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*J Immunol* 2011; 187:3488-3492; Prepublished online 31 August 2011;
doi: 10.4049/jimmunol.1101105
http://www.jimmunol.org/content/187/7/3488

Supplementary Material

http://www.jimmunol.org/content/suppl/2011/08/31/jimmunol.1101105.DC1

References

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*The Journal of Immunology* is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
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Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Cutting Edge: Virus Selectively Primes Human Langerhans Cells for CD70 Expression Promoting CD8+ T Cell Responses

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The two outermost compartments of skin are populated by different Ag-presenting dendritic cell types. Epidermal Langerhans cells (LCs) are evolutionarily adapted to the continuous presence of harmless skin commensals by the selective lack of cell surface TLRs that sense bacteria. In this article, we analyze the ability of LCs and dermal dendritic cells (DDCs) to respond to virus infection. Live virus and intracellular TLR3-agonist dsRNA commit LCs more effectively than DDCs to stimulate naive CD8+ T cell expansion and their differentiation into effector cells. This potent CD8+ T cell-promoting capacity of LCs is causally related to high levels of virus-induced CD70 expression but not to IL-12 production. These data suggest a remarkable specialization of LCs in the induction of pathogen class-specific adaptive immunity. Whereas LCs ignore bacteria, they are superior to DDCs to initiate effective CD70-mediated CD8+ T cells in response to virus stimulation. The Journal of Immunology, 2011, 187: 3488–3492.

Protective immunity against different classes of pathogens requires different classes of T cell-mediated immune responses. The development of an appropriate type of effector T cells during infection is initiated by pathogen-induced commitment of dendritic cells (DCs), resulting in the presentation of pathogen-derived peptides to naive T cells in the context of signal 3 molecules that polarize the activated T cells into specialized effector T cells (1, 2). A well recognized concept is that DCs obtain T cell-polarizing properties by sensing different classes of pathogens via different groups of cell surface-, cytoplasmatic-, or endosomal pattern-recognition receptors (PRRs) (3, 4). Accordingly, viruses are recognized by cytoplasmic and endosomal PRRs, which programs DCs for development of cytotoxic effector CD8+ T cells. Protective CD8+ T cell-mediated immunity largely depends on the ability of CD8+ T cells to expand and acquire effector molecules, including the cytotoxic molecules granzyme B (GrB) and perforin as well as antiviral cytokines such as IFN-γ and TNF-α (5, 6). DC-derived mediators that drive the development of effector CD8+ T cells include IL-12 (1, 7) and the costimulatory cell surface molecule CD70, a TNF superfamily member that ligation CD27 (8–10).

DCs form a heterogeneous population with regard to differences in lineage and localization in tissue compartments. DC subtypes can display intrinsic differences in pathogen recognition and T cell-polarizing capacity. We and others have recently shown that, in contrast to dermal DCs (DDCs), TGF-β–driven development of human epidermal Langerhans cells (LCs) is associated with a lack of expression of cell surface TLRs 2, 4, and 5, resulting in their selective inability to respond to bacteria (11, 12). This unresponsiveness probably prevents T cell-mediated pathology upon superficial injury and subsequent entry in the epidermal compartment of relatively harmless commensal bacteria that permanently adhere to healthy skin (13). However, viruses entering the epidermis are always a potential danger. Therefore, an important question is whether LCs do effectively initiate CD8+ T cell immunity. Indeed, both LCs and DDCs express PRRs that recognize important viral RNA or DNA patterns (e.g., similar levels of endosomal TLR3 (12)). Interestingly, human LCs more effectively prime allogenic CD8+ T cells in homeostatic conditions (14). In this study, we analyzed the relative role of LCs in the initiation of virus-driven CD8+ T cell responses.

Materials and Methods

Priming LCs and DDCs

Human LCs and DDCs from random healthy donors generated as previously described (12) were stimulated for 48 h with p(I:C) (20 μg/ml; Sigma-Aldrich), influenza virus (FLU) A/PR/8/34 (a gift from G. Rimmelzwaan, Erasmus Medical Center, Rotterdam, The Netherlands), or R848 (Invivogen) and analyzed by FACS, using anti–CD83-APC, anti–CD86-PE, anti–CD70-FITC, and anti–HLA-DR-PerCP (BD Biosciences), anti–HLA-ABC-FITC (Serotec), anti–ICAM-1 (Immunotech), anti–CD70 (BD Biosciences), anti–ICAM-1 (Immunotech), anti–CD70 (BD Biosciences).
and PE-conjugated goat anti-mouse (Jackson ImmunoResearch Laboratories), IL-12p70 levels in 24-h supernatants were determined by ELISA as described previously (15).

**Isolation of naive CD8+ T cells**

PBL were obtained from peripheral blood by Ficoll-Hypaque (Nycomed) and Percoll (Pharmacia) density gradients. CD8+ T cells were isolated with a MACS kit (Miltenyi Biotec). CD27hiCD45RA−CD45RO+CD8+ T cells were purified by sort using CD27-PE, CD45RA-FITC, CD45RO-allophycocyanin, and CD8-allophycocyanin-cy7 (BD Biosciences).

**T cell assays**

Primed LCs and DDCs from the same donor were counted and cocultured in a flat-bottom 96-wells plate with allogeneic naive CD8+ T cells (1:4) with Staphylococcus aureus enterotoxin B (0.1 pg/ml; Sigma-Aldrich) in the absence or presence of neutralizing mAbs (10 μg/ml) against human CD70 (16), IL-12 (20C2), or control Abs (U-Cytech). Proliferation was measured by [3H]thymidine incorporation at days 3 and/or 4. Cells were analyzed for intracellular cytokines (IFN-γ, IL-2, TNF-α, and IL-12p70 levels in 24-h supernatants were determined by ELISA as described previously (15).

Cytotoxic activity was measured by using EBV-transformed B cells (JY) labeled with [51Cr] (PerkinElmer) as target cells. Target cells and the CD8+ effector T cells induced by LCs and DDCs were counted and cocultured at indicated E:T ratios at 37˚C in the presence of S. aureus enterotoxin B (5 ng/ml). Chromium release was measured after 4 h, and specific lysis was calculated as (experimental release − spontaneous release)/total release × 100.

**Statistics**

Significance was analyzed with GraphPad (GraphPad InStat) using paired Student t test for data of LCs and DDCs from the same donor. A p value <0.05 was considered significant.

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**FIGURE 1.** LCs primed with p(I:C) or FLU are potent in activating naive CD8+ T cells. Proliferation of allogeneic naive CD8+ T cells stimulated with DDCs and LCs primed by p(I:C) (A) or whole FLU (B). Different DC:T cell ratios were tested. Data show mean ± SEMs of one representative experiment of at least 10 independent experiments. C. Intracellular IFN-γ, TNF-α, and IL-2 expression by CD8+ T cells stimulated by p(I:C)- or FLU-primed DDCs or LCs. Graphs show data of at least five independent experiments. *p < 0.05; **p < 0.01.

**FIGURE 2.** LCs primed with p(I:C) or FLU induce higher cytotoxic activity in naive CD8+ T cells than DDCs. A. GrB expression by CD8+ T cells at days 3 and 5 after stimulation by DDCs and LCs primed with p(I:C) or FLU. Data are representative for at least 10 independent experiments (B, C). Cytotoxic activity of CD8+ T cells stimulated with p(I:C)- or FLU-primed DDCs or LCs, as assessed using a [51Cr] release assay. The maximum release was set at 100%. Graphs show cytotoxic activity of activated CD8+ T cells in six independent experiments at an E:T ratio of 10:1 (*p < 0.05; (B) and cytotoxic activity with different E:T ratios (one representative experiment out of six) (C).

**Results and Discussion**

To assess to what extent LCs and DDCs initiate antiviral T cell responses, the two DC subsets were stimulated with polyinosinic-polycytidylic acid [p(I:C)], a TLR3-agonist dsRNA to mimic viral priming, or exposed to live FLU and analyzed for their ability to activate allogeneic naive CD8+ T cells. FLU was chosen as a model virus because it is recognized by various intracellular PRRs that are expressed by both LCs and DDCs and does not exploit immune evasion strategies that substantially interfere with the induction of T cell responses. LCs were much better stimulators of naive CD8+ T cell proliferation than DDCs after priming with p(I:C) or FLU (Fig. 1A, 1B), which was not associated with different sensitivity of apoptosis of these DC types (data not shown). No or only low T cell proliferation was induced by immature LCs or LCs primed by bacteria-associated TLR ligands (data not shown). This is in line with the finding that LCs do not respond to bacterial ligands (12). Next, we investigated the capacity of LCs to induce functional differentiation of naive CD8+ T cells by analyzing the expression of molecules characteristic for effector functions. The numbers of CD8+ T cells expressing the antiviral cytokines IFN-γ and TNF-α were significantly higher within the T cell population induced by LCs than by DDCs (Fig. 1C). Interestingly, LCs also more effectively induced IL-2–producing CD8+ T cells than DDCs (Fig. 1C). Because IL-2 is important for T cell proliferation, the high IL-2 production may contribute to the strong CD8+ T cell proliferation induced by LCs.

Importantly, LCs were also more potent than DDCs in inducing the production of the cytotoxic effector molecule GrB. LCs not only promoted GrB expression in naive CD8+ T cells more rapidly than DDCs, but also induced higher levels of GrB in markedly more CD8+ T cells (Fig. 2A). In line with the higher expression of effector molecules, the CD8+ T cells activated by LCs were significantly more potent...
in killing target cells (Fig. 2B) than p(I:C)- and FLU-primed DDCs at all E:T cell ratios tested (Fig. 2C). These data collectively show that p(I:C)- and FLU-primed LCs are highly efficient in the induction of cytotoxic effector CD8+ T cells that have a greater cytolytic activity on a per cell basis.

We subsequently investigated the mechanisms underlying the superior induction of CD8+ effector T cells by LCs. As shown in Fig. 3A, we found that LCs and DDCs express similar levels of the costimulatory molecules CD86, CD80 and CD83, as well as Ag-presenting MHC class I and MHC class II molecules, upon priming by p(I:C) or FLU. These cells also express similar levels of ICAM-1, which is known to promote CD8+ T cell activation (17). Furthermore, LCs and DDCs produced comparable amounts of IL-12 upon priming with p(I:C) and FLU (Fig. 3B), which is in line with our previous findings (12). Surprisingly, a major difference between LCs and DDCs is that CD70 was consistently upregulated only in LCs after stimulation with p(I:C) or FLU (Fig. 3C), which was not dose dependent (i.e., not related to a difference in poly(I:C) sensitivity between the DC subsets. In a minority of the DC donors, CD70 is also upregulated in LCs upon priming with R848, a synthetic ligand of the intracellular TLR7 and TLR8 which both recognize single stranded RNA (Supplemental Fig. 1). As reported before (12), LCs selective lack cell surface PRRs and did not respond at all to whole bacteria or to classical agonists of cell surface sensors of bacteria. Notably, CD70 was not upregulated upon stimulation of DDCs by any other cell surface or intracellular TLR ligands tested, including ligands of TLR2, 3, 4, and 7/8 (data not shown). Moreover, LCs and DDCs did not upregulate other TNF superfamily members CD134L (OX40L) or CD137L (4-1BB) in any tested condition (data not shown).

To assess the contribution of virus-induced CD70 to the capacity of LCs to generate effector T cell responses, we cocultured p(I:C)- or FLU-primed LCs with naive CD8+ T cells in the presence of neutralizing mAb against CD70. As shown in Fig. 4, neutralizing CD70 substantially and consistently reduced the proliferation, IFN-γ production and GrB expression of naive T cells induced by p(I:C)- or FLU-primed LCs (Fig. 4A) down to the same levels as observed with DDCs (Fig. 4A). As expected, the presence of blocking Abs against CD70 did not affect the CD8+ T cells induced by p(I:C)- or FLU-primed DDCs (Fig. 4A). Surprisingly, despite previous data describing a role of IL-12 in the induction of CD8+ T cell mediated responses, we did not find an effect of blocking IL-12 on the induction of CD8+ T cell proliferation, whereas GrB and cytokine production was not substantially different for both DC subsets (Fig. 4B). These data indicate that the virus-induced selective upregulation of CD70 by LCs is the critical feature that enhances their capacity to induce effector CD8+ T cell responses compared with virus-primed DDCs that lack CD70.

The importance of CD70 is in line with previous studies, in human as well as mouse models, which show a critical role for DC-associated expression of CD70 in CD8+ T cell responses

![FIGURE 3. Priming with p(I:C) or FLU induces CD70 expression by LCs but not by DDCs. A, Representative histograms for HLA-DR, HLA-ABC, CD86, CD80, CD83, and ICAM-1 expression of nonstimulated (filled histogram) and DDCs and LCs primed for 48 h with p(I:C) or FLU (open histograms). B, IL-12 production by p(I:C)- or FLU-primed DDCs and LCs measured by ELISA. C, CD70 expression by unstimulated (filled histogram) and p(I:C)- or FLU-primed DDCs and LCs (open histograms). Data are representative for at least 10 (A, C) or 3 (B) independent experiments.](http://www.jimmunol.org/)

![FIGURE 4. The superior capacity of LCs to activate CD8+ T cells upon p(I:C) or FLU priming depends on CD70. A, Proliferation (day 4), IFN-γ expression (day 5), and GrB expression (day 5) by CD8+ T cells activated by p(I:C)- or FLU-primed LCs in the absence and presence of anti-CD70. Data show mean ± SEMs of four and three independent experiments, respectively (**p < 0.05). B, Proliferation, IFN-γ expression, and GrB expression by CD8+ T cells activated by p(I:C)- or FLU-primed LCs in the absence and presence of anti-IL-12. Data show mean ± SEMs of four independent experiments.](http://www.jimmunol.org/)
are able to directly present Ags to CD8+ T cells. Which provide evidence that migratory DCs, including LCs, subsets further stress the potential of p(I:C) in promoting the unique induction of high levels of CD70 on certain DC The unique ability to express these high levels of CD70. Because of its effects on cell mediated immune responses, p(I:C) is often considered as an adjuvant in antiviral vaccines. DDCs, or ex vivo conventional DCs, do not express CD70 after stimulation through TLR3, TLR4 or CD40 (20, 21). The Journal of Immunology 3491

In conclusion, our data provide further insight in the relative roles of epidermal and dermal DCs in the initiation of T cell immunity. Whereas LCs ignore bacteria and immunity is only initiated by DDCCs when these microbes have crossed the epidermis where they are potentially pathogenic, LCs have the potential to readily initiate effective antimicrobial to challenge viruses that upon skin contact readily infect the epidermal layer. Knowledge about the capacity of different DC subsets to induce T cell responses is not only essential in understanding their roles in immunity but could also be beneficial for the development of effective immunotherapies.

Disclosures

The authors have no financial conflicts of interest.

References


Supplemental figure 1: The difference in CD70 expression by LCs and DDCs is not due to differences in sensitivity for p(I:C) and R848 stimulation results in marginally elevated CD70 expression in LCs of a minority of the donors. 

A. Monocyte-derived Langerhans cells (open areas) and dermal DCs (grey areas) stimulated for 24 hours with different concentrations p(I:C) and analysed for CD70 expression FACS.

B. Monocyte-derived Langerhans cells, stimulated (open areas) for 24 hours with R848 or p(I:C) and analysed for CD70 expression FACS. Grey areas represent expression levels of isotype-matched control Ab.