. Secretion of immunoregulatory and proinflammatory cytokines plays a pivotal role during T cell priming in the lymphoid organs. As expected, CD154 stimulation induced significant levels of secreted IL-12, IL-6, TNF-α, and IL-1β compared with those produced by unstimulated DCs (Fig. 4). Consistent with their weak effect in the MLR, LIGHT-stimulated DCs only modestly induced IL-12 and TNF-α and had no effect on IL-6 and IL-1β relative to

<table>
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<th>Expression levels of stably transfected L cells*</th>
<th>Mean Fluorescence Intensity</th>
<th>CD154</th>
<th>LIGHT</th>
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<td>CD154− L cells</td>
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<td>247 ± 47</td>
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<td>LIGHT + CD154− L cells</td>
<td>111 ± 16</td>
<td>CD154</td>
<td>132 ± 22</td>
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* L cells are labeled by anti-CD154 and anti-LIGHT mAbs followed by FITC-conjugated goat anti-mouse IgG. Mean fluorescence intensity of corresponding isotypic control is subtracted. Data represent the mean of six independent experiments.
control unstimulated DCs. This low response could not be attributed to a toxic effect of LIGHT on cytokine secretion pathways because IL-8 secretion was not inhibited (data not shown). Moreover, LIGHT cooperated with CD154 to induce significantly higher levels of IL-12, IL-6, and TNF-α than observed for CD154 alone. IL-12 production raised from 2,958 ± 1,703 pg/10⁶ cells to 17,165 ± 6,489 pg/10⁶ cells, IL-6 production raised from 1,649 ± 344 pg/10⁶ cells to 20,395 ± 14,335 pg/10⁶ cells, and TNF-α production raised from 1,646 ± 527 pg/10⁶ cells to 13,522 ± 4,961 pg/10⁶ cells (p < 0.05). In contrast, this combination had little effect on IL-1β secretion.

LIGHT and CD154 synergize in the priming of specific anti-tumor CTLs and their production of IFN-γ

Recent publications (3, 4, 23) have proposed a two-step model for the induction of cytotoxic T cell responses in which DCs play the role of “temporal bridge.” First Th cells activate the DCs via the interaction of CD154 and CD40. Then, DCs are conditioned to prime CTL responses. We developed an in vitro model of CTL priming against a tumor Ag consistent with this hypothesis. iDCs and matured DCs were pulsed with Melan-A26–35 peptide and incubated with autologous, purified CD8⁺ T cells. After two rounds of stimulation, CD8⁺ T cells were tested for their anti-Melan-A CTL activity against T2 cells (Fig. 5A). LIGHT-matured DCs, like iDCs, were unable to prime CTL activity. As predicted by the model described above, CD154-matured DCs induced Melan-A-specific lysis ranging from 8 ± 1% to 18 ± 2%, at E:T ratios of >10:1. LIGHT plus CD154-matured DCs induced a marked increase of the anti-Melan-A cytotoxic activity relative to CD154 alone, showing specific lysis from 17 ± 2% to 34 ± 1% over the same range of E:T ratios.

IFN-γ levels were measured to further assess the relative priming activity of the DCs matured in the presence of LIGHT and/or CD154. On days 13–14 after the initial DC stimulation, T cells were harvested, challenged for 48 h with autologous, irradiated Melan-A-pulsed PBMC, and tested for levels of IFN-γ secretion. Consistent with the cytotoxicity assays, LIGHT-matured DCs, much like iDCs, failed to prime T cells for IFN-γ secretion. CD8⁺ T cells obtained by coculture with CD154-matured DCs produced only a modest level of IFN-γ. In sharp contrast, CD8⁺ T cells primed by LIGHT plus CD154 DCs secreted large amounts of IFN-γ.

Discussion

In this study, we demonstrate that LIGHT, a recently identified member of the TNF superfamily, cooperates with CD154 to induce maturation of monocyte-derived DCs and increases CD154-induced DC conditioning for CTL priming. DCs are potent APCs that control the development of T cell-mediated immune responses (1). In their immature state, DCs capture Ag or apoptotic cells.
from sites of infection, inflammatory lesions, or tissue damage and
process the Ags for subsequent antigenic peptide loading to MHC
class I and class II molecules and presentation to CD8 and CD4 T
cells, respectively. Following the stage of Ag capture, they begin
to mature and migrate to the T cell areas of lymphoid organs to
initiate the adoptive immune responses. The maturation process is
a complex but sequentially highly ordered process. Among the
factors that contribute to this process are bacterial and viral prod-
ucts such as LPS and dsRNA (24), which activate DCs, resulting
in up-regulation of adhesion and costimulatory molecules and
down-regulation of endocytic activities. Inflammatory cytokines
TNF-α and IL-1β (25) or the activated T cell molecules CD154
and TNF-related activation-induced cytokine (TRANCE) (26, 27)
represent endogenous stimuli that enhance the stimulatory capacity
of DCs.

LIGHT, like CD154 and TRANCE, is a member of the TNF
family that is induced on T cells following activation (7). Upon
LIGHT stimulation, only a fraction of monocyte-derived iDCs ac-
quire the fully mature phenotype characterized by expression of
CD83 and high levels of HLA-DR. This is not due to insufficient
level of LIGHT because stimulation at increased ratios of LIGHT
L cell:DC or stimulation with L cells expressing 3-fold higher
levels of LIGHT on their surface does not improve the DC re-
sponse (data not shown). Furthermore, the LIGHT L cells used in

**FIGURE 3.** A, LIGHT reduces macropinocytic activity of DCs. On day 8, iDCs (cocultured with CD32 L cells) or CD154-, LIGHT-, and LIGHT plus CD154-matured DCs were incubated at 37°C or 4°C (negative control) for 1 h in the presence of FITC-dextran (0.5 mg/ml). Results are expressed as the percentage of positive cells and represent the mean of three independent experiments performed with different healthy blood donors. *, p < 0.05 (stimulated vs control). B, CD83-positive LIGHT-matured DCs have reduced macropinocytic activity. On day 8, LIGHT-matured DCs were incubated at 37°C or 4°C (negative control) for 1 h in the presence of FITC-dextran (0.5 mg/ml) and were then stained with CD83-PE mAb. The histograms show FITC-dextran
uptake for the negative control (dotted line), CD83-negative LIGHT-treated DCs (solid line), and CD83-positive LIGHT-treated DCs (bold line). C, LIGHT cooperates with CD154 to enhance the capacity of DCs to activate allogeneic naive T cells. A total of 10⁵ purified peripheral naive T cells
(CD4⁺CD45RA⁺CD45RO⁻) were stimulated by serial dilutions (3 × 10⁴-14 cells/well) of irradiated (25 Gy) iDCs, CD154-, LIGHT-, or LIGHT plus CD154- matured DCs on day 8. The proliferative response was measured by [³H]thymidine incorporation during the last 16 h of a 6-day culture. Background T cell proliferation was <100 cpm. Results are expressed as mean cpm ± SD and are representative of four independent experiments. **, p <
0.05 (LIGHT plus CD154-stimulated DCs vs CD154-stimulated DCs).
this study expressed similar cell surface levels to activated T cells (see Fig. 1A). Moreover, because LIGHT interaction with HVEM appears to down-modulate this receptor (7), the absence of HVEM on a majority of DCs stimulated with LIGHT (Fig. 2) indicates that most cells responded. Because LTβR is not on T and B cells or DCs (15), and because blocking anti-HVEM mAb (12) inhibits LIGHT-mediated DC maturation (Fig. 2B), LIGHT presumably stimulates DCs through its interaction with HVEM. Based on our data, even if HVEM seems to be involved in LIGHT-mediated DC maturation, there is no evidence to rule out the implication of other LIGHT receptors (e.g., LTβR and DcR3), in the phenomenon observed. Moreover, DcR3 mRNA, even if predominantly expressed

**FIGURE 4.** LIGHT synergizes with CD154 for some cytokine secretion. The iDC were stimulated for 72 h by CD32 (control), CD154-, LIGHT-, or LIGHT plus CD154-transfected L cells. Supernatants were then harvested and tested by ELISA for IL-12p75, IL-6, TNF-α, and IL-1β. Results are expressed as picograms per 10⁶ cells and are the mean ± SD of four to five experiments performed with different donors. *, p < 0.05 (stimulated vs control); **, p < 0.05 (LIGHT plus CD154-stimulated DCs vs CD154-stimulated DCs).

**FIGURE 5.** A, LIGHT and CD154 synergize for induction of specific antitumoral CTL activity. A total of 10⁶ CD8⁺ purified T cells were cultured with 2 × 10⁵ Melan-Α₂₆–₃₅ peptide-pulsed iDCs, CD154-, LIGHT-, or LIGHT plus CD154-matured DCs in the presence of IL-2 (10 U/ml) and IL-7 (5 ng/ml). On day 7, CTLs were harvested and restimulated at the initial ratio with the corresponding peptide-pulsed DCs. On days 13–14, cytotoxic activity of the resulting CTL cultures was tested at E:T ratios ranging from 100:1 to 0.4:1 in a standard 4-h ⁵¹Cr-release assay on T2 targets in the presence or absence of Melan-Α₂₆–₃₅ peptide (1 µg/ml). Results are expressed as percentage of specific lysis = the percentage of lysis in the presence of peptide – the percentage of lysis in the absence of peptide. One representative experiment of three performed is shown. *, p < 0.05 (stimulated vs control); **, p < 0.05 (LIGHT plus CD154-stimulated DCs vs CD154-stimulated DCs). B, LIGHT costimulation induces enhanced IFN-γ secretion by CTLs upon restimulation. On days 13–14, the resulting CTLs were harvested and restimulated at the initial ratio by autologous Melan-Α₂₆–₃₅-pulsed, irradiated (25 Gy) PBMC. After 48 h, supernatants were harvested and tested by ELISA for IFN-γ secretion. One representative experiment of three performed is shown.
in lung tissue and in the SW480 cell line, is weakly expressed in most hematopoietic cells (10), including DC (data not shown). Because DcR3 is a soluble decoy receptor for LIGHT, it might regulate LIGHT stimulation in our experimental system.

LIGHT-stimulated DCs are unable to secrete significant amounts of IL-12, IL-6, TNF-α, and IL-1β compared with unstimulated DCs. The weak cytokine secretion cannot be attributed to insufficient stimulation or to a different kinetics than CD154 stimulation. Although signaling via HVEM alone is inefficient at inducing cytokine production, it synergizes with CD40-mediated IL-12, IL-6, and TNF-α, but not IL-1β secretion. The molecular basis of this synergy remains to be elucidated.

Generation of specific CTL responses by the immune system may provide a therapeutic approach to cancer. Cross-presentation is a mechanism that allows exogenous Ags such as tumor, viral, or transplantation Ags to be presented to class I-restricted CTLs by the APC (28, 29). It has been demonstrated that induction of specific CD8+ CTL response by such a cross-presentation mechanism requires cognate CD4+ T cell help (30). This CD4+ T cell help is particularly necessary under noninflammatory conditions, as occur in most cancers, including noninflammatory persistent tumor vi-
ruses (human papillomavirus or EBV) (31). Recent papers (3, 4, 23) have demonstrated that the CD4+ T cell help for the cytotoxic T cell response can be bypassed by activation of APCs through CD40. APCs also function as a temporal bridge in that, once activated, APCs areconditioned to deliver a “license to kill” to CD8+ CTLs. Ridge et al. (23) have implicated DCs in this model, suggesting that the activation state of DCs is more important than the CD4+ T cell help itself (31). In our in vitro CTL induction experiments, we used phenotypically defined DC populations, as underscored by Schuurhuis et al. (32). Human monocyte-derived iDCs express low levels of HLA class II and costimulatory molecules. Upon coculture with CD154 L cells, these DCs exhibit the characteristic phenotype of mature DCs (1), i.e., expression of CD83 and high levels of HLA class II and costimulatory mole-
cules. The LIGHT plus CD154-costimulated DCs show a similar phenotype of mature DC but with down-regulated HVEM expres-
sion and with enhanced functional capacity. We developed an in vitro model of CTL differentiation against the human melanoma Ag Melan-A/Mart-1 to test the specific CTL-presentation capacity of these differentially matured DCs. CD154-matured DCs elicit mod-
erate anti-Melan-A cytotoxic activity, whereas LIGHT plus CD154-costimulated DCs are able to prime a significantly en-
hanced anti-Melan-A cytotoxic response. The difference between the similar phenotype and the CTL-presentation capacity of LIGHT plus CD154+ vs CD154-stimulated DCs is interesting, and some hypotheses can be proposed. First, costimulation molecules, particularly CD86, are expressed at higher levels on LIGHT plus CD154-stimulated DCs, allowing an increased stimulation of CTL precursor. Then, LIGHT plus CD154-stimulated DCs can produce higher levels of IL-12, IL-6, and TNF-α, which could increase the expansion of CTLs. Taken together, these data suggest that help for cytotoxic T cell responses mediated by CD40 signaling can be modulated by LIGHT costimulation. These results are consistent with recent studies examining the role of LIGHT in tumor and graft-vs-host disease models in mice (18). Transfection of LIGHT cDNA into P815 tumor cells enhanced their immunogenicity. This effect was not due to LIGHT-induced apoptosis, as has been ob-
served for the human colon carcinoma line HT29 (16) and the human breast carcinoma MDA-MB-231 (9). We conclude that, in addition to its costimulatory effect on T cell activation, LIGHT costimulates DCs by modulating CD40 signals. The DCs then achieve an activation state in which they are able to elicit an en-
hanced anti-tumoral CTL response.

CD40-independent pathways of T cell help for CTL priming have been reported (19). In addition to LIGHT, these pathways may involve other TNF/TNFRI family members. TRANCE/recep-
tor activator of NF-κB ligand is reported to enhance the adjuvant properties of DCs in a in vivo model of delayed-type hypersensitivity (33). OX40 ligand is expressed on matured DCs (34), and mice lacking this gene show an impaired APC function in both priming and effector phases of T cell activation (35). Thus, we propose that the model for priming of CTLs by Th cell-matured DCs (19) could be extended to include the LIGHT-HVEM inter-
action and perhaps other TNF/TNFRI family members. In initial studies, immunization of human subjects with autologous ex vivo-modified DCs efficiently primed and boosted CD4+ and CD8+ T cells (36). Our results suggest that LIGHT/HVEM may enhance this response and lead to applications in DC-meditated cancer immunotherapy.

Acknowledgments

At SmithKline Beecham Pharmaceuticals, we thank T. Wattam and S. Holmes (Molecular and Cell Culture) for preparation of the mAbs to LIGHT and HVEM and K. Deen for discussions in the early stages of this project. We also thank A.-M. Schmitt-Vershal (Centre d’Immunologie, Marseille-Luminy), C. Mawas, B. Gaugler, and Y. Collette (Institut Paoli Calmettes, Marseille, France) for helpful advice and for critically reading the manuscript. We thank P. Romero (Ludwig Institute for Cancer Research, Lausanne, Switzerland) for helpful advice in CTL generation and B. Barbarat, F. Mallet, and S. Just-Landi for technical assistance.

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