TLR Activation of Langerhans Cell-Like Dendritic Cells Triggers an Antiviral Immune Response

Claudia N. Renn, David Jesse Sanchez, Maria Teresa Ochoa, Annaliza J. Legaspi, Chang-Keun Oh, Philip T. Liu, Stephan R. Krutzik, Peter A. Sieling, Genhong Cheng and Robert L. Modlin

J Immunol 2006; 177:298-305; doi: 10.4049/jimmunol.177.1.298
http://www.jimmunol.org/content/177/1/298

**Why The JI?**

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

*average

**References**  
This article cites 56 articles, 23 of which you can access for free at:  
http://www.jimmunol.org/content/177/1/298.full#ref-list-1

**Subscription**  
Information about subscribing to The Journal of Immunology is online at:  
http://jimmunol.org/subscription

**Permissions**  
Submit copyright permission requests at:  
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**  
Receive free email-alerts when new articles cite this article. Sign up at:  
http://jimmunol.org/alerts
TLR Activation of Langerhans Cell-Like Dendritic Cells Triggers an Antiviral Immune Response

Claudia N. Renn,* David Jesse Sanchez,† Maria Teresa Ochoa,* Annaliza J. Legaspi,* Chang-Keun Oh,‡ Philip T. Liu,* Stephan R. Krutzik,* Peter A. Sieling,* Genhong Cheng, † and Robert L. Modlin2*†

Langerhans cells (LC) are a unique subset of dendritic cells (DC), present in the epidermis and serving as the first line of defense against pathogens invading the skin. To investigate the role of human LCs in innate immune responses, we examined TLR expression and function of LC-like DCs derived from CD34+ progenitor cells and compared them to DCs derived from peripheral blood monocytes (monocyte-derived DC; Mo-DC). LC-like DCs and Mo-DCs expressed TLR1–10 mRNAs at comparable levels. Although many of the TLR-induced cytokine patterns were similar between the two cell types, stimulation with the TLR3 agonist poly(I:C) triggered significantly higher amounts of the IFN-inducible chemokines CXCL9 (monokine induced by IFN-γ) and CXCL11 (IFN-γ-inducible T cell α chemoattractant) in LC-like DCs as compared with Mo-DCs. Supernatants from TLR3-activated LC-like DCs induced intracellular replication of vesicular stomatitis virus in a type I IFN-dependent manner. Finally, CXCL9 colocalized with LCs in skin biopsy specimens from viral infections. Together, our data suggest that LCs exhibit a direct antiviral activity that is dependent on type I IFN as part of the innate immune system. The Journal of Immunology, 2006, 177: 298–305.

Materials and Methods

In vitro differentiation of LC-like DCs and monocyte-derived DCs (Mo-DC)

LC-like DCs derived from human cord blood were prepared as described previously (14, 15). Briefly, cultures of CD34+ cells (enriched with RosetteSep; StemCell Technologies) were established in the presence of stem cell factor (25 ng/ml; R&D Systems), GM-CSF (200 U/ml; Berlex), and TNF-α (2.5 ng/ml; BioSource International). At day 8, cultures were repleted in the presence of GM-CSF (200 U/ml; Berlex) and TGF-β1 (1 ng/ml; R&D Systems) to increase CD1a expression. LC-like DCs were harvested at day 12–14.

PBMC were isolated from human peripheral blood as described previously (16). Monocytes were enriched using Percoll (Amersham Biosciences) and were adhered onto culture dishes for 2 h in RPMI 1640 containing 1% FBS (HyClone). The cells were washed with 1× PBS (In-vitrogen Life Technologies), and the adherent cells were cultured with a combination of recombinant human GM-CSF (200 U/ml) and recombinant human IL-4 (100 U/ml) (PeproTech) for 72 h as described previously (17).

TLR ligands

Cells were stimulated with TLR ligands for 24 h. Flagellin was a gift from Kelly Smith (University of Washington, Seattle, WA). Other TLR ligands were purchased as follows: 19-kDa lipopeptide (EMC Microcollections), PGN (Sigma-Aldrich), macrophage-activating lipopeptide-2 (MALP-2; Alexis), poly(I:C) (Amersham Biosciences), Imiquimod (Sequioa Research), R-848 (GLS Synthesis), and CpG (Invitrogen Life Technologies). LPS (Sigma-Aldrich) was purified as described previously (18, 19).

The following concentrations were used: 19 kDa (TLR2/1, 5 μg/ml), PGN (TLR2, 5 μg/ml), MALP-2 (TLR2/6, 10 ng/ml), poly(I:C) (TLR3, 1 μg/ml), LPS (TLR4, 10 ng/ml), flagellin (TLR5, 100 ng/ml), Imiquimod...
RNA was isolated from LC-like DCs and Mo-DCs after stimulation with TLR ligands or medium control for 24 h using TRIzol Reagent (Invitrogen Life Technologies), and cDNA was synthesized using the iSCRIPT cDNA Synthesis Kit (Bio-Rad). The following primers were designed using Primer Express (Applied Biosystems): TLR1 (forward (F)), 5′-TCT AGT GTG CTC CCA ATT GCT C-3′ and TLR1 (reverse (R)), 5′-GAA TTC CGA CTC AGG AGT CAC C-3′; TLR2 (F), 5′-CAT TCC CTC AGG GCT CAC AG-3′ and TLR2 (R), 5′-TTG GTC GAG TCG ACC TCA GTG-3′; TLR3 (F), 5′-ACC CGA TGA TCT ACC CAC AAA C-3′ and TLR3 (R), 5′-GGT GCC GGC TGG TAA TCT TC-3′; TLR4 (F), 5′-AGG ATG AGG ACT GGG TAA GGA AT-3′ and TLR4 (R), 5′-TGA AGG CAG AGC TGA AAT GGA-3′; TLR5 (F), 5′-CTT GAG CTT GAT CAG TTG GC-3′ and TLR5 (R), 5′-CGT GCC CAT CCT CCT ATG GTG AG-3′; TLR6 (F), 5′-TGC TGC CCT CTT ATG TTG AGG CTT-3′ and TLR6 (R), 5′-CTC TAC AGA TGG GCA GCA G-3′; TLR7 (F), 5′-TCA CCA GAC TGT GCT ATG ATG C-3′ and TLR7 (R), 5′-CAG CCA AAA CCC ACT CGG T-3′; TLR8 (F), 5′-TAT TAC GAT GCT GCC CCG-3′ and TLR8 (R), 5′-AAT ATG CTG TGG ATA ACT CCC CTT-3′; TLR9 (F), 5′-CTC TGA AGA CTT GAC GCC CA-3′ and TLR9 (R), 5′-CAG GCT TAC CAG GGT TGT CC-3′; TLR10 (F), 5′-ATG ACA AAC ATG GCA CAG C-3′ and TLR10 (R), 5′-AAA TGG TGC GAA TGC GAC AT-3′; INF-α (F), 5′-TGG CCC TCC TGC TGC TC-3′ and INF-α (R), 5′-TTG TGG TTT GGC GCA GAT CA-3′; INF-β (F), 5′-CAG CAA TTT TCA GTG TCA GAA GCT-3′ and INF-β (R), 5′-TCA TCC CCT TGA GGC AAC AT-3′; INF-γ (F), 5′-CCA CAA AGG ACA AGC AAT ACA TGA-3′ and INF-γ (R), 5′-CAG ACC TCC CCT GTC TTA GCT GC-3′. SYBR Green reactions were conducted with the IQ SYBR Green mix (Bio-Rad). Reactions were run on the MJR Opticon Continuous Fluorescence detector (Bio-Rad) and analyzed with Opticon Monitor Software 1.08 (Bio-Rad). The relative quantities of the gene tested per sample were calculated using the ΔΔCT formula as described previously (20).

Patients and clinical specimens
Blood samples for isolation of PBMCs were obtained from venipuncture from healthy donors with informed consent (University of California, Los Angeles (UCLA) Institutional Review Board no. 92-10-591-31). PBMCs were isolated using Ficoll-Hypaque gradient centrifugation (Ficoll-Paque; Pharmacia Biotech) and served as a source of Mo-DC. Samples of human cord blood were obtained from Santa Monica-UCLA Medical Center (Santa Monica, CA). CD34+ human progenitor cells were isolated using RosetteSep (StemCell Technologies) according to the supplier’s instructions.

After informed consent was obtained, skin tissues were collected from human volunteers using skin biopsy according to the Declaration of Helsinki Principles and the Institutional Review Board at Pusan National University Hospital.

Detection of cytokines
LC-like DCs and Mo-DCs were activated with TLR ligands for 24 h. IL-12 and TNF-α level in the supernatants were measured by ELISA (BD Pharmingen). For additional chemokine and cytokine array testing, supernatants were examined using Searchlight Cytokine Arrays (Pierce Biotechnology).

Abs and cytokines
Abs for cell surface molecules were as follows: CD1a (DakoCytomation), CD207 Langerin (BD Pharmingen), CXCL9/MIG (monokine induced by INF-γ) (R&D Systems), and IgG controls (Sigma-Aldrich).

Viral infection studies
Human fibroblasts (2TGH) were plated in 12-well dishes to 80% confluency in DMEM supplemented with 10% FBS and penicillin/streptomycin. Three hours before infection, cell medium was exchanged with a mixture of equal parts TLR ligand-stimulated LC-like DC/Mo-DC supernatant and DMEM supplemented with 5% heat-inactivated FBS and penicillin/streptomycin. At 0 h, cells were infected with vesicular stomatitis virus (VSV) at a multiplicity of infection of 1.0. Infected cells were grown in a 37°C incubator for 1 h. After an additional 6 h of incubation, the cells were washed once with PBS, trypsinized, washed again with PBS, and fixed in a solution of PBS with 2% paraformaldehyde. The solution was diluted with PBS to a final concentration of paraformaldehyde of 1% before flow cytometry. Cells were analyzed on a BD Biosciences FACSCalibur, and the level of GFP expressed in cells was quantitated.

Two-color immunofluorescence staining of cryostat sections
Double immunofluorescence was performed by serial incucation of cryostat tissue sections with mouse anti-human mAbs of different isotypes (e.g., NA1/34 (anti-CD1a, IgG2a; DakoCytomation) or CXC1L/MIG (IgG1; R&D Systems)), followed by incubation with isotype-specific goat anti-mouse IgG Abs (Molecular Probes) labeled with fluorochrome (Alexa 488 or Alexa 568). Controls included staining with isotype-matched irrelevant Abs, as well as staining with anti-CD1a or anti-CXCL9 followed by secondary Abs mismatched to the primary Ab isotype to demonstrate the isotype specificity of the secondary-labeled Ab. Images were obtained using confocal laser microscopy (UCLA core facility). Immunofluorescence was examined with a Leica TCS SP inverted confocal laser scanning microscope (Leica Microsystems).

Statistical analysis
Statistical comparison between cell types in ELISA studies were made using Student’s t-test. Values of p < 0.05 were considered significant and are indicated in the figures.

Results
TLR expression and function in LC-like DCs and Mo-DCs
To determine whether LCs represent a specific functional subset of DCs with regard to innate immune responses, we examined the spectrum of TLR expression and function in both LC-like DCs and Mo-DCs; the latter representing the standard peripheral blood DCs used for in vitro study. First, we analyzed the expression levels of TLR1–10 in LC-like DCs (n = 6, except TLR 3/7/9 n = 5, TLR10 n = 4) and Mo-DCs (n = 5, except TLR2/3/5 n = 4) using qPCR (Fig. 1). LC-like DCs expressed mRNAs for TLR1–10, with high expression of TLR2 mRNA, intermediate expression of TLR8, TLR4, TLR3, and TLR10 mRNAs, and low expression of TLR1, TLR5, TLR6, TLR7, and TLR9 mRNAs. Mo-DCs showed a TLR expression pattern similar to LC-like DCs (21).

To assess the ability of these DC populations to sense invading pathogens, we stimulated LC-like DCs (n = 4–5) and Mo-DCs (n = 4–6) for 24 h with known ligands for specific TLRs: Mycobacterium tuberculosis 19-kDa lipopeptid (TLR2/1), PGN (TLR2), MALP-2 (TLR2/6), poly(I:C) (TLR3), LPS (TLR4), flagellin (TLR5), Imiquimod (TLR7), Resiquimod/R-848 (TLR7/8), and bacterial CpG (TLR9). Dose titrations for each ligand were performed to determine the most suitable concentration to use in our assays (Fig. 2A and data not shown). We then stimulated LC-like DCs and Mo-DCs and measured cytokine and chemokine production by ELISA. In both DC populations, ligands for TLR2

FIGURE 1. Expression of TLR1–10 in LC-like DCs and Mo-DCs. Expression of TLR1–10 in LC-like DCs and Mo-DCs (n = 4–6) was assessed using qPCR. LC-like DCs were generated from umbilical vein blood, and Mo-DCs were derived from freshly isolated PBMC from healthy donors as described previously. Data are represented as mean arbitrary units (AU) ± SEM.
(PGN) and TLR7/8 (R-848) were the most potent stimulators of cytokine and chemokine secretion. To help us identify potential functional differences between DC populations based on relative amounts secreted from LC-like DCs vs Mo-DCs, we grouped the cytokines/chemokines. LC-like DCs produced slightly higher quantities of IL-12p40 (2.3 ± 0.1 ng/ml), CCL3 (MIP-1α; 1.7 ± 0.1 ng/ml), and CCL4 (MIP-1β; 1.5 ± 0.1 ng/ml) after TLR2/1 stimulation than Mo-DCs. In contrast, Mo-DCs secreted more TNF-α, IL-8, and CCL2 (MCP-1; 6 ± 0.2 ng/ml) than LC-like DCs in response to PGN (Fig. 2C). In addition, after R-848 stimulation, Mo-DCs secreted more TNF-α (3.2 ± 0.2 ng/ml), IL-8 (1.6 ± 0.1 ng/ml), and CCL2 (MCP-1; 4.4 ± 0.3 ng/ml) than LC-like DCs. These results suggest that LC-like DCs and Mo-DCs differ quantitatively in their capacity to secrete cytokines and chemokines in response to TLR ligands.

LC-like DCs produce CXCL9 (MIG) and CXCL11 (IFN-γ-inducible T cell chemoattractant; I-TAC) after TLR3 stimulation

TLR3 recognizes dsRNA, an intermediate in many viral replication cycles. TLR3 stimulation by poly(I:C) in LC-like DCs and Mo-DCs did not induce the production of many of the cytokines and chemokines detected after activation with TLR2 and TLR7/8 ligands (Fig. 2). Because we were interested in antiviral immune responses, the striking result, however, was that after activation via TLR3, LC-like DCs (n = 5) produced significantly higher amounts of the chemokine CXCL9 compared with Mo-DCs (LC-like DCs: 21.9 ± 5.0 ng/ml; Mo-DCs: 5.0 ± 4.0 ng/ml) and CXCL11 (LC-like DCs: 6.3 ± 1.3 ng/ml; Mo-DCs: 2.1 ± 0.9 ng/ml) (both p < 0.05; n = 4–6) (Fig. 3A). TLR3 activation in Mo-DCs resulted in production of CXCL10 (IFN-γ-inducible protein (IP)-10) (Mo-DCs: 31.7 ± 7.8 ng/ml; LC-like DCs: 5.6 ± 2.1 ng/ml; p < 0.05) and CCL5 (RANTES) (Mo-DCs 5.5 ± 2.5 ng/ml; LC-like DCs 0.6 ± 0.18 ng/ml) (Fig. 3B).

Given that CXCL9, CXCL10, and CXCL11 belong to the group of IFN-inducible chemokines (22–25), we investigated whether the DC subsets up-regulate type I IFNs. After activation via TLR3, LC-like DCs and Mo-DCs significantly up-regulate IFN-α gene expression in both LC-like DCs (13-fold) and Mo-DCs (12-fold) greater than with stimulation of TLR2/1 (LC-like DCs: 1.4-fold/Mo-DCs: 2.4-fold) (Fig. 4). However, stimulation of LC-like DCs with the TLR7/8 agonist R-848 resulted in less IFN-β gene up-regulation than in the Mo-DCs (LC-like DCs: 0.8-fold/Mo-DCs: 10.6-fold). TLR activation of IFN-α gene expression was considerably less than IFN-β (≤5-fold in all the samples tested).

Viral infection model

Release of type I IFN by TLR3-activated LC-like DCs suggested the possibility of an antiviral gene program. We therefore tested whether TLR3-activated LC-like DCs mediate an antiviral response using an in vitro infection model. LC-like DCs or Mo-DCs were stimulated with poly(I:C) or a control, and the cell culture supernatants were collected at 24 h. Human fibroblasts (2fTGH...
cell line) were incubated with these supernatants followed by infection with VSV. The virus contains the GFP open reading frame in the viral genome and produces detectable levels of GFP after a productive infection has occurred (26). Viral replication was assessed by flow cytometry of LC-like DCs after exposure to VSV.

Preincubation of fibroblasts with supernatants from poly(I:C)-stimulated LC-like-DCs and Mo-DCs resulted in a marked decrease in the replication of VSV (Fig. 5A). This reduction was similar to preincubating fibroblasts with type I IFN and subsequently infecting them with VSV (data not shown). In contrast, preincubation of fibroblasts with supernatants from TLR2/1 or TLR7/8-stimulated DC subpopulations did not significantly reduce cell infection. Furthermore, the reduction in viral infection was partially reversed (48% in LC-like DCs, 78% in Mo-DCs) through the addition of a type I IFN receptor (IFNAR) blocking Ab (Fig. 5B). Thus, in this model of viral infection, CXCL9 was expressed not only by keratinocytes but also in and around LCs.

Discussion

DCs represent a key component of the innate immune system, well known for their potent Ag-presenting function; however, their contribution to antimicrobial function is not fully understood. One type of DCs, LCs, are specific to the skin and mucosa and function as an immunological barrier to invading pathogens. We report in this study that TLR3 activation of human LCs induces the type I IFN-dependent production of chemokines including CXCL9 (MIG) and CXCL11 (I-TAC). Furthermore, TLR3-activated LCs trigger an antiviral activity in vitro, which was dependent on type I IFN activation. Finally, in human lesions of viral infection, CXCL9 was expressed not only by keratinocytes but also in and around LCs.

CXCL9 (MIG) colocalizes with LCs in the epidermis

To determine whether LCs have the capacity to express the type I IFN-inducible chemokine CXCL9 (MIG) in vivo, immunohistology was performed in skin biopsy specimens of cutaneous viral infections and normal skin specimen. These experiments indicated that in two different viral skin diseases (molluscum contagiosum and verruca vulgaris) the chemokine CXCL9 is found in and around the keratinocytes of the epidermis. Furthermore, CXCL9 colocalizes in the epidermis with CD1a from LCs in molluscum contagiosum specimen (Fig. 6A). CXCL9 could also be localized inside LCs in verruca vulgaris specimen (Fig. 6B; three different patients each). Skin specimen of normal human skin showed minimal expression of CXCL9 and no colocalization with LCs (Fig. 6C).

FIGURE 3. Activation of LC-like DCs with TLR3 triggers production of IFN-inducible chemokines. A, After TLR3 stimulation, LC-like DCs (n = 5) produce 4.4-fold more CXCL9 (MIG) and 3.0-fold more CXCL11 (I-TAC) compared with Mo-DCs. B, TLR3 stimulation in Mo-DCs resulted in the production of 5.7-fold more CXCL10 (IP-10) and 10.6-fold more CCL5 (RANTES) compared with LC-like DCs. Statistics were performed to compare TLR-induced cytokine production of LC-like DCs and Mo-DCs, and p < 0.05 is indicated.

FIGURE 4. IFN-α mRNA (Fold change) and IFN-β mRNA (Fold change) expression after TLR3 stimulation in LC-like DCs and Mo-DCs. LC-like DCs and Mo-DCs were stimulated with TLR ligands or medium for 24 h, and IFN-α and IFN-β gene expression were determined by real-time PCR. Activation with TLR3 ligand induces up-regulation of IFN-β gene expression in both LC-like DCs (13-fold) and Mo-DCs (12-fold). Stimulation of LC-like DCs with the TLR7/8 agonist R-848 resulted in less IFN-β gene up-regulation of the Mo-DCs compared with TLR3 stimulation. (LC-like DCs: 0.8-fold/ Mo-DCs: 10.6-fold). Data represent mean ± SEM.
One striking finding of the present study was that LC-like DCs, in response to TLR3 activation, produce specific chemokines that are associated with an antiviral innate defense program. Following activation with a TLR3 ligand, LC-like DCs specifically secrete the IFN-inducible chemokines CXCL9 (MIG) and CXCL11 (I-TAC) in higher amounts than Mo-DCs. In contrast, Mo-DCs produce higher amounts of the chemokines CXCL10 (IP-10) and CCL5 (RANTES) after TLR3 activation. Despite these differences in chemokine patterns, there was no difference in the gene expression levels of TLR3-induced type I IFNs between LC-like DCs and Mo-DCs. Further studies will need to examine whether LC-like DCs and Mo-DCs are differentially primed to respond to type I

**FIGURE 5.** LC-like DCs induce an antiviral effect after TLR3 stimulation. We tested the ability of LC-like DCs and Mo-DCs to trigger antiviral activity. A, 2TGH cells were preincubated with supernatants from LC-like DCs or Mo-DCs stimulated with ligands for TLR2/1, TLR3, and TLR7/8. The cells were then infected with VSV that expresses GFP after infection. After 7 h of infection, cells were analyzed by flow cytometry for the level of GFP, and the percentage of GFP-positive cells was quantitated. VSV infection is blocked only when the target cells were preincubated with supernatant from LC-like DCs or Mo-DCs stimulated with the TLR3 ligand poly(I:C). Data represent mean ± SEM. B, To determine whether TLR3-mediated antiviral activity is induced by type I IFN, we added either control IgG Abs or Abs against the IFNAR before addition of culture supernatants. The cells were infected, and the percentage of GFP-positive cells was quantitated as in A. VSV infection is partially restored when the TLR3-stimulated supernatant is preincubated with blocking Abs directed against the IFNAR and not control Abs. Data represent mean ± SEM.

**FIGURE 6.** CXCL9 (MIG) colocalizes with LCs in vivo. The chemokine CXCL9 colocalizes with CD1a from LCs in the epidermis in viral skin diseases compared with normal skin specimen. Expression of CXCL9 (MIG) (left panels), CD1a (middle panels), and merge (right panels) in the epidermis of skin lesion of patients with molluscum contagiosum (A), verruca vulgaris (B), and normal skin specimen (C). The images represent sections from the lesion of one patient showing the same region of the epidermis. Original magnification, ×200/insert ×630. The dotted line indicates the margin between the epidermis (top) and the dermis (bottom).
IFNs, have inherent differences in chemokine gene programs, or that other TLR3-induced cytokines are involved in regulating the expression of these chemokines. Furthermore, although plasmacytoid DC are known to be potent producers of type I IFNs, our data suggest that LC-like DC and Mo-DC are also able to produce type I IFNs and respond through production of type I IFN-inducible chemokines. LC cells may function in immunologic synergy with plasmacytoid DC in skin.

The induction of CXCL9 in LC-like DCs was corroborated by detection of this chemokine in LCs of skin biopsy specimens of viral diseases in humans. We found that in two different viral skin diseases (molluscum contagiosum and verruca vulgaris) the chemokine CXCL9 colocalized with LCs of the epidermis. Although TLR3 is canonically thought of as a receptor involved in detection of RNA virus replication, it has been argued that it is critical in DNA virus replication. Many DNA viruses produce overlapping transcripts and transcripts with secondary RNA structure that may be ligands for TLR3 or intracellular receptor as well as proteins assumed to avoid dsRNA-induced signaling (27–29). Therefore, these data suggest a specific role for human LCs in the innate immune response to viral skin infections.

So far macrophages, monocytes and activated keratinocytes are known to be the main producers of the IFN-inducible CXC chemokines CXCL9, CXCL10, and CXCL11 (MIG, IP-10, and I-TAC) (30); however, our data provide evidence that CXCL9 and CXCL11 are potentially induced in LC-like DCs after TLR3 ligation, and expressed by LCs in vivo during viral infection. Given that the ligand for TLR3 is dsRNA, LCs have the ability to respond to both RNA and DNA viruses. The location of LCs in the epidermis is critical, because many viruses attack this layer of skin, including HSV-1 (31), Posvridae (molluscum contagiosum virus), human papillomavirus, and HIV (32–34). In this manner, LC can cooperate with KC in the local antiviral response in the epidermis.

A key aspect of the TLR3-induced response is the induction of type I IFN, both in LC-like DCs and Mo-DCs. The induction of IFN-β gene expression was more robust than IFN-α gene expression in both DC subsets. Previous studies have shown that IFN-β and a small subset of IFN-α genes are induced shortly after TLR3 triggering, whereas the majority of IFN-α genes are induced later (35–39). In general, the induction of IFN-β is considered an IFN regulatory factor-3-dependent, primary response to TLR3 triggering, especially by viral infection (40, 41). Secondary gene products such as many of the IFN-α genes are induced after, and often by the help of, the primary response genes such as IFN-β signaling (40, 41). Thus, the high level induction of IFN-β vs IFN-α is indicative of a bona fide response to TLR3 triggering.

Based on the expression of type I IFN, we examined whether LC-like DCs have direct antiviral activity. In the VSV model of viral infection, supernatants from poly(I:C)-stimulated LC-like DCs, as well as Mo-DCs, elicited an IFN-dependent direct antiviral activity against VSV. The TLR3-specific reduction in viral infection was partially reversible through the addition of an IFNAR-blocking Ab (Fig. 5B).

Although both LC-like DCs and Mo-DCs can be triggered by TLR3 to induce type I IFN, only LC-like DCs specifically produce the IFN-dependent chemokines CXCL9 (MIG) and CXCL11 (I-TAC) after a viral stimulus. These chemokines are known to be of importance in innate immunity because they also exhibit (receptor-independent) defense-in-like, antimicrobial function (42). Studies with SIV and vaccinia virus infections show that CXCL9 expressed in vivo at sites of viral replication can produce also an antiviral effect. This suggests that CXCL9 plays a role in IFN-mediated antiviral responses in vivo (43, 44). Recent studies with the CCL5/RANTES chemokine, for example, have shown that exposure to topical N\(^2\)-(n-nonanoyl)-des-Ser\(^1\)-γ-thioprolnine, L-α-cyclohexyl-glycine\(^1\)-RANTES can completely prevent subsequent intravaginal infection by a CCR5-using SIV isolate in female rhesus macaques (45).

Although these CXC chemokines may be important for the antiviral activity, they also may instruct the adaptive immune response. Clues to the functional consequence of LC secretion of CXCL9 and CXCL11 can be garnered from the distribution of their receptor CXCR3. This receptor is expressed in inflammatory sites by a majority of both CD4\(^+\) and CD8\(^+\)-infiltrating T cells, suggesting a functional interaction between locally produced chemokines and CXCR3-expressing T cells. CXCL9 (MIG) and CXCL11 (I-TAC) are thought to be chemoattractant for disease-promoting T cells by mobilization of intracellular Ca\(^2+\) and induction of chemotaxis (46–48) in skin inflammatory diseases like psoriasis, lichen planus, mycosis fungoides, and allergic contact dermatitis (30, 49). Interestingly, MIG-specific Abs inhibit long-term T cell infiltration and promote survival of MHC separate skin allografts (50). Therefore, LCs serve as sentinel cells for the skin, and, by producing IFN-inducible chemokines, they have the ability to directly target and inactivate viral (and bacterial) pathogens while also recruiting leukocytes to the infected tissue to defend against the invading virus. This complements the ability of LCs to serve as potent APCs to T cells.

Our results indicate that the expression of TLR1–10 on human LC-like DCs was comparable to Mo-DCs, suggesting that LCs are fully equipped immune cells to recognize different pathogen-associated molecular patterns. This was in contrast to studies on epidermal mouse LCs indicating that they have lower TLR expression (51–56), yet these cells are known to decrease their expression of cell surface receptors during extraction and in vitro culture. Nevertheless, we found that human LC-like DCs and Mo-DCs responded equally to ligands for TLR2 (PGN) and TLR7/8 (R-848). They are potent inducers of cytochrome and chemokine secretion, both inducing high levels of IL-12, IL-8, CCL3 (MIP-1α), CCL4 (MIP-1β), and TNF-α. Furthermore, similar to murine epidermal LCs (53), human LC-like DCs responded to TLR3 stimulation and produced the chemokines CCL5 and CXCL10, although to lower levels than Mo-DCs. These data indicate that LCs have the ability to respond to a wide range of bacterial and viral pathogens in innate immune defense in the skin.

Because human primary LCs are difficult to obtain in appreciable numbers or purity, we studied LC-like DCs derived from CD34\(^+\) progenitors (57) and compared their responses to Mo-DCs generated by cytokine treatment of PBMC. These LC-like DCs express all of the classical features of LCs, including CD1a and the lectin Langerin/CD207, an endocytic receptor that induces Birbeck granules formation (14, 15). The finding that LCs in situ express the same chemokines produced by TLR3-activated LC-like DCs provides additional evidence that LC-like DCs represent an appropriate model for studying LC function.

In summary, we present novel evidence for the ability of LCs to exhibit direct antiviral activity in the early stages of the antiviral immune response. Because this activity was triggered by TLR3 activation, natural or synthetic ligands for TLR3 might prove useful as pharmacologic agents for the treatment of viral infections in the skin. Finally, our data suggest that DC subsets localizing to a particular anatomic site are engendered with specific immune functions to combat local invading pathogens.
Acknowledgments

We thank Matthew Schibler and the Carol Moss Spivak Cell Imaging Facility in the UCLA Brain Research Institute for the use of the confocal laser microscope, and the UCLA Flow Cytometry Core Laboratory for the use of their facilities. We acknowledge PIERCE Biotechnology for measurement of chemokines and cytokines using SearchLight technology. We thank T. Renn for help with the figures and C. J. Hertz for critical comments.

Disclosures

The authors have no financial conflict of interest.

References


Downloaded from http://www.jimmunol.org/ on October 23, 2017