UCSF UC San Francisco Previously Published Works

Title

Plasmacytoid dendritic cell-specific receptor ILT7-Fc epsilonRI gamma inhibits Toll-like receptor-induced interferon production.

Permalink https://escholarship.org/uc/item/0n31028j

Journal The Journal of experimental medicine, 203(6)

ISSN 0022-1007

Authors

Cao, Wei Rosen, David B Ito, Tomoki <u>et al.</u>

Publication Date 2006-06-12

Peer reviewed

Plasmacytoid dendritic cell–specific receptor ILT7–Fc ε RI γ inhibits Toll-like receptor–induced interferon production

Wei Cao,¹ David B. Rosen,^{3,4} Tomoki Ito,¹ Laura Bover,¹ Musheng Bao,¹ Gokuran Watanabe,¹ Zhengbin Yao,⁵ Li Zhang,² Lewis L. Lanier,^{3,4} and Yong-Jun Liu¹

⁵Tanox, Inc., Houston, TX 77025

Immunoglobulin–like transcripts are a family of inhibitory and stimulatory cell surface immune receptors. Transcripts for one member of this family, ILT7, are selectively expressed in human plasmacytoid dendritic cells (pDCs). We demonstrate here that ILT7 protein associates with the signal adapter protein $Fc\epsilon Rl\gamma$ to form a receptor complex. Using an anti-ILT7 monoclonal antibody, we show that ILT7 is expressed specifically on human pDCs, but not on myeloid dendritic cells or other peripheral blood leukocytes. Cross-linking of ILT7 resulted in phosphorylation of Src family kinases and Syk kinase and induced a calcium influx in freshly isolated pDCs, which was blocked by Src family and Syk kinases inhibitors, thus indicating the activation of an immunoreceptor-based tyrosine activation motif– mediated signaling pathway. ILT7 cross-linking on CpG or influenza virus-stimulated primary pDCs inhibited the transcription and secretion of type I interferon and other cytokines. Therefore, the ILT7–Fc $\epsilon Rl\gamma$ receptor complex negatively regulates the innate immune functions of human pDCs.

Plasmacytoid DCs (pDCs) are a distinct population of DCs in the peripheral blood and secondary lymphoid organs and are characterized by their plasma cell-like morphology and unique surface receptor phenotype (1). These cells play an important role in innate antiviral immunity by rapidly secreting abundant type I IFNs (IFN α , β , ω , λ) after exposure to various DNA and RNA viruses (1, 2). Type I IFNs produced by pDCs promote the function of NK cells, B cells, T cells, and myeloid DCs (mDCs) during the initial immune response (3-5). After activation, pDCs differentiate into a unique type of mature DCs, capable of directing T cell responses with considerable flexibility (3, 4). Thus, pDCs represent a critical link between innate and adaptive immune responses.

The unique ability of pDCs to sense and respond rigorously to microbes by rapidly producing large amounts of type I IFN is underlined by their expression, in contrast with

The online version of this article contains supplemental material.

mDCs and other immune cells, of a selective set of toll-like receptors (TLRs), in particular TLR7 and TLR9 (6). Recent studies have revealed an intracellular multiprotein complex that likely includes TLR9/7-MyD88-IRAK1/4-TRAF6-IRF7 and a complicated spatiotemporal signaling scheme in pDCs (7, 8). Because both TLR7 and TLR9 are located in the endosomal compartment of pDCs, how these cells sense the external microenvironment by surface receptors has remained elusive. We, therefore, performed a global gene expression analysis of human pDCs, in comparison with the other major human immune cell types. Human pDCs selectively express ILT7 (also named CD85g and LILRA4) transcripts as well as IL-3R (CD123) and BDCA-2, as previously reported (9-11).

Downloaded from www.jem.org on June 15, 2006

ILT7 is a member of the immunoglobulinlike transcripts (ILTs), or leukocyte immunoglobulin-like receptor (LIR) gene family (12), which comprises at least 13 loci. ILTs are predominantly expressed on the surface of

CORRESPONDENCE Wei Cao: wcao@mdacc.tmc.edu OR Yong-Jun Liu: yiliu@mdacc.tmc.edu

1399

¹Department of Immunology and ²Department of Biostatistics, University of Texas M.D. Anderson Cancer Center, Houston, TX 77030

³Department of Microbiology and Immunology, the Biomedical Sciences Graduate Program and ⁴Cancer Research Institute, University of California at San Francisco, San Francisco, CA 94143

JEM

myelomonocytic cells, including macrophages and DCs. Although the extracellular Ig domains are responsible for ligand binding, the residues within the transmembrane and cytoplasmic domains define two functional classes of ILTs: the inhibitory ILTs contain the immunoreceptor tyrosine-based inhibition motifs (ITIMs) in the cytoplasmic domain, whereas the activating ILTs lack any intrinsic signaling motifs and rely on association with transmembrane adapter proteins bearing immunoreceptor-based tyrosine activation motif (ITAM). Certain ILTs, such as ILT2 and ILT4, bind to classical and nonclassical MHC class I proteins (13). The ITIM-containing ILT2 inhibits signaling through the TCR in T cells (14) and enhances the inhibitory effects of killer cell Ig-like receptors (KIRs) in NK cells (13). In contrast, ILT1 associates with $Fc \in RI\gamma$ and activates eosinophils to release cytotoxic granule proteins, cytokines, and lipid mediators (15).

ILT7 encodes a surface receptor that is preferentially transcribed by human pDCs. This molecule contains four extracellular Ig domains and has a positively charged residue within the transmembrane region, which potentially allows it to associate with membrane-anchored adapter proteins. In this study, we report that ILT7 and Fc ϵ RI γ form a receptor complex that is specific for human pDCs and transduces ITAM-mediated signals that negatively modulate TLR-induced type I IFN production by human pDCs.

RESULTS AND DISCUSSION

ILT7 mRNA is specifically expressed by pDCs

To determine the expression profile of ILT7 in human leukocytes, we searched our established expression database, which included the major immune cell types in peripheral blood. Strikingly, ILT7 transcripts were expressed abundantly and exclusively by human pDCs (Fig. 1 A). pDCs also expressed ILT2 and ILT3; however, these receptors were also expressed by other cell types (Fig. 1 A). To verify this finding, we performed quantitative RT-PCR analysis on several cell types from multiple healthy donors. Consistently, human pDCs uniquely expressed ILT7 mRNA (Fig. 1 B).

ILT7 uses Fc ε Rl γ as an adapter

The positively charged arginine residue at position 449 is located within the predicated transmembrane segment of the ILT7 protein. To look for the adapter proteins interacting with ILT7, we examined the expression of the transmembrane



total PBMCs. The median expression is marked by a horizontal bar. (C) The expression levels of known transmembrane signaling adapters in pDCs were plotted using the values extracted from the gene expression database. (D) The relative expression of FceRI γ , DAP12, and DAP10 was determined from three healthy donors by quantitative RT-PCR analysis. The expression was normalized with S18 and multiplied by 1,000. The median expression is marked by a horizontal bar.

adapters in human pDCs from the expression database (Fig. 1 C). Although lacking the expression of ITAM-bearing components found in TCR and B cell receptors (BCRs), pDCs expressed two ITAM-bearing adapters (i.e., Fc ϵ RI γ and DAP12). DAP10, a non-ITAM adapter, which signals via a YINM motif permitting activation of PI3 kinase, was also transcribed in pDCs (Fig. 1 C). We further confirmed the expression of these adapters by RT-PCR analysis of three healthy donors (Fig. 1 D).

To determine which adapter pairs with ILT7, we used an "adapter trap" reporter cell system in which mouse BaF/3 pro–B cells were stably transfected with Fc ϵ RI γ , DAP12, or DAP10. ILT7 stabilized the surface expression of Fc ϵ RI γ , but neither DAP12 nor DAP10 (Fig. 2 A). Similarly, Fc ϵ RI γ enhanced the cell surface expression of ILT7 (Fig. 2 A). As a



Figure 2. ILT7 associates with FccRly. (A) BaF/3 cells stably transfected with FLAG-FccRly (top), -DAP12 (middle), or -DAP10 (bottom) were transduced with HA-ILT7 (center column) or with the vector alone (left column). Cells were stained for FLAG and HA and analyzed by flow cytometry. HA-tagged human KIR2DL4 or a mouse NKG2D variant was transduced into the BaF/3 cells as positive controls (right column). (B) Lysate from parental BaF/3 cells or cells expressing ILT7-HA and FccRly-FLAG was precipitated with IgG1, anti-HA, or anti-FLAG mAbs. Western blot (WB) was performed with anti-HA or anti-FccRly Ab. A fraction of the original lysate was run as control. Notably, ILT7-HA ran as two bands, likely reflecting differentially processed isoforms.

positive control for DAP10 and DAP12, we used a mouse NKG2D variant that could pair with either DAP12 or DAP10 (16). Additionally, ILT7 and Fc ϵ RI γ were coimmunoprecipitated from a lysate of BaF/3 cells transfected with HA-tagged ILT7 and FLAG-tagged Fc ϵ RI γ (Fig. 2 B). Thus, our results demonstrate that ILT7 associates with the ITAM-containing signaling adapter Fc ϵ RI γ to form a stable receptor complex.

ILT7 is a pDC-specific receptor

To confirm the expression of the ILT7 protein, we generated a mAb that recognizes a mouse T cell line transfected with human ILT7 (Fig. 3 A). In total PBMCs, anti-ILT7 mAb stained a rare cell population of non–T, non–B, nonmonocyte, and non–NK cells (unpublished data). When double stained with mAb against pDC marker BDCA2, almost all the ILT7⁺ cells were BDCA2^{high} (Fig. 3 B), suggesting that the circulating blood pDCs preferentially express ILT7. When activated by CpG or treated with IL-3, pDCs expressed lower levels of ILT7 (Fig. 3 C), consistent with the reported downregulation of ILT7 mRNA in activated pDCs (10, 11).

ILT7 triggers ITAM signaling

To reveal the cellular signals transduced by ILT7, we introduced a human ILT7–Fc ϵ RI γ complex into a mouse T cell hybridoma line that contained an intracellular NFAT–GFP construct (Fig. 4 A). Cross-linking ILT7 with immobilized anti-ILT7 mAb resulted in GFP expression, indicating that ILT7–Fc ϵ RI γ is able to activate NFAT, similar to the effect of TCR activation. The immobilized isotype-matched control antibody did not activate the cells under the same conditions (Fig. 4 A). When the same experiment was conducted in NFAT–GFP reporter cells expressing ILT7 alone (without Fc ϵ RI γ), cross–linking ILT7 did not induce GFP expression, which illustrated again the requirement of Fc ϵ RI γ for ILT7 to signal (unpublished data).

To evaluate the signaling events in primary human pDCs induced by ILT7 activation, we cross-linked ILT7 on freshly isolated pDCs and analyzed the phosphorylation status of two key types of protein tyrosine kinases (PTKs) by Western blot analysis (Fig. 4 B). After ITAM-mediated activation, two core tyrosine residues within the ITAMs are phosphorylated by PTKs of the Src family kinases. The tyrosine-phosphorylated ITAMs associate with the Src homology 2 (SH2) domains of spleen tyrosine kinase (Syk)-family kinases to initiate the well-orchestrated cascade of downstream events. Human pDCs express several members of the Src family kinases and Syk, but not ZAP70 (unpublished data). Shortly after ILT7 activation, both Src family kinases and Syk were phosphorylated (Fig. 4 B), indicating the onset of ITAM-mediated signaling in pDCs. In contrast, cross-linking with neither the isotype-matched control antibody nor the mAb against BDCA4, another surface molecule expressed on pDCs, phosphorylated these kinases under the same conditions.

Next, we analyzed one of the important cellular activation events that occurs downstream of ITAM-mediated signaling: calcium influx. Cross-linking of ILT7 effectively triggered prominent intracellular calcium mobilization in



Figure 3. Anti-ILT7 mAb specifically stains peripheral blood pDCs. (A) Mouse 2B4 T cell hybridoma expressing human FceRly (top) was transduced with HA-ILT7 (bottom). The cells were stained with an isotype-matched control Ab (IgG1), anti-HA, or anti-ILT7 mAb. (B) Total PBMCs were stained with IgG1 (left) or anti-ILT7 (right) and anti-BDCA2 mAb. Representative results from the analysis of multiple healthy donors are shown. (C) Surface ILT7 was measured on freshly isolated, IL-3-treated, or CpG-treated pDCs at various times after culture. Mean fluorescence intensity of each sample is indicated (top right).

human primary pDCs (Fig. 4 C). This activity was greatly reduced by PP2, a compound that interferes with the function of Src family kinases, but was not affected by an inactive control compound PP3 (Fig. 4 C). In addition, an inhibitor specific to Syk kinase completely abolished the intracellular calcium activity in ILT7–cross-linked pDCs (Fig. 4 C). Therefore, the ILT7–FcεRIγ complex is capable of activating the ITAM pathways in human pDCs.



Figure 4. Cross-linking of ILT7/Fcc RI γ activates ITAM-mediated signaling. (A) 2B4 T cell hybridoma expressing Fcc RI γ and ILT7 was cross-linked with mouse IgG1 or anti-ILT7 mAb and analyzed for NFAT-activated GFP expression. Cells cultured in medium alone were also analyzed. (B) Freshly isolated human pDCs were cross-linked with mouse IgG1, anti-ILT7, or anti-BDCA4 mAb and the cell lysates were analyzed by Western blotting. (C) The kinetics of intracellular calcium flux in human pDCs when cross-linked (arrow) by control (IgG1) or anti-ILT7 Ab. As indicated, cells were preincubated for 30 min with 50 μ M of PP2, 50 μ M of PP3, or 10 μ M of Syk inhibitor (EMD Biosciences) before cross-linking.

ILT7–Fc ε RI γ inhibits pDCs' TLR responses

An important role of the ILT molecules is to modulate the function of other immune receptors (12). pDCs expressing TLR9 and TLR7 respond to CpGs and viruses by producing large amounts of type I IFN, as well as other proinflammatory cytokines (6). We examined how ILT7 triggering influences TLR responses in human pDCs. When ILT7 was cross-linked, pDCs stimulated by the TLR9 ligand CpG oligonucleotide (ODN) produced less IFN α and TNF α , in comparison with medium control or isotype-matched control Ab (Fig. 5 A). However, ILT7 cross-linking did not affect the surface maturation phenotype of pDCs, as measured by CD80 and CD86 expression (Fig S1, available at http://www.jem.org/cgi/ content/full/jem.20052454/DC1). Cross-linking of surface BDCA4 under the same conditions did not alter the TLR responses (Fig. 5 A), consistent with a published report (17). As a positive control, preincubation of pDCs with anti-BDCA2 mAb (5 µg/ml) dramatically reduced the TLR-mediated responses. Interestingly, ILT7 cross-linking effectively reduced TLR response even after pDCs were preexposed to CpG up to 1 h, which was analogous to the effects of BDCA2 cross-linking (Fig. 5 B). Moreover, when we activated pDCs with inactivated influenza virus (Flu), a ligand for TLR7 (18), ILT7 cross-linking similarly inhibited the production of both IFN α and TNF α (Fig. 5 A).

To confirm this observation, we examined the intracellular IFN α and TNF α levels in the CpG-activated pDCs that were cross-linked under similar conditions. Consistently, ILT7 cross-linking specifically decreased the amount of intracellular IFN α as well as TNF α (Fig. 5 C). Last, we



Figure 5. ILT7 cross-linking inhibits TLR responses by pDCs. (A) Human pDCs were cross-linked with various Abs before stimulation by CpG (left) or Flu virus (right). The levels of the secreted IFN α and TNF α in a triplicate assay from a representative donor are shown. (B) Purified pDCs were cultured with CpG for 1 h (left) or 30 min (second panel from left) at 37°C before being cross-linked. Alternatively, CpG was added at the same time as (second panel from right) or 30 min after (right) cells

evaluated the amount of type I IFN transcripts from both CpG and Flu-activated pDCs that were cross-linked with anti-ILT7 or control mAbs. ILT7 cross-linking consistently reduced the transcription of all four subtypes of type I IFN analyzed (i.e., IFN α 1, IFN α 2, IFN α 4 and IFN β) (Fig. 5 D). Thus, ILT7 activation by mAb cross-linking significantly down-regulated the TLR-induced IFN and cytokine responses in pDCs.

We have shown that the ILT7–Fc ϵ R1 γ complex represents the first human pDC-specific ITAM-containing receptor complex. Cross-linking of the surface ILT7–Fc ϵ R1 γ complex on pDCs elicited activating signals, including phosphorylation of Src family kinases and Syk kinase, and induced a robust intracellular calcium mobilization. However, rather were cross-linked by various mAbs. The amounts of IFN α and TNF α in the supernatant after 18 h of culture are shown. (C) The amounts of IFN α and TNF α were determined by intracellular staining of CpG-activated pDCs. The percentage of the double-positive cell population is indicated. (D) The amounts of type I IFN transcripts were determined by quantitative RT-PCR analysis. The expression is shown as the relative level of transcription compared with unstimulated pDCs.

than enhancing the production of cytokines induced by stimulation of TLR, ILT7 functioned as a negative regulator of these TLR-mediated responses. Inhibition of TLR-induced human pDC activation by ITAM-receptor signaling has been documented in several prior studies. For example, cross-linking of the high affinity IgE receptor, which signals via Fc ϵ RI γ , also inhibits CpG-induced type I IFN production by human pDCs (19, 20). Additionally, Fuchs et al. reported that crosslinking NKp44, a receptor signaling through the ITAMbearing DAP12 adapter, inhibited CpG-induced IFN- α production (20, 21). In mice, mAb cross-linking of Siglec-H, another DAP12-associated receptor, reduced type I IFN production by pDCs in vitro and in vivo (22). These findings support and extend the prior studies reporting that

JEM

ITAM-mediated signaling can suppress the response of mouse macrophages to TLR ligands in vivo and in vitro (22, 23).

It is not clear how ITAM and TLR pathways intersect at the molecular level in human pDCs. Schroeder et al. demonstrated that IgE receptor cross-linking inhibited TLR9 expression in pDCs, which may explain in part the reduced CpG responses by these cells (19). Alternately, one possible mechanism may involve a bifunctional role of the ITAM of FcERIy. Specifically, under different conditions, FcERIy may function as an activating molecule by recruiting Syk, or alternatively act as an inhibitory receptor by recruiting SHP-1 and subsequently impairing protein tyrosine phosphorylation in the absence of sustained receptor aggregation (23, 24). Similarly, the downstream adapter protein LAB/NTAL may play either positive or negative roles in regulating FcERIymediated signaling under different conditions (25, 26). It will be important to examine in detail the biochemical mechanism of this inhibitory effect by ITAMs on TLR signaling.

pDCs are critically responsible for the massive and rapid type I IFN production elicited by viral infections. Controlling the type and the magnitude of this response is important in resolving disease without harming healthy tissue. Elevated levels of IFN α and up-regulated expression of IFN-responsive genes were found in the peripheral blood of systemic lupus erythematosus patients, where pDCs were presumably activated by chromatin-containing immune complexes (27). Therefore, the effective inhibition of pDCs and down-regulation of their constitutive type I IFN production, possibly through the ILT7–Fc ϵ RI γ receptor complex, may present a plausible therapeutic approach.

MATERIALS AND METHODS

Establishment of human leukocyte expression database. This study was approved by the institutional review board for human research at the M.D. Anderson Cancer Center. Human peripheral DCs, B cells, T cells, monocytes, and monocyte-derived DCs were prepared as described previously (28). CD56⁺CD4⁻CD14⁻CD19⁻CD69⁻ NK cells were isolated by flow cytometry. Neutrophils, eosinophils, basophils, and blood CD34⁺ hematopoietic progenitor cells (HPCs) were isolated as described previously (29). RNA preparation and microarray hybridization was performed as described previously (27, 28). The Positional Dependent Nearest Neighbor model (30) was used to estimate the gene expression values from the probe intensity values. The final expression output was normalized with the numerical value of one representing the estimated threshold of basal expression.

Quantitative RT-PCR analysis. Reverse transcription and quantitative PCR was performed as described previously (27, 28). Oligonucleotide primers used are listed in Table S1 (available at http://www.jem.org/cgi/content/full/jem.20052454/DC1).

Cloning and expression of ILT7 and transmembrane adapters. ILT7 was cloned from pDC cDNAs. A retroviral vector expressing ILT7 with an HA tag was used to transduce BaF/3 cells expressing FLAG-tagged human $Fc\epsilon RI\gamma$, DAP12, or DAP10 (16).

Generation of ILT7 mAb and PBMC staining. 6–8-wk-old BALB/c mice were immunized with ILT7–FcεRIγ-transfected 2B4 cells. Hybridoma clones secreting mAb that specifically stained ILT7⁺ cells were established. mAb 17G10.2 against ILT7 (IgG1) was purified and fluorochrome conjugated using mAb-labeling kits obtained from Invitrogen. PBMCs **NFAT-GFP reporter assay.** HA-tagged ILT7 was transduced into the 2B4 NFAT-GFP reporter cells (H. Arase, Osaka University, Osaka, Japan) with or without mouse $Fc\epsilon R1\gamma$. The cells were cultured on Ab-coated plates (10 µg/ml) for 16 h and analyzed for GFP expression.

Western blot analysis of protein tyrosine phosphorylation. pDCs isolated using BDCA4 cell isolation kit (Miltenyi Biotec) were incubated with mouse IgG1 (eBioscience), anti-ILT7, or anti-BDCA4 mAb (IgG1; Miltenyi Biotec) and cross-linked with $F(ab')_2$ goat anti-mouse IgG (Jackson ImmunoResearch Laboratories) for 2 min. Western blot was performed with an anti-phospho–Src family Ab, anti-phospho–Syk Ab, anti-nonphospho– Src family Ab, anti-Syk Ab (Cell Signaling Technology), and anti- β -actin mAb (Sigma-Aldrich).

Calcium influx assay. pDCs preloaded with Fluo4- and Fura Red-AM (Invitrogen) were cross-linked as described in the previous paragraph and analyzed on a FACSAria (BD Biosciences) and by FlowJo software (Tree Star).

ILT7 cross-linking pDCs on pDCs in culture. pDCs isolated by flow cytometry (CD4⁺CD11c⁻CD3⁻CD14⁻CD16⁻CD19⁻CD56⁻) were incubated in the Ab-coated wells (10 μ g/ml) for 30 min before stimulation with 0.1 μ M of CpG 2216 or heat-inactivated Flu at a multiplicity of infection of 3, unless otherwise specified. Cells and supernatants were harvested 18 h later for RT-PCR and ELISA. ELISA kits used were human IFN α (Bender MedSystems) and TNF α (R&D Systems). Intracellular cytokine staining was performed after 6 h of culture with CpG as described previously (28). Cells were stained with anti–IFN- α –FITC Ab (Chromaprobe), anti-CD123–PE Ab (eBioscience), and anti-TNF α –allophycocyanin Ab (BD Biosciences).

Online supplemental material. Fig. S1 shows that ILT7 cross-linking does not affect pDC maturation. Surface CD80 and CD86 expression was measured by flow cytometry on pDCs cultured with CpG or IL-3. Table S1 lists all the oligonucleotide primers used in this study. Fig. S1 and Table S1 are available at http://www.jem.org/cgi/content/full/jem.20052454/DC1.

We appreciate the service of M.D. Anderson Immunology Hybridoma Core facility, especially the work by J. Wygant, R. Munoz, and Dr. B. McIntyre, and are grateful to K. Ramirez, Z. He, and E. Wieder for cell sorting and technical support. We thank Y. Deng and Dr. B. Su for technical help.

This study was supported by a grant from the M.D. Anderson Cancer Foundation (to Y.-J. Liu) and National Institutes of Health grant Al068129 (to LL. Lanier and D.B. Rosen). LL. Lanier is an American Cancer Society Research Professor. D.B. Rosen is supported by a Genentech Graduate Student Fellowship.

The authors have no conflicting financial interests.

Submitted: 8 December 2005 Accepted: 23 April 2006

REFERENCES

- Siegal, F.P., N. Kadowaki, M. Shodell, P.A. Fitzgerald-Bocarsly, K. Shah, S. Ho, S. Antonenko, and Y.-J. Liu. 1999. The nature of the principal type 1 interferon-producing cells in human blood. *Science*. 284:1835–1837.
- Coccia, E., M. Severa, E. Giacomini, D. Monneron, M. Remoli, I. Julkunen, M. Cella, R. Lande, and G. Uze. 2004. Viral infection and Toll-like receptor agonists induce a differential expression of type I and λ interferons in human plasmacytoid and monocyte-derived dendritic cells. *Eur. J. Immunol.* 34:796–805.
- Liu, Y.-J. 2005. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. *Annu. Rev. Immunol.* 23:275–306.
- 4. Colonna, M., G. Trinchieri, and Y.J. Liu. 2004. Plasmacytoid dendritic cells in immunity. *Nat. Immunol.* 5:1219–1226.

- 5. Asselin-Paturel, C., and G. Trinchieri. 2005. Production of type I interferons: plasmacytoid dendritic cells and beyond. J. Exp. Med. 202:461-465.
- 6. Kadowaki, N., S. Ho, S. Antonenko, R. de Waal Malefyt, R.A. Kastelein, F. Bazan, and Y.-J. Liu. 2001. Subsets of human dendritic cell precursors express different Toll-like receptors and respond to different microbial antigens. J. Exp. Med. 194:863-870.
- 7. Honda, K., H. Yanai, A. Takaoka, and T. Taniguchi. 2005. Regulation of the type I IFN induction: a current view. Int. Immunol. 17:1367-1378.
- 8. Kawai, T., and S. Akira. 2006. Innate immune recognition of viral infection. Nat. Immunol. 7:131-137.
- 9. Dzionek, A. 2001. BDCA-2, a novel plasmacytoid dendritic cell-specific type II C-type lectin, mediates antigen capture and is a potent inhibitor of interferon α/β induction. J. Exp. Med. 194:1823–1834.
- 10. Ju, X.-S., C. Hacker, B. Scherer, V. Redecke, T. Berger, G. Schuler, H. Wagner, G.B. Lipford, and M. Zenke. 2004. Immunoglobulinlike transcripts ILT2, ILT3 and ILT7 are expressed by human dendritic cells and down-regulated following activation. Gene. 331:159-164.
- 11. Rissoan, M.C., T. Duhen, J.M. Bridon, N. Bendriss-Vermare, C. Pâeronne, B. de Saint Vis, F. Briáere, and E.E. Bates. 2002. Subtractive hybridization reveals the expression of immunoglobulin-like transcript 7, Eph-B1, granzyme B, and 3 novel transcripts in human plasmacytoid dendritic cells. Blood. 100:3295-3303.
- 12. Brown, D., J. Trowsdale, and R. Allen. 2004. The LILR family: modulators of innate and adaptive immune pathways in health and disease. Tissue Antigens, 64:215–225.
- 13. Navarro, F., M. Llano, T. Bellón, M. Colonna, D.E. Geraghty, and M. López-Botet. 1999. The ILT2(LIR1) and CD94/NKG2A NK cell receptors respectively recognize HLA-G1 and HLA-E molecules coexpressed on target cells. Eur. J. Immunol. 29:277-283.
- 14. Dietrich, J., M. Cella, and M. Colonna. 2001. Ig-like transcript 2 (ILT2)/leukocyte Ig-like receptor 1 (LIR1) inhibits TCR signaling and actin cytoskeleton reorganization. J. Immunol. 166:2514-2521.
- 15. Tedla, N., C. Bandeira-Melo, P. Tassinari, D.E. Sloane, M. Samplaski, D. Cosman, L. Borges, P.F. Weller, and J.P. Arm. 2003. Activation of human eosinophils through leukocyte immunoglobulin-like receptor 7. Proc. Natl. Acad. Sci. USA. 100:1174-1179.
- 16. Rosen, D.B., M. Araki, J.A. Hamerman, T. Chen, T. Yamamura, and L.L. Lanier. 2004. A structural basis for the association of DAP12 with mouse, but not human, NKG2D. J. Immunol. 173:2470-2478.
- 17. Dzionek, A., Y. Inagaki, K. Okawa, J. Nagafune, J. Réock, Y. Sohma, G. Winkels, M. Zysk, Y. Yamaguchi, and J. Schmitz. 2002. Plasmacytoid

dendritic cells: from specific surface markers to specific cellular functions. Hum. Immunol. 63:1133-1148.

- Diebold, S.S., T. Kaisho, H. Hemmi, S. Akira, and C. Reis e Sousa. 18. 2004. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science. 303:1529-1531.
- 19. Schroeder, J.T., A.P. Bieneman, H. Xiao, K.L. Chichester, K. Vasagar, S. Saini, and M.C. Liu. 2005. TLR9- and FcERI-mediated responses oppose one another in plasmacytoid dendritic cells by down-regulating receptor expression. J. Immunol. 175:5724-5731.
- 20. Novak, N., J.-P. Allam, T. Hagemann, C. Jenneck, S. Laffer, R. Valenta, J. Kochan, and T. Bieber. 2004. Characterization of FcERI-bearing CD123⁺ blood dendritic cell antigen-2⁺ plasmacytoid dendritic cells in atopic dermatitis. J. Allergy Clin. Immunol. 114:364-370.
- 21. Fuchs, A., M. Cella, T. Kondo, and M. Colonna. 2005. Paradoxic inhibition of human natural interferon-producing cells by the activating receptor NKp44. Blood. 106:2076-2082.
- 22. Blasius, A.L., M. Cella, T. Takai, and M. Colonna. 2006. Siglec-H is an IPC-specific receptor that modulates type I IFN secretion through DAP12. Blood. 107:2474-2476.
- 23. Hamerman, J.A., N.K. Tchao, C.A. Lowell, and L.L. Lanier. 2005. Enhanced Toll-like receptor responses in the absence of signaling adaptor DAP12. Nat. Immunol. 6:579-586.
- 24. Pasquier, B., P. Launay, Y. Kanamaru, I. Moura, S. Pfirsch, C. Ruffie, D. Henin, M. Benhamou, M. Pretolani, U. Blank, and R. Monteiro. 2005. Identification of FcaRI as an inhibitory receptor that controls inflammation: dual role of FcR y ITAM. Immunity. 22:31-42.
- 25. Zhu, M., Y. Liu, S. Koonpaew, O. Granillo, and W. Zhang. 2004. Positive and negative regulation of FcERI-mediated signaling by the adaptor protein LAB/NTAL. J. Exp. Med. 200:991-1000.
- 26. Volna, P., P. Lebduska, L. Draberova, S. Simova, P. Heneberg, M. Boubelik, V. Bugajev, B. Malissen, B.S. Wilson, V. Horejsi, et al. 2004. Negative regulation of mast cell signaling and function by the adaptor LAB/NTAL. J. Exp. Med. 200:1001-1013.
- 27. Baechler, E.C., P.K. Gregersen, and T.W. Behrens. 2004. The emerging role of interferon in human systemic lupus erythematosus. Curr. Opin. Immunol. 16:801-807.
- Ito, T., H. Kanzler, O. Duramad, W. Cao, and Y.J. Liu. 2006. 28. Specialization, kinetics, and repertoire of type 1 interferon responses by human plasmacytoid predendritic cells. Blood. 107:2423-2431.
- 29. Coligan, J.E. 2001. Current Protocols in Immunology. John Wiley & Sons, New York. Units 7.23, 7.24, 7.31.
- 30. Zhang, L., M.F. Miles, and K.D. Aldape. 2003. A model of molecular interactions on short oligonucleotide microarrays. Nat. Biotechnol. 21:818-821.