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Unraveling the functions of plasmacytoid dendritic cells during viral infections, autoimmunity, and tolerance

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Summary

Plasmacytoid dendritic cells (pDCs) are bone marrow-derived cells that secrete large amounts of type I interferon (IFN) in response to viruses. Type I IFNs are pleiotropic cytokines with antiviral activity that also enhance innate and adaptive immune responses. Viruses trigger activation of pDCs and type I IFN responses mainly through the Toll-like receptor pathway. However, a variety of activating and inhibitory pDC receptors fine tune the amplitude of type I IFN responses. Chronic activation and secretion of type I IFN in the absence of infection can promote autoimmune diseases. Furthermore, while activated pDCs promote immunity and autoimmunity, resting or alternatively activated pDCs may be tolerogenic. The various roles of pDCs have been extensively studied *in vitro* and *in vivo* with depleting antibodies. However, depleting antibodies cross-react with other cell types that are critical for eliciting protective immunity, potentially yielding ambiguous phenotypes. Here we discuss new approaches to assess pDC functions *in vivo* and provide preliminary data on their potential roles during viral infections. Such approaches would also prove useful in the more specific evaluation of how pDCs mediate tolerance and autoimmunity. Finally, we discuss the emergent role of pDCs and one of their receptors, tetherin, in human immunodeficiency virus pathogenesis.

Keywords

plasmacytoid dendritic cell; interferon; virus; HIV; tetherin; autoimmunity; tolerance

pDCs: immune cells with potential impact on innate and adaptive host responses to viruses

Plasmacytoid dendritic cells (pDCs) are a small population of bone marrow-derived cells that specialize in the secretion of type I interferons (IFNs), i.e. IFN- α , - β , - ω and - λ (1, 2). These cells were first described in humans as plasmacytoid T cells or plasmacytoid monocytes because of their plasma cell-like morphology, expression of CD4 (3), and their expression of monocytic markers such as the α chain of the interleukin-3 receptor (IL-3R) (also called CD123) and CD68 (4, 5). Although the function of these cells was initially unknown, the presence of a large endoplasmic reticulum suggested a potential function in cytokine secretion. Later, it was shown that plasmacytoid T cells/plasmacytoid monocytes could differentiate into a subset of antigen-presenting cells called pre-DC2, now referred to as pDCs (6, 7). Eventually, pDCs were found to secrete type I IFN and to correspond to the human natural IFN-producing cells (NIPCs) (8, 9), a subset of blood cells that had been previously shown to produce high amounts of type I IFN in response to *in vitro* incubation

with bacteria and several viruses (8–14). Mouse cells with phenotypic and functional characteristics of human pDCs were finally identified in 2001 (15–17).

The phenotypic characterization of pDCs is quite extensive. Human pDCs can be distinguished from other blood cells based on the selective expression of blood dendritic cell antigen-2 (BDCA-2) (also called CD303) (18, 19) and ILT7 (20, 21). Human pDCs also express CD4, MHC class II, CD123 and CD2. CD2 is a cell adhesion molecule that distinguishes two human pDC subsets (22). One of these subsets (CD2^{hi}) expresses lysozyme and displays cytolytic capacity. Additionally, human pDCs express two intracellular proteins, granzyme B (20, 23) and the CD2-associated protein (CD2AP) (24). In contrast, human pDCs lack the lineage markers CD3, CD19, CD14, CD16 and the DC marker CD11c.

In mice, pDCs selectively express Siglec-H (25, 26), although there is evidence that certain macrophage subsets in the spleen, lymph nodes (LNs), and brain are also Siglec-H⁺ (26). Bone marrow stromal antigen-2 (BST-2), also known as CD317 or tetherin, is another marker that is selectively expressed on pDCs and plasma cells in naive mice, but is upregulated on most cell types following exposure to type I IFN or IFN- γ (27). Lymphocyte activation marker 3 (LAG-3) is a molecule highly, although not exclusively, expressed by mouse pDCs (28). Additional pDC markers in mice include B220, Ly6C, CD11c (low amounts), CD8 α (variable amounts), whereas CD11b is either not expressed or present in very low amounts (15). Mouse pDCs also express Ly49Q (29–31) and the gut homing chemokine receptor CCR9 (32–34). Prior to the generation of an anti-Siglec-H antibody, mouse pDCs were often identified and sorted based on the co-expression of B220 and CD11c. However, since activated natural killer (NK) cells express CD11c and B220, we now know that B220⁺CD11c⁺ cells are actually a heterogeneous population comprised of pDCs, NK cells, and some B cells (35–37).

pDCs originate in the bone marrow from myeloid and lymphoid precursors and require flt3-like kinase 3 ligand (Flt3L) for development. pDC development has been extensively reviewed elsewhere (38, 39) and is not addressed here. Under homeostatic conditions, pDCs are released into the blood from the bone marrow and are found in small numbers mainly in T-cell areas of LNs and spleen, mucosal-associated lymphoid tissues, thymus, and liver. A marked increase of pDCs is evident in several infectious and non-infectious human pathologies, particularly in lymph nodes of patients affected by sarcoidosis, *Mycobacterium tuberculosis* infection (40), Kikuchi's disease (41) and in the skin of patients affected by systemic lupus erythematosus (SLE) (42). Additionally, pDCs infiltrate LNs that drain sites of infection or inflammation in response to inflammatory chemokines (43, 44).

pDCs can secrete high amounts of type I IFNs, which induce a strong antiviral state (45–47) by augmenting the expression of numerous antiviral molecules that make cells resistant to viral infections and promote apoptosis of virally infected cells. Type I IFN production by pDCs has been shown to be largely independent of IFN- α/β receptor (IFNAR) feedback signaling (48). However, recent studies suggest that pDCs sense type I IFNs generating an autocrine circuit through IFNAR that amplifies type I IFN secretion (49, 50) and makes pDCs refractory to viral infections. Besides producing type I IFNs, pDCs also secrete IL-12, IL-6, TNF- α , and inflammatory chemokines. Through secretion of these cytokines and type I IFNs, pDCs may influence both innate and adaptive immune responses. Type I IFN and IL-12 promote multiple T cell functions including long-term T-cell survival and memory (51–54), T-helper 1 (Th1) polarization of CD4⁺ T cells (55), CD8⁺ T-cell cytolytic activity, and IFN- γ production (54). Moreover, type I IFN and IL-12 increase NK cell-mediated cytotoxicity and IFN- γ production *in vitro* and *in vivo* (56). IFN- α and IFN- β also promote

the differentiation and maturation of DCs allowing them to effectively present and cross-present antigen to naive T cells (57–59).

By producing IL-6 and type I IFN, pDCs can induce the differentiation of B cells into immunoglobulin-secreting plasma cells (60–62) and instruct plasma cells to preferentially secrete IgG rather than IgM. By producing chemokines such as CXCL9 (MIG), CXCL10 (IP-10), CCL3 (MIP-1 α), CCL4 (MIP-1 β), and CCL5 (RANTES) (63), mouse and human pDCs can attract activated CD4⁺ and CD8⁺ T cells to sites of infection (64, 65). pDCs express MHC class II and the costimulatory molecules CD80 and CD86 and present antigens to CD4⁺ T cells and cross-present antigens to CD8⁺ T cells, although less efficiently than classical DCs. The role of pDCs in antigen presentation has been extensively reviewed by Villadangos *et al.* (66) and therefore is not addressed here. In conclusion, evidence so far accumulated, which is largely based on *in vitro* studies, indicates that pDCs have the potential to impact multiple aspects of innate and adaptive immune responses to viruses. The present challenge is to understand the *in vivo* role of pDCs in immunity and pathology.

Pathways that trigger pDCs secretion of type I IFN in response to viruses

pDCs sense viruses through Toll-like receptor 9 (TLR9) and TLR7 (1, 2, 67). TLR9 senses double-stranded (ds) DNA viral genomes rich in unmethylated CpG sequences as well as synthetic CpG oligodeoxyribonucleotides (CpG ODN). TLR7 detects viral single-stranded (ss) RNA and synthetic oligoribonucleotides (ORN). TLR9 and TLR7 are synthesized in the endoplasmic reticulum and transported into endosomes where TLR9 is converted into an active form by proteolytic cleavage (68, 69). Viruses like VSV and Sendai virus (SeV), which enter pDCs by fusion, generate replicative intermediates that are redirected from the cytosol into TLR9/7⁺ endosomes by autophagy (70). In this process, a fraction of the cytosol is encircled by a membrane to generate a vacuole that can fuse to TLR9/7⁺ endosomes. Other viruses, like herpes simplex virus (HSV) and Coxsackievirus B (CVB), are internalized through receptor-mediated endocytosis and delivered to TLR9/7⁺ endosomes (71–73). The delivery of viral nucleic acids into intracellular endosomes containing active TLR9 and TLR7 requires a tight spatiotemporal regulation of membrane trafficking (74, 75).

pDCs also express two cytosolic sensors for viral RNA, RIG-I and MDA5. RIG-I has been shown to preferentially recognize ssRNA that is phosphorylated at the 5' end and dsRNA molecules that are relatively short. In contrast, MDA5 recognizes long dsRNAs but does not discern 5' phosphorylation (76–79). Since these viral RNA intermediates are generated during active viral replication and pDCs are rather refractory to viral replication due to the autocrine effect of type I IFNs, RIG-I and MDA5 do not play a major role in pDCs activation, unless type I IFN signaling is impaired (50). Unlike conventional DC, pDCs do not express TLR2, TLR4, TLR5, or TLR3, which accounts for why they do not respond to peptidoglycans, lipopolysaccharide, flagellin, or double-stranded RNA (67).

Upon engagement with nucleic acid ligands, TLR9 and TLR7 deliver intracellular signals through the cytosolic adapter MyD88, which acts as a docking site for IRAK1/4, TRAF3, IKK α , and IRF7 (80). IRF7 is then phosphorylated and translocated into the nucleus to induce the transcriptional activation of the type I IFN genes (81). IRF7 is positively controlled by the PI(3)K-mTOR-p70S6K pathway (82) and translationally repressed by 4E-BPs (83). MyD88 also recruits IRF5, a transcription factor that cooperates with nuclear factor- κ B (NF- κ B) in activating the transcription of genes encoding proinflammatory cytokines and chemokines (84). TLR7 and TLR9 signaling pathways also induce the maturation of pDCs, which consists of increased expression of MHC class II and

costimulatory molecules (CD80, CD86 and CD40), resulting in the capacity to present antigens. Engagement of TLR7/9 in early endosomes leads to type I IFN production while engagement of TLR7/9 in late endosomes results in pDC maturation and cytokine production (85, 86). In addition to TLR7 and 9, pDCs can be activated via CD40, a member of the TNF receptor superfamily that signals through the alternative NF- κ B pathway (6, 87). CD40 is a weaker inducer of type I IFN as compared to TLR7 and TLR9. However, it can effectively promote the maturation of pDCs and their antigen presenting capacity.

A role for ITAM and ITIM signaling in type I IFN responses of pDCs

pDCs express several cell surface receptors that regulate the amplitude of type I IFN responses to TLR9 and TLR7 ligands (86, 88–90) (Table 1). These receptors can be divided into two major groups. The first group includes cell surface receptors that do not signal on their own but deliver intracellular signals through an associated adaptor, usually the DNAX activation protein 12 (DAP12) or the γ chain of Fc receptors (FcR γ). These adaptors contain an intracellular tyrosine-based activation motif (ITAM), which, upon engagement of the associated receptor, becomes phosphorylated and recruits the protein tyrosine kinase Syk. The second group of receptors includes cell surface molecules that contain intracellular tyrosine-based inhibitory motifs (ITIM). Upon receptor engagement, ITIMs are phosphorylated and recruit protein tyrosine phosphatases SHP-1/2 and/or phosphatidylinositol phosphatase SHIP1/2.

pDC receptors that signal through ITAMs or ITIMs control the amplitude of type I IFN secretion in response to TLR ligands as well as the degree of pDCs activation. Although receptors that signal through ITAMs usually activate cells, whereas ITIM-bearing receptors inhibit cell activation, this rule is not always true in pDCs; in fact, it is frequently reversed. In mice, these receptors include Siglec-H, PDC-TREM, and Ly49Q (Table 1). Siglec-H is a member of the immunoglobulin (Ig)-superfamily that signals through DAP12 (25, 26). Engagement of Siglec-H with a specific antibody inhibits pDC secretion of IFN- α in response to CpG (25). Attempts to identify the ligand for Siglec-H have so far been unsuccessful. In contrast to other Siglec family members, Siglec-H does not appear to recognize sialic acids or other carbohydrates (26). Since Siglec-H mediates endocytosis and is down-regulated during viral infections it is speculated that it may bind to viral or microbial components and function as an antigen capturing molecule. PDC-TREM is a member of the Ig-superfamily that, like Siglec-H, associates with the adapter DAP12 (91). It is exclusively expressed by activated mouse pDCs and as such, it is a useful marker for identifying and monitoring activated pDCs *in vitro* and *in vivo*. In contrast to Siglec-H, PDC-TREM enhances type I IFN secretion induced by TLR-signaling. Ly49Q is a C-type lectin that belongs to the Ly49 family of NK cell receptors (29–31). Like other Ly49 receptors, Ly49Q binds MHC class I and has a cytoplasmic ITIM. However, despite the presence of the ITIM, Ly49Q is a positive regulator of type I IFN secretion (92). Accordingly, receptor cross-linking using an immobilized anti-Ly49Q monoclonal antibody or recombinant H-2K^b ligand (K^b:Ig/OVA) greatly enhances CpG-dependent IFN- α production by pDCs. Moreover, pDCs obtained from Ly49Q^{-/-} mice are impaired in their ability to produce cytokines in response to TLR7 and TLR9 ligands both *in vitro* and *in vivo*. It was also reported that Ly49Q^{-/-} pDCs produce less IFN- α after infection with mouse cytomegalovirus (MCMV) *in vitro*; however, no differences in systemic IFN- α levels were observed after MCMV infection *in vivo*, suggesting that pDCs are not the only source of IFN- α during host responses to MCMV. In fact, during MCMV infection, conventional DC produce IFN- α , activate NK cells, and limit MCMV replication *in vivo* (93).

Human pDCs receptors that signal through ITAMs or ITIMs include BDCA-2, ILT7, NKp44, DCIR, Fc γ RIIa (CD32), and CD300a/c (Table I). BDCA-2 is a C-type lectin that

associates with FcR γ (18, 19, 94, 95). Cross-linking BDCA-2 inhibits type I IFN production in response to TLR agonists and DNA/anti-DNA immunocomplexes (19). BDCA-2-specific Abs are rapidly internalized by pDCs and processed into peptides that are presented on MHC class II to T cells, suggesting that BDCA-2 may also be involved in the capture and presentation of yet unknown BDCA-2 ligands (19). Immunoglobulin-like transcript 7 (ILT7) is a pDC-specific surface receptor of the Ig-superfamily that, like BDCA-2, associates with FcR γ (21). When cross-linked with an antibody, ILT7 suppresses the secretion of IFN- α and TNF- α after TLR7 and TLR9 engagement but has no effect on pDC maturation (21). NKp44 is an Ig-like receptor that signals through DAP12. NKp44 is constitutively expressed on mucosal NK cells (96) and tonsil pDCs (97) and is induced on blood NK cells and pDCs by IL-2 and IL-3 (97), respectively. Although NKp44 functions as an activating receptor on human NK cells, cross-linking of NKp44 on human pDCs inhibits the secretion of type I IFN induced by CpG. Dendritic cell immunoreceptor (DCIR) is an ITIM-containing C-type lectin receptor expressed by human pDCs (98). Cross-linking of DCIR inhibits the production of TLR9-induced IFN- α but has no effect on TLR9-induced pDCs maturation. Additionally, DCIR is internalized from the cell surface after antibody-mediated cross-linking, and antigens targeted to pDCs via DCIR are presented to T cells. Thus, DCIR may function as an antigen-capturing molecule.

In contrast to BDCA-2, ILT7, NKp44, and DCIR, the low affinity receptor for IgG, Fc γ RIIa, enhances type I IFN responses of human pDCs (99). In SLE, Fc γ RIIa binds and internalizes immune complexes that contain nucleic acids derived from apoptotic cells, facilitating the delivery of nucleic acids to TLR7 and TLR9, which trigger the inappropriate stimulation of pDCs, the release of type I IFN and, ultimately autoimmunity (100–102). Finally, CD300a/c are two related members of the Ig-superfamily expressed on a variety of leukocytes including monocytes, macrophages, granulocytes, DCs, T lymphocytes, NK cells, and pDCs (103). CD300a contains three ITIMs, while CD300c contains no obvious signaling motifs and probably signals through a yet unidentified ITAM-containing adapter. Antibody-mediated cross-linking of CD300a/c suppresses TNF- α , IL-6, and HLA-DR expression and increases IFN- α production by pDCs in response to CpG. Because the available antibody does not discriminate between CD300a and CD300c, it is unclear whether these effects depend on ITIM or ITAM signaling.

Recent studies have identified some of the intracellular signaling molecules downstream of pDC cell surface receptors that modulate type I IFN production (88–90). Cao *et al.* and Röck *et al.* showed that pDCs express several signaling molecules previously found in B cells, like Lyn kinase, spleen tyrosine kinase (Syk), Bruton's tyrosine kinase (Btk), the adapter B-cell linker (BLNK) (also called SLP65), and the phospholipase PLC γ 2 (94, 95). Triggering of the inhibitory BDCA-2 receptor results in the phosphorylation of Syk, BLNK, and PLC γ 2. PLC γ 2 generates the second messengers DAG and IP3, which induce protein kinase C (PKC) activity and calcium mobilization, respectively. While the PKC activator phorbol myristate acetate (PMA) inhibits pDC type I IFN responses in a dose-dependent manner, the calcium ionophore ionomycin has no effect (95). These results suggest that PKC activity and not calcium mobilization is responsible for type I IFN inhibition after BDCA-2 engagement.

The molecular mechanisms by which cell surface molecules and their downstream signaling components regulate the production of type I IFN in pDCs still remain somewhat elusive. It is interesting to note that many of these molecules undergo internalization from the cell surface upon ligand binding and therefore are involved in membrane trafficking. Moreover, the ITAM and ITIM signaling pathways initiated by these cell surface receptors have a profound impact on actin cytoskeleton rearrangement, microtubule polarization and vesicle trafficking (104, 105). Since pDC activation and secretion of type I IFN depend on membrane trafficking and, in particular, on the timely intersection of TLR7- and TLR9-

containing vesicles with endosomes containing RNA and DNA ligands (74), it has been proposed that receptors which signal through ITAMs and ITIMs, as well as receptors that traffic back and forth between the cell surface and the intracellular compartments, may influence pDC activation by altering the tight spatiotemporal regulation of TLR9 and TLR7 activation (75).

Generation of pDCs-less mice for the analysis of pDCs functions *in vivo*

Because of their capacity to produce type I IFNs, pDCs have been proposed to provide an initial line of defense against viral infections. However, it is essential to demonstrate such function *in vivo*. One fundamental approach to ascertain pDC functions *in vivo* is to analyze host responses in the absence of pDCs. The first attempts to deplete pDCs involved the administration of the antibody Gr-1 (RB6-8C5) (15, 106), which cross-reacts with both Ly6C on pDCs and plasma cells (107) and Ly6G on granulocytes (Table 2). The major drawback of this approach is that injecting Gr-1 not only eliminates pDCs but also depletes granulocytes. Antibodies specific for BST-2 have been routinely used to deplete pDCs *in vivo* (Table 2). These antibodies include 120G8 (108), mouse plasmacytoid dendritic cell antigen-1 (mPDCA-1) (109) and 927 (27). In naive mice, the expression of BST-2 is restricted to pDCs and plasma cells but is induced on most cell types following stimulation with type I IFN or IFN- γ (27). This activation-induced promiscuous expression of BST-2 has important implications for the use of anti-BST-2 antibodies in the depletion of pDCs, as these antibodies may also deplete additional cell types during a viral infection and the subsequent immune response. Therefore, it is important to corroborate the results obtained with monoclonal antibody-mediated depletion with alternative approaches.

One approach that has been extensively used to deplete conventional DCs in the mouse is based on the generation of transgenic mice that selectively express the diphtheria toxin receptor (DTR) under the control of the CD11c promoter (110). Thus, administration of diphtheria toxin (DT) to CD11c-DTR transgenic mice results in the selective elimination of classical DCs. pDCs are not eliminated in this system because they express low levels of CD11c compared to classical DC. We have followed a similar approach to express DTR under a pDC-specific promoter. The promoters of both murine Siglec-H and human BDCA-2 genes were considered, but, due to the potential expression of Siglec-H by certain macrophage subsets (26), we opted for the BDCA-2 promoter. Although BDCA-2 is not expressed in mouse, we assumed that its promoter contains target sequences for pDC-specific transcription factors that are likely to be conserved across species. Indeed, this assumption was correct as Cisse *et al.* demonstrated that the basic helix-loop-helix transcription factor (E protein) E2-2/Tcf4, which is a specific regulator of pDCs development (111), interacts with elements in the BDCA-2 promoter (112). Hence, we succeeded in generating a BDCA-2-DTR transgenic mouse on a C57BL/6 background in which pDCs can be depleted with high efficacy and specificity after DT administration (Fig. 1). The DT-induced depletion persists for 2–3 days after which pDCs numbers are gradually restored; however, pDCs can be depleted for longer periods of time because repeated DT administration does not appear to be fatal. This transgenic mouse represents a unique and cutting edge tool to investigate the function of pDCs in a variety of models. In the two following sections we summarize our results in two models of viral infections and draw some preliminary conclusions on the roles of pDCs in host responses to viral infections.

pDCs enhance anti-VSV CD8⁺ T-cell responses *in vivo*

We first chose to assess the role of pDCs using our BDCA-2-DTR mice during a viral infection with a recombinant VSV expressing ovalbumin (VSV-OVA). VSV belongs to the family *Rhabdoviridae* and is a single-stranded negative sense RNA virus that encodes five

major proteins: glycoprotein, large protein, phosphoprotein, matrix protein, and nucleoprotein. Several reports have implicated pDCs and/or pDCs-derived type I IFN in host responses against VSV. First, IFN- α/β R^{-/-} mice are highly susceptible to VSV (113); second, pDCs have been described as the major producers of IFN- α during systemic VSV infection (48); third, mice deficient in TLR7 or MyD88 have reduced responses to VSV (114); fourth, VSV infects pDCs and reaches TLR7⁺ endosomes by autophagy (70); finally, IRF-7^{-/-} pDCs are unable to produce type I IFN in response to VSV (81). Moreover, since VSV induces a strong antibody response that is necessary and sufficient for protection and a strong cytotoxic T-cell (CTL) response in some but not all H-2 haplotypes (115), we felt that this model would permit us to examine the impact of pDCs on both B and T-cell responses during a viral infection.

BDCA-2-DTR mice were injected with PBS or DT on days -1 and 3 post infection. Serum was collected at various timepoints after infection and spleens from surviving mice were analyzed on day 7. Spleens from PBS-injected mice were moderately larger than spleens from DT-treated animals and contained more cells (Fig. 2A). Closer examination of the OVA-specific CD8⁺ T cells from both groups of mice by staining with H-2K^b/OVA₂₅₇₋₂₆₄ peptide tetramers revealed a reduction in the frequencies and numbers of Ag-specific CD8⁺ T cells in pDCs-depleted animals compared to controls (Fig. 2B). These observations led us to question why T-cell numbers were reduced in pDC-depleted mice relative to controls. Because CD8⁺ T-cell expansion and survival are largely dependent on type I IFN and/or IL-12 (51-54), we investigated whether pDC-depleted mice had lower levels of one or both of these cytokines following VSV-OVA infection. Surprisingly, both control and depleted animals exhibited similar serum levels of IFN- α at 6, 12, 24, and 72 h post-infection while IL-12 was slightly reduced in the depleted mice after 24 h (Fig. 2C).

pDCs may contribute to CD8⁺ T-cell expansion by activating bystander DCs, as suggested by a study of a model of HSV infection (116). Thus, we tested if the decrease in OVA-specific CD8⁺ T cells in pDCs-depleted mice was due to a reduction in DC activation. Twenty-four hours after infection, splenic CD11c^{hi} DCs from control and depleted animals were stained with a panel of antibodies. DCs from both groups of mice expressed similar levels of CD80, CD86, MHC II, and CD40 molecules (Fig. 2D). pDCs from non-depleted mice stained with the same panel of antibodies showed an upregulation of CD80, CD86, and CD40 (data not shown). It was also possible that pDCs directly cross-present OVA to CD8⁺ T cells and therefore the lack of pDCs in the DT-treated mice would result in less antigen presentation and a diminished CD8⁺ T-cell response. This was not the case though (Fig. 2E). When we sorted DC subsets from VSV-infected mice, only the CD8 α ⁺ DCs elicited IFN- γ in a dose-dependent manner from CD8⁺ T cells purified from OT-1 TCR transgenic mice (Fig. 2E). We also observed no major differences in the levels of IFN- γ detected in culture supernatants from CD8⁺ T cells cultured with CD8 α ⁺ DCs from control versus pDCs-depleted VSV-infected mice.

In addition to secreting type I IFN, IL-12, TNF- α , and IL-6, pDCs also produce the chemokines MIP-1 α and MIP-1 β . Both of these chemokines have been reported to recruit naive CD8⁺ T cells to sites of CD4⁺ T cell:DC interactions (117). Thus, it is possible that pDC depletion affects the recruitment of CD8⁺ T cells to the spleens of VSV-infected mice. Interestingly, we detected a modest but statistically significant reduction of MIP-1 β in the serum of pDC-depleted VSV-infected mice compared to controls (Fig. 2F). A similar finding was obtained when splenocytes from control or depleted mice were incubated with VSV-OVA or VSV-GFP *ex vivo* (data not shown). These results support a defect in CD8⁺ T-cell recruitment in pDC-depleted mice infected with VSV-OVA. This hypothesis is supported by a recent study of influenza virus infection in mice with a hypomorphic mutation of the transcription factor Ikaros (Ikaros^{L/L}), which impairs pDC development.

During early infection, accumulation of T cells in the bronchoalveolar space was delayed, suggesting a role of pDCs in promoting the recruitment of influenza-specific CD8⁺ T cells (118). Additional approaches to corroborate these observations are currently ongoing.

Neutralizing antibodies are critical for the clearance of systemic VSV (113), and pDC-derived type I IFN and IL-6 have been shown to promote plasma cell generation and antibody production (60–62). Thus, we also looked at B-cell responses in pDC-depleted mice infected with VSV-OVA. Like IFN- α , serum IL-6 levels were similar in control and depleted mice 24 and 72 h post-infection (Fig. 2G). Furthermore, B-cell activation in the spleen as judged by the expression of CD69, CD80, and CD86 at different timepoints post-infection and the frequencies and absolute numbers of plasma cells on day 7 did not suggest a defect in B-cell responses in either group of mice (data not shown). We next evaluated the levels of OVA-specific antibodies and virus-neutralizing antibodies in VSV-infected mice. Both control and pDCs-depleted mice had detectable levels of anti-OVA IgM in their serum by day 7 and had high levels of antibodies capable of neutralizing VSV-OVA (Fig. 2H). Based on these findings, we conclude that pDCs depletion does not affect antibody responses to VSV.

Perhaps the most pertinent and obvious question related to these studies is whether depleting pDCs had any effect on viral titers or survival. Viral titers in the spleen, liver, lung and brain were examined on days 1, 3, and 7 post-infection. Virus was only detected in the brain and while no major differences were evident at early timepoints, titers were slightly higher in the brains of DT-treated mice on day 7 (Fig. 2I). A true survival experiment that extended beyond day 7 was never conducted, but we did record the number of deaths in each group of mice during individual experiments. VSV has a strong tropism for olfactory receptor neurons, using them for entry into the CNS and severe infection presents as hind limb paralysis followed by death. In our hands, this typically occurred on days 4–6, respectively, in both groups of mice. On day 7 the survival rate in the PBS-injected group was 66% compared to 60% in the DT-injected group.

These experiments suggest that when pDCs are depleted during VSV-OVA infection, the systemic IFN- α response is unaffected in the absence of pDCs. This finding is somewhat surprising, given that pDCs are generally assumed to be the primary producers of type I IFN during viral infections. However, since VSV is detected through TLR7 (114) and TLR7 is expressed in multiple cells, including classical DCs, it is likely that type I IFN produced by other TLR7⁺ cells compensate for the lack of pDCs. In contrast, we observed that the absence of pDCs resulted in a moderate but significant decrease in the numbers of OVA-specific CD8⁺ T cells, whereas the generation of plasma cells and production of virus-specific antibodies were unaffected. Although the reduction in antigen-specific CD8⁺ T cells had a modest impact on viral titers and survival, previously published data have illustrated that antibody responses are necessary and sufficient for VSV eradication, whereas a CTL response is not essential for VSV clearance but provides limited help to control viral spread (119).

Lack of pDCs does not impact anti-LCMV CTL responses *in vivo*

It is possible, therefore, that pDCs are more critical during viral infections where viral clearance is highly dependent on CTL responses. To test this possibility, we explored the role of pDCs during infection with lymphocytic choriomeningitis virus (LCMV). LCMV is a non-cytopathic, single-stranded negative sense RNA virus whose clearance relies heavily on a robust type I IFN-dependent CTL response (53, 120). Jung *et al.* previously reported that pDCs are a major source of IFN- α in LCMV infection (121). However, TLR7^{-/-}TLR9^{-/-} mice infected with LCMV showed no major impairment in IFN- α production or CTL

responses compared to wildtype mice (121). Additionally, the depletion of pDCs with anti-Gr-1 did not reduce IFN- α responses to LCMV infection (106). Thus, the role of pDCs in the control of LCMV infection is unclear. To address this, BDCA-2-DTR mice were infected with a low or high dose of the Armstrong strain of LCMV (LCMV-ARM) after PBS or DT administration. Serum was collected at 24 and 72 h post-infection and IFN- α and pro-inflammatory cytokines were measured by ELISA and cytometric bead array, respectively. pDC depletion had no effect on the levels of IFN- α , MCP-1, IL-6, or TNF- α at either time point with either dose of virus (Fig. 3A). On days 10 and 11 post infection, CTL responses were examined. Both control and pDC-depleted mice exhibited vigorous CTL responses as determined by tetramer stainings, intracellular IFN- γ stainings and cytotoxicity assays (Fig. 3B). These data imply that while pDCs may produce IFN- α during LCMV infection, they are by no means the only source. Moreover, their overall contribution to CTL responses against LCMV appears minimal, in opposition to the noticeable contribution of pDCs to anti-VSV CTL responses. It has been shown that LCMV extensively replicates in the host, eliciting very strong CTL responses that are independent of CD4⁺ T-cell help (122); in contrast, since VSV replicates poorly in mice (123), anti-VSV CTL responses require CD4⁺ T-cell help (115) and costimulation (124). Therefore, these results suggest that pDCs may be required for enhancing relatively weak CTL responses. Experiments are currently ongoing to establish the impact of pDCs depletion on CD4⁺ T-cell helper responses. While LCMV-ARM establishes an acute infection that is completely eradicated by host CTL responses, LCMV-clone 13 establishes a chronic infection that leads to CTL exhaustion (125–127). It will be important to assess whether pDCs can help control chronic viral infection by preventing or limiting CTL exhaustion. In summary, we envision that pDCs strengthen CTL responses against viruses that replicate slowly or inefficiently and therefore depend on T cell help and costimulation. Furthermore, pDCs may be particularly important in secreting chemokines that attract CD4⁺ and CD8⁺ T cells, facilitating the generation of antigen-specific T cells (Fig. 4).

pDCs and human immunodeficiency virus

In parallel to studies in the mouse, intense focus has been placed on the function of pDCs in human viral infections, particularly human immunodeficiency virus (HIV). The potential role of pDCs in host responses against HIV gained a great deal of attention when it was observed that pDCs numbers were reduced in the blood of patients chronically infected with HIV. Loss of pDCs correlates with high viral loads, decreased numbers of CD4⁺ T cells, and the onset of opportunistic infections (128–136). Moreover, some studies have shown that pDC loss is often sustained and that pDC function and numbers are not completely restored in patients receiving highly active antiretroviral therapy (HAART) (130, 133, 137, 138). At present, the causes of this drop in pDC frequency are unclear. Human pDCs might be targets of HIV because they express the HIV receptors CD4, CXCR4, and CCR5 (139). Alternatively, HIV could induce changes in the migratory properties of pDCs. Evidence for pDC recruitment stems from the finding that pDCs exposed to HIV upregulate the LN homing receptor CCR7 (140). Additionally, pDCs express a yet unidentified receptor for the HIV p17 matrix protein, which induces pDCs to upregulate CCR7 and migrate in response to CCL19 into secondary lymphoid tissues, where they may undergo apoptosis (141). Accordingly, it has been shown that in rhesus macaques infected with simian immunodeficiency virus (SIV), reduced pDC numbers in the blood during the acute phase correlate with a rapid influx of pDCs into peripheral LNs where they undergo cell death (142, 143).

An open-ended question is the whether pDC activation and type I IFN production during HIV infection is advantageous or detrimental to the host (Fig. 5). *In vitro*, pDCs secrete high amounts of IFN- α and other immune mediators in response to HIV (140, 144) and can

cross-present HIV-derived antigens to CD8⁺ T cells (145). HIV activation of pDCs is dependent on endocytosis of the virus through envelope-CD4 interactions followed by the engagement of TLRs in the endosomal compartment with viral RNA (144). HIV-activated pDCs may also contribute to host responses by inducing the maturation of classical DC through the secretion of IFN- α and TNF- α (140). Furthermore, pDC-derived type I IFN has been shown to limit HIV replication in CD4⁺ T cells (146, 147). These *in vitro* data suggests that in the short-term pDCs may be capable of mounting a protective response to HIV *in vivo*.

However, there is also evidence that chronic pDC activation may promote HIV pathogenesis and disease progression. Prolonged pDC activation and type I IFN production have been associated with the upregulation of activation markers on CD8⁺ T cells, the suppression of functional T-cell responses by T-regulatory cells (Tregs) and the progressive depletion of CD4⁺ T cells through apoptotic mechanisms such as TRAIL (tumor necrosis factor-related apoptosis-inducing ligand)/DR5 (death receptor 5) or Fas/Fas ligand (148–151). HIV-activated pDCs express the enzyme indoleamine-2,3-dioxygenase (IDO), which promotes tryptophan catabolism and induces Tregs from naive CD4⁺ T cells (152, 153) that suppress T-cell proliferation (152) and impair DC maturation (153). Interestingly, it was recently reported that pDCs isolated from women produce more IFN- α in response to HIV than pDCs isolated from men. Moreover, CD8⁺ T cells from HIV-infected women displayed a more activated phenotype than CD8⁺ T cells from men with a comparable viral load (154). Taken together, these findings suggest that extensive pDC activation potentially explains why HIV-infected women progress faster to AIDS. In summary, pDCs and type I IFN production might be important for maintaining lower viral loads early during HIV infection but chronic immune activation i.e. type I IFN and the expression of IDO by pDCs may potentiate disease progression by exerting apoptotic and suppressive effects on T cells (148–151).

The impact of BST-2 (tetherin) in pDCs functions and control of viral infections

BST-2 (now referred to as tetherin because most of the papers in the field use this nomenclature) is a type II transmembrane protein expressed by pDCs (27) and plasma cells (155), which is induced on virtually all cell types following exposure to type I IFNs and IFN- γ . Tetherin is a rather unique protein with atypical membrane topology (156). It consists of an N-terminal transmembrane domain, extracellular coiled-coiled domain and a C-terminal glycosylphosphatidylinositol (GPI) anchor. Tetherin is found on the cell surface in cholesterol-rich lipid microdomains (lipid rafts) and also in the transgolgi network following internalization via clathrin-dependent endocytosis. These localization studies and the biased expression of tetherin in plasma cells, which secrete immunoglobulins, and pDCs, which produce type I IFNs, have suggested that it might be involved in the trafficking of secreted proteins. Tetherin may also have a role in the trafficking and sorting of membrane proteins on the cell surface or in vesicles (27). Cross-linking tetherin on mouse pDCs with the anti-BST-2 antibody 927 reduces the secretion of IFN- α to CpG suggesting it may function as a modulator of IFN release (27). Furthermore, it was recently discovered that human tetherin interacts with ILT7, triggering an ILT7-FcR γ signaling pathway that leads to the reduction of type I IFN and proinflammatory cytokine secretion by pDCs to TLR7/9 ligands (157). The endogenous ligand for tetherin in mice has not yet been identified; however, it is tempting to speculate that BST-2 might interact with one of the paired immunoglobulin like receptors (PirA or PirB), the mouse orthologues of human ILT proteins (158).

In addition to the role of tetherin in regulating intracellular trafficking and secretion of type I IFN, there is a growing literature illustrating its function in limiting viral spread and infection. Tetherin was recently shown to inhibit the release of HIV-1 (159, 160) and other viruses from infected cells (161). Underscoring the importance of tetherin in blocking viral spreading, several viruses have evolved mechanisms to counteract the antiviral activity of tetherin in a species-specific manner. The HIV genome encodes a viral accessory protein, Vpu, that antagonizes tetherin and downregulates it from the cell surface (159, 160, 162–170). HIV-2 and SIV isolates do not carry a Vpu gene; however, these viruses are also capable of antagonizing and decreasing cell surface levels of tetherin via envelope glycoprotein (171) and Nef (negative regulatory factor) protein (172, 173). Ebola glycoprotein (174) and the Kaposi's sarcoma herpes virus K5/MIR2 (175, 176) also counteract the function of tetherin.

Does tetherin benefit pDCs during viral infections? Perhaps the constitutive expression of tetherin is one reason why, in addition to their innate ability to synthesize and secrete high levels of type I IFN, pDCs themselves are poorly permissive to viral replication. The constant expression of tetherin may help retain viruses at sites where they are easily degraded or in endosomes where they can be sensed by TLRs. Other cell types, conversely, might not have the opportunity to up-regulate tetherin before getting infected, thereby allowing the virus to spread. This scenario would almost always ensure that pDCs are able to respond to the viruses they internalize and that viruses, even before pDCs can secrete sufficient levels of type I IFN, are not able to efficiently replicate in or spread to other neighboring cells.

pDCs in autoimmunity

Although pDCs activation and type I IFN secretion may be important for antiviral responses, recent studies indicate that chronic pDC activation and secretion of type I IFN in the absence of infection may result in autoimmune diseases such as SLE and psoriasis vulgaris (89, 101, 177–181). In patients with SLE, pDCs infiltrate skin and secrete IFN- α/β in response to DNA/RNA/immunocomplexes that are internalized through Fc receptors and stimulate TLR7 and TLR9. SLE patients show increased serum levels of IFN- α/β and an IFN- α/β signature in the transcriptome of peripheral blood mononuclear cells (182–185). Activation of the IFN- α/β system in SLE correlates with disease activity and severity. Furthermore, long-term IFN- α/β treatment of patients afflicted with tumors or chronic viral infections induces SLE-like syndromes. Anti-TNF- α therapy for Crohn's disease and rheumatoid arthritis (RA) can also induce SLE-like syndromes in patients due to a cross-regulation of TNF- α and type I IFN. TNF- α inhibits the generation of pDCs from CD34⁺ hematopoietic progenitors and suppresses pDC production of IFN- α/β in response to influenza virus (186). pDCs also infiltrate psoriatic lesions and secrete IFN- α . Using a xenograft model of human psoriasis, Nestle *et al.* (187) showed that blocking IFN- α signaling or preventing pDCs production of IFN- α hindered the T-cell-dependent development of psoriasis and IFN- α reconstitution experiments demonstrated that pDC-derived IFN- α was necessary to drive the development of psoriasis *in vivo*. The antimicrobial peptide LL-37 has been shown to mediate pDC activation in psoriasis by converting self-DNA into structures that are sequestered in endosomal compartments where they trigger type I IFN production through TLR9 (188).

In a study by Li *et al.* (189), pDC-derived type I IFN was implicated in the initiation of type I diabetes in nonobese diabetic (NOD) mice. This study demonstrated that pDCs are present in pancreatic lymph nodes and secrete IFN- α when pancreatic β -cell death is thought to peak. These data suggest that pDCs are stimulated by apoptotic β -cell debris containing ssRNA and dsDNA that bind to TLR7 and TLR9, respectively. The resulting secretion of

type I IFN eventually activates DCs and autoreactive T cells giving rise to autoimmunity. Accordingly, the authors found that blocking IFNAR early on resulted in increased numbers of immature DCs in pancreatic LNs, enhanced production of IL-4 and IL-10 by CD4⁺ T cells, and delayed onset of disease in NOD mice.

Recently it was proposed that pDCs may have a pathogenic role in the development of experimental autoimmune encephalomyelitis (EAE) by facilitating the induction of myelin oligodendrocyte glycoprotein (MOG)-specific Th17 T cells (190). In this study, the depletion of pDCs with mPDCA-1 antibody at the time of MOG immunization resulted in reduced numbers of MOG-specific Th17 cells in spleens and less severe clinical and histopathological signs of EAE. In contrast, it has been shown that eliminating pDCs with mPDCA-1 antibody during the peak of EAE exacerbates disease (190, 191).

A potential explanation for these opposing results is that treating mice with depleting antibody at the time of immunization may result not only in the depletion of pDCs but also the depletion of other cell types including antigen-specific T cells. In the study by Isaksson *et al.*, mice were immunized with MOG in complete Freund's adjuvant followed by pertussis toxin. This immunization strategy would upregulate BST-2 on multiple cell types in addition to pDCs perhaps resulting in their elimination. In contrast, injecting depleting antibody at later timepoints, after T-cell priming has already occurred and the antigen/adjuvant has been cleared, might result in the depletion of cells that are actively producing and responding to type I IFNs, thereby impairing the protective effects of type I IFN. IFN- β , which is used to treat patients with multiple sclerosis (MS), constrains the development of EAE by limiting Th17 development and autoimmune inflammation (192) and IFNAR signaling in myeloid cells is critical for modulating the effector phase of EAE thereby limiting disease severity and lethality (193). PDC-less mice will be helpful to address these issues, and, in general the role of pDCs in autoimmunity.

Bacterial infections represent another situation where type I IFN appears to be more detrimental than beneficial. Several studies found that IFNAR^{-/-} mice are less susceptible to *Listeria monocytogenes* (LM) infection than wildtype mice, as indicated by lower bacterial burdens and infective lesions with fewer apoptotic cells (194–196). This deleterious effect of type I IFN prompted us to question whether pDCs were a major source of IFN during LM infection. To address this question, we depleted pDCs in BDCA-2-DTR mice and then infected them with the EGD strain of LM. As shown in Fig. 6, depleting pDCs had no effect on the colony-forming units in either the spleen or liver compared to non-depleted mice, suggesting that pDC-derived type I IFN is not responsible for the pathology described above. These data are consistent with a recent publication showing that CD11b⁺CD11c⁻ macrophages are the primary source of type I IFN during LM infection (197).

Tolerogenic properties of pDCs

While pDCs activated through TLR7 and TLR9 act as immunogenic cells, there is increasing evidence supporting the notion that unstimulated or alternatively stimulated pDCs can act as tolerogenic cells promoting tolerance to tumor cells, harmless antigens, and alloantigens (Table 3). This dual behavior of pDCs is reminiscent of the immunogenic and tolerogenic functions of mature and immature DC, respectively (198). In 2001, Zou *et al.* (199) showed that malignant human ovarian epithelial tumor cells attract pDCs to ascites by producing stromal-derived factor-1 (SDF-1). pDCs exposed to the tumor microenvironment induced IL-10-producing Tregs rather than T-cell activation, thus promoting tolerance instead of anti-tumor immunity. In 2004, it was found that pDCs mediate tolerance to harmless inhaled antigen by inducing Tregs that suppress the generation of effector T cells by DCs (200). To arrive at this conclusion, the authors depleted pDCs with anti-Gr-1

antibody or anti-BST-2 antibody (120G8) and looked at asthmatic responses to OVA. pDC-depleted mice developed the cardinal features of asthma while non-depleted mice were tolerized. Another study showed that systemic depletion of pDCs with antibodies prevented oral tolerance induced by antigen feeding (201). Interestingly, the suppression of T-cell responses was selectively mediated by liver or mesenteric LN pDCs as splenic pDCs failed to induce T-cell tolerance. These results strongly indicate that the tolerogenic function of pDCs may be dependent on their anatomical location. Consistent with this, Contractor *et al.* (202) found that pDCs isolated from Peyer's patches (PPs) are unable to produce type I IFN after TLR stimulation in contrast to splenic pDCs. This 'conditioned' phenotype exhibited by PP pDCs was recapitulated with splenic pDCs treated with IL-10, TGF- β , or prostaglandin E (PGE), three regulatory factors associated with mucosal tissues. The tolerogenic potential of pDCs was also demonstrated in a transplantation model where the depletion of pDCs or the blockade of pDCs homing to the LN inhibited the generation of Tregs and graft survival (203).

The mechanisms by which pDCs induce tolerance have been actively investigated (Table 3). pDCs express the immunoregulatory enzyme indoleamine-2,3-dioxygenase (IDO), which promotes tryptophan catabolism, depleting the tryptophan pool that T cells need to generate effective responses. pDCs were initially shown to express IDO after stimulation through B7 or CD200R with CTLA4-Ig (204) or CD200-Ig (205), respectively. In 2004, Munn *et al.* (206) reported that a fraction of pDCs express IDO in tumor draining LNs (TDLN). It is unclear from this study whether the IDO⁺ cells in the TDLN are actually pDCs, because they express CD19, which is a B-cell marker not typically found on pDCs. Therefore, additional studies to define the population of IDO⁺ cells using pDCs-specific markers should be conducted. Nonetheless, these IDO⁺ cells activated Tregs in the TDLN (207), whereas inhibition of IDO converted Tregs into Th17 cells (208, 209). Human pDCs incubated *in vitro* with HIV also express IDO and promote the differentiation of naïve CD4⁺ T cells into Tregs (152, 153) that suppress CD4⁺ T-cell proliferation (152) and prevent the maturation of bystander DCs (153).

In addition to IDO, the expression of inducible costimulator ligand (ICOS-L) on activated human pDCs can promote the generation of IL-10-producing Tregs from naive T cells (210). Type I IFN secreted by pDCs may enhance this process (211). Another mechanism that pDCs utilize to suppress T-cell proliferation is the secretion of the granzyme B (212). This activity is enhanced by IL-3 and IL-10 and strongly inhibited by TLR or CD40L signaling. It is unlikely that granzyme B mediates cytotoxicity, since pDCs, in contrast to NK cells, do not produce perforin, which allows the entrance of granzyme B into target cells. Finally, human pDCs can influence T and NKT cell responses via interaction between OX40L and OX40, which are members of the TNFR/TNF superfamily. After culture in IL-3, human pDCs upregulate OX40L and induce Th2 polarization. After stimulation with SeV, pDCs produce type I IFN, which overcomes the OX40L Th2 polarizing effect, resulting in Th1 rather than Th2 responses (213). Since the tolerogenic functions of pDCs have been mainly demonstrated either *in vitro* or by depleting pDCs *in vivo* with cross-reactive antibodies, it will be important to re-evaluate the impact of pDCs on tolerance using pDCs-less mice.

Concluding remarks and future perspectives

The generation of BDCA-2-DTR mice has allowed us to make the following observations and conclusions about the contributions of pDCs to antiviral immunity. First, pDCs are only one of many sources of type I IFN during viral infections. Accordingly, Kumagai *et al.* (214) demonstrated that alveolar macrophages (AM) are the primary source of type I IFN during pulmonary infection with Newcastle disease virus (NDV), while pDCs only produce type I IFN when AM are depleted or after NDV is administered intravenously. Second, because

pDCs are mainly found in secondary lymphoid organs, their role is probably more vital during systemic viral infections or during viral infections where the virus has tropism for secondary lymphoid organs. Third, regarding adaptive responses, pDCs may be more important for promoting CTL responses that require help or costimulation rather than antibody production.

Studies utilizing anti-BST-2 antibodies to deplete pDCs during viral infections and perhaps other immune responses should be interpreted with caution (Table 2). There is no doubt that these antibodies eliminate pDCs quite well, but our data suggest that other cells, critical for eliciting protective immunity, may also be reduced or eliminated potentially yielding ambiguous phenotypes. Even though depletion of pDCs with DT in BDCA-2-DTR mice appears to be highly specific for pDCs, one may argue that this system also has its disadvantages, for example the length and efficacy of depletion. Under steady-state conditions one injection of toxin eliminates the majority of pDCs in the periphery, BM, and spleen for up to 48 h at which time the numbers and frequencies of pDCs begin to return to normal; however, during an immune response, the turnover rate might be faster. Next, one cannot be entirely sure that the depletion approaches 90–100% in all organs. When applicable, we calculate the frequencies and numbers of pDCs in the spleens of depleted mice at various timepoints post-infection but it is possible that, while pDCs might be absent in the spleen, some are still present in other organs. One way to really address the function of pDCs is to examine mice that are constitutively lacking pDCs. In 2006, it was reported that mice expressing low levels of the transcription factor Ikaros have a block in pDC development and essentially no pDCs in peripheral tissues (215). Upon infection with MCMV, these mice fail to secrete detectable levels IFN- α and show increased frequencies of infected splenic DCs. MCMV-infected Ikaros^{L/L} mice also secreted slightly less IFN- γ and slightly more IL-12 than wildtype mice, but these findings were not statistically significant. Cisse *et al.* (112) described another mouse that lacks pDCs. This study showed that pDCs development is highly dependent on the transcription factor E2-2 (112). Constitutive or conditional deletion of E2-2 blocks the development of pDCs but not cells of other lineages and eliminates IFN responses to unmethylated DNA.

Alternative approaches to those mentioned above would be to generate pDC-less mice using the diphtheria toxin A (DTA) subunit under the control of a pDC-specific promoter. Alternatively, mice expressing the Cre recombinase under the control of the BDCA-2 promoter could be crossed with mice that express DTA under the control of a loxP-flanked neomycin resistance cassette from the ROSA26 locus. DTA would be exclusively expressed by pDCs when the neomycin cassette is removed and the cells would be depleted. This technology has already been described for Langerin-Cre and CD11c-Cre mice. In Langerin-Cre mice, Cre-mediated removal of the neo cassette leads to DTA expression and continuous loss of Langerhans cells, resulting in enhanced contact hypersensitivity (216, 217). Using CD11c-Cre mice, conventional DCs, pDCs, and Langerhans cells have been deleted, resulting in spontaneous fatal autoimmunity (218) or myeloproliferative disorder (219). In addition to being useful for assessing the roles of pDCs during viral infections, mice constitutively lacking pDCs will provide us with a tremendous amount of information regarding their tolerogenic properties and dysfunction in autoimmune diseases.

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References

1. Colonna M, Trinchieri G, Liu YJ. Plasmacytoid dendritic cells in immunity. *Nat Immunol.* 2004; 5:1219–1226. [PubMed: 15549123]
2. Liu YJ. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. *Annu Rev Immunol.* 2005; 23:275–306. [PubMed: 15771572]
3. Muller-Hermelink HK, Stein H, Steinmann G, Lennert K. Malignant lymphoma of plasmacytoid T-cells. Morphologic and immunologic studies characterizing a special type of T-cell. *Am J Surg Pathol.* 1983; 7:849–862. [PubMed: 6606989]
4. Lennert K, Remmele W. [Karyometric research on lymph node cells in man. I. Germinalblasts, lymphoblasts & lymphocytes.]. *Acta Haematol.* 1958; 19:99–113. [PubMed: 13520253]
5. Facchetti F, de Wolf-Peeters C, Mason DY, Pulford K, van den Oord JJ, Desmet VJ. Plasmacytoid T cells. Immunohistochemical evidence for their monocyte/macrophage origin. *Am J Pathol.* 1988; 133:15–21. [PubMed: 3052093]
6. Grouard G, Risoan MC, Filgueira L, Durand I, Banchereau J, Liu YJ. The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. *J Exp Med.* 1997; 185:1101–1111. [PubMed: 9091583]
7. Risoan MC, et al. Reciprocal control of T helper cell and dendritic cell differentiation. *Science.* 1999; 283:1183–1186. [PubMed: 10024247]
8. Siegal FP, et al. The nature of the principal type 1 interferon-producing cells in human blood. *Science.* 1999; 284:1835–1837. [PubMed: 10364556]
9. Cella M, et al. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat Med.* 1999; 5:919–923. [PubMed: 10426316]
10. Ronnblom L, Forsgren A, Alm GV. Characterization of interferons induced by bacteria and interferon-producing leukocytes in human peripheral blood. *Infect Immun.* 1983; 40:126–132. [PubMed: 6403464]
11. Perussia B, Fanning V, Trinchieri G. A leukocyte subset bearing HLA-DR antigens is responsible for in vitro alpha interferon production in response to viruses. *Nat Immun Cell Growth Regul.* 1985; 4:120–137. [PubMed: 2995798]
12. Fitzgerald-Bocarsly P, Feldman M, Mendelsohn M, Curl S, Lopez C. Human mononuclear cells which produce interferon-alpha during NK(HSV-FS) assays are HLA-DR positive cells distinct from cytolytic natural killer effectors. *J Leukoc Biol.* 1988; 43:323–334. [PubMed: 2832497]
13. Fitzgerald-Bocarsly P. Human natural interferon-alpha producing cells. *Pharmacol Ther.* 1993; 60:39–62. [PubMed: 8127923]
14. Ferbas JJ, Toso JF, Logar AJ, Navratil JS, Rinaldo CR Jr. CD4+ blood dendritic cells are potent producers of IFN-alpha in response to in vitro HIV-1 infection. *J Immunol.* 1994; 152:4649–4662. [PubMed: 7908920]
15. Asselin-Paturel C, et al. Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. *Nat Immunol.* 2001; 2:1144–1150. [PubMed: 11713464]
16. Nakano H, Yanagita M, Gunn MD. CD11c(+)B220(+)Gr-1(+) cells in mouse lymph nodes and spleen display characteristics of plasmacytoid dendritic cells. *J Exp Med.* 2001; 194:1171–1178. [PubMed: 11602645]
17. Bjorck P. Isolation and characterization of plasmacytoid dendritic cells from Flt3 ligand and granulocyte-macrophage colony-stimulating factor-treated mice. *Blood.* 2001; 98:3520–3526. [PubMed: 11739152]
18. Dzionek A, et al. BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. *J Immunol.* 2000; 165:6037–6046. [PubMed: 11086035]
19. Dzionek A, et al. BDCA-2, a novel plasmacytoid dendritic cell-specific type II C-type lectin, mediates antigen capture and is a potent inhibitor of interferon alpha/beta induction. *J Exp Med.* 2001; 194:1823–1834. [PubMed: 11748283]
20. Risoan MC, et al. Subtractive hybridization reveals the expression of immunoglobulin-like transcript 7, Eph-B1, granzyme B, and 3 novel transcripts in human plasmacytoid dendritic cells. *Blood.* 2002; 100:3295–3303. [PubMed: 12384430]

21. Cao W, et al. Plasmacytoid dendritic cell-specific receptor ILT7-Fc epsilonRI gamma inhibits Toll-like receptor-induced interferon production. *J Exp Med*. 2006; 203:1399–1405. [PubMed: 16735691]
22. Matsui T, et al. CD2 distinguishes two subsets of human plasmacytoid dendritic cells with distinct phenotype and functions. *J Immunol*. 2009; 182:6815–6823. [PubMed: 19454677]
23. Facchetti F, Vermi W, Santoro A, Vergoni F, Chilosi M, Doglioni C. Neoplasms derived from plasmacytoid monocytes/interferon-producing cells: variability of CD56 and granzyme B expression. *Am J Surg Pathol*. 2003; 27:1489–1492. author reply 92–3. [PubMed: 14576486]
24. Marafioti T, et al. Novel markers of normal and neoplastic human plasmacytoid dendritic cells. *Blood*. 2008; 111:3778–3792. [PubMed: 18218851]
25. Blasius AL, Cella M, Maldonado J, Takai T, Colonna M. Siglec-H is an IPC-specific receptor that modulates type I IFN secretion through DAP12. *Blood*. 2006; 107:2474–2476. [PubMed: 16293595]
26. Zhang J, et al. Characterization of Siglec-H as a novel endocytic receptor expressed on murine plasmacytoid dendritic cell precursors. *Blood*. 2006; 107:3600–3608. [PubMed: 16397130]
27. Blasius AL, Giurisato E, Cella M, Schreiber RD, Shaw AS, Colonna M. Bone marrow stromal cell antigen 2 is a specific marker of type I IFN-producing cells in the naive mouse, but a promiscuous cell surface antigen following IFN stimulation. *J Immunol*. 2006; 177:3260–3265. [PubMed: 16920966]
28. Workman CJ, et al. LAG-3 regulates plasmacytoid dendritic cell homeostasis. *J Immunol*. 2009; 182:1885–1891. [PubMed: 19201841]
29. Toyama-Sorimachi N, et al. Inhibitory NK receptor Ly49Q is expressed on subsets of dendritic cells in a cellular maturation- and cytokine stimulation-dependent manner. *J Immunol*. 2005; 174:4621–4629. [PubMed: 15814685]
30. Kamogawa-Schifter Y, Ohkawa J, Namiki S, Arai N, Arai K, Liu Y. Ly49Q defines 2 pDCs subsets in mice. *Blood*. 2005; 105:2787–2792. [PubMed: 15598811]
31. Omatsu Y, et al. Development of murine plasmacytoid dendritic cells defined by increased expression of an inhibitory NK receptor, Ly49Q. *J Immunol*. 2005; 174:6657–6662. [PubMed: 15905504]
32. Wendland M, et al. CCR9 is a homing receptor for plasmacytoid dendritic cells to the small intestine. *Proc Natl Acad Sci U S A*. 2007; 104:6347–6352. [PubMed: 17404233]
33. Hadeiba H, Sato T, Habtezion A, Oderup C, Pan J, Butcher EC. CCR9 expression defines tolerogenic plasmacytoid dendritic cells able to suppress acute graft-versus-host disease. *Nat Immunol*. 2008; 9:1253–1260. [PubMed: 18836452]
34. Segura E, Wong J, Villadangos JA. Cutting edge: B220+CCR9- dendritic cells are not plasmacytoid dendritic cells but are precursors of conventional dendritic cells. *J Immunol*. 2009; 183:1514–1517. [PubMed: 19570827]
35. Chan CW, et al. Interferon-producing killer dendritic cells provide a link between innate and adaptive immunity. *Nat Med*. 2006; 12:207–213. [PubMed: 16444266]
36. Taieb J, et al. A novel dendritic cell subset involved in tumor immunosurveillance. *Nat Med*. 2006; 12:214–219. [PubMed: 16444265]
37. Blasius AL, Barchet W, Cella M, Colonna M. Development and function of murine B220+CD11c +NK1.1+ cells identify them as a subset of NK cells. *J Exp Med*. 2007; 204:2561–2568. [PubMed: 17923504]
38. Shortman K, Naik SH. Steady-state and inflammatory dendritic-cell development. *Nat Rev Immunol*. 2007; 7:19–30. [PubMed: 17170756]
39. Naik SH, et al. Development of plasmacytoid and conventional dendritic cell subtypes from single precursor cells derived in vitro and in vivo. *Nat Immunol*. 2007; 8:1217–1226. [PubMed: 17922015]
40. Facchetti F, De Wolf-Peters C, De Vos R, van den Oord JJ, Pulford KA, Desmet VJ. Plasmacytoid monocytes (so-called plasmacytoid T cells) in granulomatous lymphadenitis. *Hum Pathol*. 1989; 20:588–593. [PubMed: 2656499]

41. Facchetti F, de Wolf-Peeters C, van den Oord JJ, de Vos R, Desmet VJ. Plasmacytoid monocytes (so-called plasmacytoid T-cells) in Kikuchi's lymphadenitis. An immunohistologic study. *Am J Clin Pathol.* 1989; 92:42–50. [PubMed: 2787597]
42. Farkas L, Beiske K, Lund-Johansen F, Brandtzaeg P, Jahnsen FL. Plasmacytoid dendritic cells (natural interferon- alpha/beta-producing cells) accumulate in cutaneous lupus erythematosus lesions. *Am J Pathol.* 2001; 159:237–243. [PubMed: 11438470]
43. Yoneyama H, et al. Evidence for recruitment of plasmacytoid dendritic cell precursors to inflamed lymph nodes through high endothelial venules. *Int Immunol.* 2004; 16:915–928. [PubMed: 15159375]
44. Diacovo TG, Blasius AL, Mak TW, Cella M, Colonna M. Adhesive mechanisms governing interferon-producing cell recruitment into lymph nodes. *J Exp Med.* 2005; 202:687–696. [PubMed: 16147979]
45. Nagano Y, Kojima Y. [Immunizing property of vaccinia virus inactivated by ultraviolet rays.]. *C R Seances Soc Biol Fil.* 1954; 148:1700–1702. [PubMed: 14364998]
46. Lindenmann J, Burke DC, Isaacs A. Studies on the production, mode of action and properties of interferon. *Br J Exp Pathol.* 1957; 38:551–562. [PubMed: 13479681]
47. Pestka S, Krause CD, Walter MR. Interferons, interferon-like cytokines, and their receptors. *Immunol Rev.* 2004; 202:8–32. [PubMed: 15546383]
48. Barchet W, Cella M, Odermatt B, Asselin-Paturel C, Colonna M, Kalinke U. Virus-induced interferon alpha production by a dendritic cell subset in the absence of feedback signaling in vivo. *J Exp Med.* 2002; 195:507–516. [PubMed: 11854363]
49. Asselin-Paturel C, et al. Type I interferon dependence of plasmacytoid dendritic cell activation and migration. *J Exp Med.* 2005; 201:1157–1167. [PubMed: 15795237]
50. Kumagai Y, Kumar H, Koyama S, Kawai T, Takeuchi O, Akira S. Cutting Edge: TLR-Dependent viral recognition along with type I IFN positive feedback signaling masks the requirement of viral replication for IFN- α production in plasmacytoid dendritic cells. *J Immunol.* 2009; 182:3960–3964. [PubMed: 19299691]
51. Marrack P, Kappler J, Mitchell T. Type I interferons keep activated T cells alive. *J Exp Med.* 1999; 189:521–530. [PubMed: 9927514]
52. Le Bon A, Tough DF. Links between innate and adaptive immunity via type I interferon. *Curr Opin Immunol.* 2002; 14:432–436. [PubMed: 12088676]
53. Kolumam GA, Thomas S, Thompson LJ, Sprent J, Murali-Krishna K. Type I interferons act directly on CD8 T cells to allow clonal expansion and memory formation in response to viral infection. *J Exp Med.* 2005; 202:637–650. [PubMed: 16129706]
54. Mescher MF, et al. Signals required for programming effector and memory development by CD8+ T cells. *Immunol Rev.* 2006; 211:81–92. [PubMed: 16824119]
55. Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol.* 2003; 3:133–146. [PubMed: 12563297]
56. Biron CA. Interferons alpha and beta as immune regulators--a new look. *Immunity.* 2001; 14:661–664. [PubMed: 11420036]
57. Blanco P, Palucka AK, Gill M, Pascual V, Banchereau J. Induction of dendritic cell differentiation by IFN- α in systemic lupus erythematosus. *Science.* 2001; 294:1540–1543. [PubMed: 11711679]
58. Santini SM, Di Pucchio T, Lapenta C, Parlato S, Logozzi M, Belardelli F. The natural alliance between type I interferon and dendritic cells and its role in linking innate and adaptive immunity. *J Interferon Cytokine Res.* 2002; 22:1071–1080. [PubMed: 12513906]
59. Le Bon A, et al. Cross-priming of CD8+ T cells stimulated by virus-induced type I interferon. *Nat Immunol.* 2003; 4:1009–1015. [PubMed: 14502286]
60. Le Bon A, Schiavoni G, D'Agostino G, Gresser I, Belardelli F, Tough DF. Type I interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. *Immunity.* 2001; 14:461–470. [PubMed: 11336691]
61. Jegou G, Palucka AK, Blanck JP, Chalouni C, Pascual V, Banchereau J. Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. *Immunity.* 2003; 19:225–234. [PubMed: 12932356]

62. Poeck H, et al. Plasmacytoid dendritic cells, antigen, and CpG-C license human B cells for plasma cell differentiation and immunoglobulin production in the absence of T-cell help. *Blood*. 2004; 103:3058–3064. [PubMed: 15070685]
63. Penna G, Vulcano M, Roncari A, Facchetti F, Sozzani S, Adorini L. Cutting edge: differential chemokine production by myeloid and plasmacytoid dendritic cells. *J Immunol*. 2002; 169:6673–6676. [PubMed: 12471096]
64. Krug A, et al. IFN-producing cells respond to CXCR3 ligands in the presence of CXCL12 and secrete inflammatory chemokines upon activation. *J Immunol*. 2002; 169:6079–6083. [PubMed: 12444109]
65. Vanbervliet B, et al. The inducible CXCR3 ligands control plasmacytoid dendritic cell responsiveness to the constitutive chemokine stromal cell-derived factor 1 (SDF-1)/CXCL12. *J Exp Med*. 2003; 198:823–830. [PubMed: 12953097]
66. Villadangos JA, Young L. Antigen-presentation properties of plasmacytoid dendritic cells. *Immunity*. 2008; 29:352–361. [PubMed: 18799143]
67. Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. *Nat Immunol*. 2004; 5:987–995. [PubMed: 15454922]
68. Park B, Brinkmann MM, Spooner E, Lee CC, Kim YM, Ploegh HL. Proteolytic cleavage in an endolysosomal compartment is required for activation of Toll-like receptor 9. *Nat Immunol*. 2008; 9:1407–1414. [PubMed: 18931679]
69. Ewald SE, et al. The ectodomain of Toll-like receptor 9 is cleaved to generate a functional receptor. *Nature*. 2008; 456(7222):658–662. [PubMed: 18820679]
70. Lee HK, Lund JM, Ramanathan B, Mizushima N, Iwasaki A. Autophagy-dependent viral recognition by plasmacytoid dendritic cells. *Science*. 2007; 315:1398–1401. [PubMed: 17272685]
71. Lund J, Sato A, Akira S, Medzhitov R, Iwasaki A. Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. *J Exp Med*. 2003; 198:513–520. [PubMed: 12900525]
72. Hochrein H, et al. Herpes simplex virus type-1 induces IFN-alpha production via Toll-like receptor 9-dependent and -independent pathways. *Proc Natl Acad Sci U S A*. 2004; 101:11416–11421. [PubMed: 15272082]
73. Wang JP, Asher DR, Chan M, Kurt-Jones EA, Finberg RW. Cutting Edge: Antibody-mediated TLR7-dependent recognition of viral RNA. *J Immunol*. 2007; 178:3363–3367. [PubMed: 17339429]
74. Honda K, et al. Spatiotemporal regulation of MyD88-IRF-7 signalling for robust type-I interferon induction. *Nature*. 2005; 434:1035–1040. [PubMed: 15815647]
75. Yoshizaki M, et al. Spatiotemporal regulation of intracellular trafficking of Toll-like receptor 9 by an inhibitory receptor, Ly49Q. *Blood*. 2009; 114:1518–1527. [PubMed: 19528537]
76. Pichlmair A, et al. RIG-I-mediated antiviral responses to single-stranded RNA bearing 5'-phosphates. *Science*. 2006; 314:997–1001. [PubMed: 17038589]
77. Hornung V, et al. 5'-Triphosphate RNA is the ligand for RIG-I. *Science*. 2006; 314:994–997. [PubMed: 17038590]
78. Pichlmair A, Reis e Sousa C. Innate recognition of viruses. *Immunity*. 2007; 27:370–383. [PubMed: 17892846]
79. Ishii KJ, Koyama S, Nakagawa A, Coban C, Akira S. Host innate immune receptors and beyond: making sense of microbial infections. *Cell Host Microbe*. 2008; 3:352–363. [PubMed: 18541212]
80. Kawai T, Akira S. Toll-like receptor and RIG-I-like receptor signaling. *Ann N Y Acad Sci*. 2008; 1143:1–20. [PubMed: 19076341]
81. Honda K, et al. IRF-7 is the master regulator of type-I interferon-dependent immune responses. *Nature*. 2005; 434:772–777. [PubMed: 15800576]
82. Cao W, et al. Toll-like receptor-mediated induction of type I interferon in plasmacytoid dendritic cells requires the rapamycin-sensitive PI(3)K-mTOR-p70S6K pathway. *Nat Immunol*. 2008; 9:1157–1164. [PubMed: 18758466]
83. Colina R, et al. Translational control of the innate immune response through IRF-7. *Nature*. 2008; 452:323–328. [PubMed: 18272964]

84. Honda K, Yanai H, Takaoka A, Taniguchi T. Regulation of the type I IFN induction: a current view. *Int Immunol*. 2005; 17:1367–1378. [PubMed: 16214811]
85. Guiducci C, et al. Properties regulating the nature of the plasmacytoid dendritic cell response to Toll-like receptor 9 activation. *J Exp Med*. 2006; 203:1999–2008. [PubMed: 16864658]
86. Cao W, Liu YJ. Innate immune functions of plasmacytoid dendritic cells. *Curr Opin Immunol*. 2007; 19:24–30. [PubMed: 17113765]
87. Cella M, Facchetti F, Lanzavecchia A, Colonna M. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization. *Nat Immunol*. 2000; 1:305–310. [PubMed: 11017101]
88. Swiecki MK, Colonna M. Running to stand still: BCR-like signaling suppresses type I IFN responses in pDCs. *Eur J Immunol*. 2007; 37:3327–3329. [PubMed: 18050162]
89. Gilliet M, Cao W, Liu YJ. Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. *Nat Rev Immunol*. 2008; 8:594–606. [PubMed: 18641647]
90. Cao W. Molecular characterization of human plasmacytoid dendritic cells. *J Clin Immunol*. 2009; 29:257–264. [PubMed: 19266271]
91. Watarai H, Sekine E, Inoue S, Nakagawa R, Kaisho T, Taniguchi M. PDC-TREM, a plasmacytoid dendritic cell-specific receptor, is responsible for augmented production of type I interferon. *Proc Natl Acad Sci U S A*. 2008; 105:2993–2998. [PubMed: 18287072]
92. Tai LH, et al. Positive regulation of plasmacytoid dendritic cell function via Ly49Q recognition of class I MHC. *J Exp Med*. 2008; 205:3187–3199. [PubMed: 19075287]
93. Andoniou CE, et al. Interaction between conventional dendritic cells and natural killer cells is integral to the activation of effective antiviral immunity. *Nat Immunol*. 2005; 6:1011–1019. [PubMed: 16142239]
94. Cao W, et al. BDCA2/Fc epsilon RI gamma complex signals through a novel BCR-like pathway in human plasmacytoid dendritic cells. *PLoS Biol*. 2007; 5:e248. [PubMed: 17850179]
95. Rock J, et al. CD303 (BDCA-2) signals in plasmacytoid dendritic cells via a BCR-like signalosome involving Syk, Slp65 and PLCgamma2. *Eur J Immunol*. 2007; 37:3564–3575. [PubMed: 18022864]
96. Cella M, et al. A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. *Nature*. 2009; 457:722–725. [PubMed: 18978771]
97. Fuchs A, Cella M, Kondo T, Colonna M. Paradoxical inhibition of human natural interferon-producing cells by the activating receptor NKp44. *Blood*. 2005; 106:2076–2082. [PubMed: 15941912]
98. Meyer-Wentrup F, et al. Targeting DCIR on human plasmacytoid dendritic cells results in antigen presentation and inhibits IFN-alpha production. *Blood*. 2008; 111:4245–4253. [PubMed: 18258799]
99. Bave U, Magnusson M, Eloranta ML, Perers A, Alm GV, Ronnblom L. Fc gamma RIIa is expressed on natural IFN-alpha-producing cells (plasmacytoid dendritic cells) and is required for the IFN-alpha production induced by apoptotic cells combined with lupus IgG. *J Immunol*. 2003; 171:3296–3302. [PubMed: 12960360]
100. Means TK, Latz E, Hayashi F, Murali MR, Golenbock DT, Luster AD. Human lupus autoantibody-DNA complexes activate DCs through cooperation of CD32 and TLR9. *J Clin Invest*. 2005; 115:407–417. [PubMed: 15668740]
101. Banchereau J, Pascual V. Type I interferon in systemic lupus erythematosus and other autoimmune diseases. *Immunity*. 2006; 25:383–392. [PubMed: 16979570]
102. Marshak-Rothstein A. Toll-like receptors in systemic autoimmune disease. *Nat Rev Immunol*. 2006; 6:823–835. [PubMed: 17063184]
103. Ju X, Zenke M, Hart DN, Clark GJ. CD300a/c regulate type I interferon and TNF-alpha secretion by human plasmacytoid dendritic cells stimulated with TLR7 and TLR9 ligands. *Blood*. 2008; 112:1184–1194. [PubMed: 18535206]
104. Amigorena S, Bonnerot C. Fc receptor signaling and trafficking: a connection for antigen processing. *Immunol Rev*. 1999; 172:279–284. [PubMed: 10631953]

105. Barrow AD, Trowsdale J. You say ITAM and I say ITIM, let's call the whole thing off: the ambiguity of immunoreceptor signalling. *Eur J Immunol.* 2006; 36:1646–1653. [PubMed: 16783855]
106. Dalod M, et al. Interferon alpha/beta and interleukin 12 responses to viral infections: pathways regulating dendritic cell cytokine expression in vivo. *J Exp Med.* 2002; 195:517–528. [PubMed: 11854364]
107. Wrammert J, Kallberg E, Agace WW, Leanderson T. Ly6C expression differentiates plasma cells from other B cell subsets in mice. *Eur J Immunol.* 2002; 32:97–103. [PubMed: 11754008]
108. Asselin-Paturel C, Brizard G, Pin JJ, Briere F, Trinchieri G. Mouse strain differences in plasmacytoid dendritic cell frequency and function revealed by a novel monoclonal antibody. *J Immunol.* 2003; 171:6466–6477. [PubMed: 14662846]
109. Krug A, et al. TLR9-dependent recognition of MCMV by IPC and DC generates coordinated cytokine responses that activate antiviral NK cell function. *Immunity.* 2004; 21:107–119. [PubMed: 15345224]
110. Jung S, et al. In vivo depletion of CD11c(+) dendritic cells abrogates priming of CD8(+) T cells by exogenous cell-associated antigens. *Immunity.* 2002; 17:211–220. [PubMed: 12196292]
111. Nagasawa M, Schmidlin H, Hazekamp MG, Schotte R, Blom B. Development of human plasmacytoid dendritic cells depends on the combined action of the basic helix-loop-helix factor E2-2 and the Ets factor Spi-B. *Eur J Immunol.* 2008; 38:2389–2400. [PubMed: 18792017]
112. Cisse B, et al. Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development. *Cell.* 2008; 135:37–48. [PubMed: 18854153]
113. Steinhoff U, Muller U, Schertler A, Hengartner H, Aguet M, Zinkernagel RM. Antiviral protection by vesicular stomatitis virus-specific antibodies in alpha/beta interferon receptor-deficient mice. *J Virol.* 1995; 69:2153–2158. [PubMed: 7884863]
114. Lund JM, et al. Recognition of single-stranded RNA viruses by Toll-like receptor 7. *Proc Natl Acad Sci U S A.* 2004; 101:5598–5603. [PubMed: 15034168]
115. Bachmann, MF. Evaluation of vesicular stomatitis virus-specific cytotoxic T cell responses. In: Zinkernagel, RM.; Hengartner, H., editors. *Immunology Methods Manual.* Academic Press Ltd; 1996. p. 1897–1907.
116. Yoneyama H, et al. Plasmacytoid DCs help lymph node DCs to induce anti-HSV CTLs. *J Exp Med.* 2005; 202:425–435. [PubMed: 16061729]
117. Castellino F, Huang AY, Altan-Bonnet G, Stoll S, Scheinecker C, Germain RN. Chemokines enhance immunity by guiding naive CD8+ T cells to sites of CD4+ T cell-dendritic cell interaction. *Nature.* 2006; 440:890–895. [PubMed: 16612374]
118. Wolf AI, et al. Plasmacytoid dendritic cells are dispensable during primary influenza virus infection. *J Immunol.* 2009; 182:871–879. [PubMed: 19124730]
119. Huneycutt BS, Bi Z, Aoki CJ, Reiss CS. Central neuropathogenesis of vesicular stomatitis virus infection of immunodeficient mice. *J Virol.* 1993; 67:6698–6706. [PubMed: 8105106]
120. Zuniga EI, Hahn B, Oldstone MB. Type I interferon during viral infections: multiple triggers for a multifunctional mediator. *Curr Top Microbiol Immunol.* 2007; 316:337–357. [PubMed: 17969455]
121. Jung A, et al. Lymphocytoid choriomeningitis virus activates plasmacytoid dendritic cells and induces a cytotoxic T-cell response via MyD88. *J Virol.* 2008; 82:196–206. [PubMed: 17942529]
122. Bachmann, MF. Evaluation of lymphocytic choriomeningitis virus-specific cytotoxic T cell responses. In: Zinkernagel, RM.; Hengartner, H., editors. *Immunology Methods Manual.* Academic Press Ltd; 1996. p. 1919–1933.
123. Wagner, RR. *The Rhabdoviruses.* New York: Plenum Press; 1987.
124. Kundig TM, et al. Duration of TCR stimulation determines costimulatory requirement of T cells. *Immunity.* 1996; 5:41–52. [PubMed: 8758893]
125. Oldstone MB. Biology and pathogenesis of lymphocytic choriomeningitis virus infection. *Curr Top Microbiol Immunol.* 2002; 263:83–117. [PubMed: 11987822]
126. Zuniga EI, McGavern DB, Pruneda-Paz JL, Teng C, Oldstone MB. Bone marrow plasmacytoid dendritic cells can differentiate into myeloid dendritic cells upon virus infection. *Nat Immunol.* 2004; 5:1227–1234. [PubMed: 15531885]

127. Liou LY, Blasius AL, Welch MJ, Colonna M, Oldstone MB, Zuniga EI. In vivo conversion of BM plasmacytoid DC into CD11b+ conventional DC during virus infection. *Eur J Immunol*. 2008; 38:3388–3394. [PubMed: 18979509]
128. Siegal FP, Spear GT. Innate immunity and HIV. *Aids*. 2001; 15(Suppl 5):S127–S137. [PubMed: 11816160]
129. Feldman S, et al. Decreased interferon-alpha production in HIV-infected patients correlates with numerical and functional deficiencies in circulating type 2 dendritic cell precursors. *Clin Immunol*. 2001; 101:201–210. [PubMed: 11683579]
130. Pacanowski J, et al. Reduced blood CD123+ (lymphoid) and CD11c+ (myeloid) dendritic cell numbers in primary HIV-1 infection. *Blood*. 2001; 98:3016–3021. [PubMed: 11698285]
131. Donaghy H, et al. Loss of blood CD11c(+) myeloid and CD11c(–) plasmacytoid dendritic cells in patients with HIV-1 infection correlates with HIV-1 RNA virus load. *Blood*. 2001; 98:2574–2576. [PubMed: 11588058]
132. Soumelis V, et al. Depletion of circulating natural type 1 interferon-producing cells in HIV-infected AIDS patients. *Blood*. 2001; 98:906–912. [PubMed: 11493432]
133. Chehimi J, et al. Persistent decreases in blood plasmacytoid dendritic cell number and function despite effective highly active antiretroviral therapy and increased blood myeloid dendritic cells in HIV-infected individuals. *J Immunol*. 2002; 168:4796–4801. [PubMed: 11971031]
134. Barron MA, Blyveis N, Palmer BE, MaWhinney S, Wilson CC. Influence of plasma viremia on defects in number and immunophenotype of blood dendritic cell subsets in human immunodeficiency virus 1-infected individuals. *J Infect Dis*. 2003; 187:26–37. [PubMed: 12508143]
135. Kanga I, et al. Type I interferon production is profoundly and transiently impaired in primary HIV-1 infection. *J Infect Dis*. 2005; 192:303–310. [PubMed: 15962225]
136. Lopez C, Fitzgerald PA, Siegal FP. Severe acquired immune deficiency syndrome in male homosexuals: diminished capacity to make interferon-alpha in vitro associated with severe opportunistic infections. *J Infect Dis*. 1983; 148:962–966. [PubMed: 6606691]
137. Zhang Z, et al. Differential restoration of myeloid and plasmacytoid dendritic cells in HIV-1-infected children after treatment with highly active antiretroviral therapy. *J Immunol*. 2006; 176:5644–5651. [PubMed: 16622034]
138. Finke JS, Shodell M, Shah K, Siegal FP, Steinman RM. Dendritic cell numbers in the blood of HIV-1 infected patients before and after changes in antiretroviral therapy. *J Clin Immunol*. 2004; 24:647–652. [PubMed: 15622449]
139. Penna G, Sozzani S, Adorini L. Cutting edge: selective usage of chemokine receptors by plasmacytoid dendritic cells. *J Immunol*. 2001; 167:1862–1866. [PubMed: 11489962]
140. Fonteneau JF, et al. Human immunodeficiency virus type 1 activates plasmacytoid dendritic cells and concomitantly induces the bystander maturation of myeloid dendritic cells. *J Virol*. 2004; 78:5223–5232. [PubMed: 15113904]
141. Fiorentini S, et al. HIV-1 matrix protein p17 induces human plasmacytoid dendritic cells to acquire a migratory immature cell phenotype. *Proc Natl Acad Sci U S A*. 2008; 105:3867–3872. [PubMed: 18310327]
142. Malleret B, et al. Primary infection with simian immunodeficiency virus: plasmacytoid dendritic cell homing to lymph nodes, type I interferon, and immune suppression. *Blood*. 2008; 112:4598–4608. [PubMed: 18787223]
143. Brown KN, Wijewardana V, Liu X, Barratt-Boyes SM. Rapid influx and death of plasmacytoid dendritic cells in lymph nodes mediate depletion in acute simian immunodeficiency virus infection. *PLoS Pathog*. 2009; 5:e1000413. [PubMed: 19424421]
144. Beignon AS, et al. Endocytosis of HIV-1 activates plasmacytoid dendritic cells via Toll-like receptor-viral RNA interactions. *J Clin Invest*. 2005; 115:3265–3275. [PubMed: 16224540]
145. Hoeffel G, et al. Antigen crosspresentation by human plasmacytoid dendritic cells. *Immunity*. 2007; 27:481–492. [PubMed: 17869134]
146. Gurney KB, Colantonio AD, Blom B, Spits H, Uittenbogaart CH. Endogenous IFN-alpha production by plasmacytoid dendritic cells exerts an antiviral effect on thymic HIV-1 infection. *J Immunol*. 2004; 173:7269–7276. [PubMed: 15585849]

147. Meyers JH, et al. Impact of HIV on cell survival and antiviral activity of plasmacytoid dendritic cells. *PLoS One*. 2007; 2:e458. [PubMed: 17520017]
148. Herbeuval JP, et al. Differential expression of IFN-alpha and TRAIL/DR5 in lymphoid tissue of progressor versus nonprogressor HIV-1-infected patients. *Proc Natl Acad Sci U S A*. 2006; 103:7000–7005. [PubMed: 16632604]
149. Herbeuval JP, Shearer GM. HIV-1 immunopathogenesis: how good interferon turns bad. *Clin Immunol*. 2007; 123:121–128. [PubMed: 17112786]
150. Boasso A, Shearer GM. Chronic innate immune activation as a cause of HIV-1 immunopathogenesis. *Clin Immunol*. 2008; 126:235–242. [PubMed: 17916442]
151. Boasso A, Hardy AW, Anderson SA, Dolan MJ, Shearer GM. HIV-induced type I interferon and tryptophan catabolism drive T cell dysfunction despite phenotypic activation. *PLoS One*. 2008; 3:e2961. [PubMed: 18698365]
152. Boasso A, et al. HIV inhibits CD4+ T-cell proliferation by inducing indoleamine 2,3-dioxygenase in plasmacytoid dendritic cells. *Blood*. 2007; 109:3351–3359. [PubMed: 17158233]
153. Manches O, et al. HIV-activated human plasmacytoid DCs induce Tregs through an indoleamine 2,3-dioxygenase-dependent mechanism. *J Clin Invest*. 2008; 118:3431–3439. [PubMed: 18776940]
154. Meier A, et al. Sex differences in the Toll-like receptor-mediated response of plasmacytoid dendritic cells to HIV-1. *Nat Med*. 2009; 15:955–959. [PubMed: 19597505]
155. Ishikawa J, et al. Molecular cloning and chromosomal mapping of a bone marrow stromal cell surface gene, BST2, that may be involved in pre-B-cell growth. *Genomics*. 1995; 26:527–534. [PubMed: 7607676]
156. Kupzig S, Korolchuk V, Rollason R, Sugden A, Wilde A, Banting G. Bst-2/HM1.24 is a raft-associated apical membrane protein with an unusual topology. *Traffic*. 2003; 4:694–709. [PubMed: 12956872]
157. Cao W, et al. Regulation of TLR7/9 responses in plasmacytoid dendritic cells by BST2 and ILT7 receptor interaction. *J Exp Med*. 2009; 206:1603–1614. [PubMed: 19564354]
158. Takai T. Paired immunoglobulin-like receptors and their MHC class I recognition. *Immunology*. 2005; 115:433–440. [PubMed: 16011512]
159. Neil SJ, Zang T, Bieniasz PD. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature*. 2008; 451:425–430. [PubMed: 18200009]
160. Van Damme N, et al. The interferon-induced protein BST-2 restricts HIV-1 release and is downregulated from the cell surface by the viral Vpu protein. *Cell Host Microbe*. 2008; 3:245–252. [PubMed: 18342597]
161. Jouvenet N, et al. Broad-spectrum inhibition of retroviral and filoviral particle release by tetherin. *J Virol*. 2009; 83:1837–1844. [PubMed: 19036818]
162. Miyagi E, Andrew AJ, Kao S, Strebel K. Vpu enhances HIV-1 virus release in the absence of Bst-2 cell surface down-modulation and intracellular depletion. *Proc Natl Acad Sci U S A*. 2009; 106:2868–2873. [PubMed: 19196977]
163. McNatt MW, et al. Species-specific activity of HIV-1 Vpu and positive selection of tetherin transmembrane domain variants. *PLoS Pathog*. 2009; 5:e1000300. [PubMed: 19214216]
164. Goffinet C, et al. HIV-1 antagonism of CD317 is species specific and involves Vpu-mediated proteasomal degradation of the restriction factor. *Cell Host Microbe*. 2009; 5:285–297. [PubMed: 19286137]
165. Dube M, et al. Suppression of Tetherin-restricting activity upon human immunodeficiency virus type 1 particle release correlates with localization of Vpu in the trans-Golgi network. *J Virol*. 2009; 83:4574–4590. [PubMed: 19244337]
166. Mitchell RS, et al. Vpu antagonizes BST-2-mediated restriction of HIV-1 release via beta-TrCP and endo-lysosomal trafficking. *PLoS Pathog*. 2009; 5:e1000450. [PubMed: 19478868]
167. Gupta RK, Hue S, Schaller T, Verschoor E, Pillay D, Towers GJ. Mutation of a single residue renders human tetherin resistant to HIV-1 Vpu-mediated depletion. *PLoS Pathog*. 2009; 5:e1000443. [PubMed: 19461879]

168. Douglas JL, Viswanathan K, McCarroll MN, Gustin JK, Fruh K, Moses AV. Vpu directs the degradation of the human immunodeficiency virus restriction factor BST-2/Tetherin via a {beta}TrCP-dependent mechanism. *J Virol.* 2009; 83:7931–7947. [PubMed: 19515779]
169. Rong L, et al. The transmembrane domain of BST-2 determines its sensitivity to down-modulation by human immunodeficiency virus type 1 Vpu. *J Virol.* 2009; 83:7536–7546. [PubMed: 19474106]
170. Mangeat B, Gers-Huber G, Lehmann M, Zufferey M, Luban J, Piguet V. HIV-1 Vpu neutralizes the antiviral factor Tetherin/BST-2 by binding it and directing its beta-TrCP2-dependent degradation. *PLoS Pathog.* 2009; 5:e1000574. [PubMed: 19730691]
171. Le Tortorec A, Neil SJ. Antagonism and intracellular sequestration of human tetherin by the HIV-2 envelope glycoprotein. *J Virol.* 2009
172. Jia B, et al. Species-specific activity of SIV Nef and HIV-1 Vpu in overcoming restriction by tetherin/BST2. *PLoS Pathog.* 2009; 5:e1000429. [PubMed: 19436700]
173. Zhang F, et al. Nef proteins from simian immunodeficiency viruses are tetherin antagonists. *Cell Host Microbe.* 2009; 6:54–67. [PubMed: 19501037]
174. Kaletsky RL, Francica JR, Agrawal-Gamse C, Bates P. Tetherin-mediated restriction of filovirus budding is antagonized by the Ebola glycoprotein. *Proc Natl Acad Sci U S A.* 2009; 106:2886–2891. [PubMed: 19179289]
175. Barteel E, McCormack A, Fruh K. Quantitative membrane proteomics reveals new cellular targets of viral immune modulators. *PLoS Pathog.* 2006; 2:e107. [PubMed: 17238276]
176. Mansouri M, et al. Molecular mechanism of BST2/Tetherin downregulation by K5/MIR2 of Kaposi's sarcoma herpesvirus. *J Virol.* 2009
177. Ronnblom L, Eloranta ML, Alm GV. Role of natural interferon-alpha producing cells (plasmacytoid dendritic cells) in autoimmunity. *Autoimmunity.* 2003; 36:463–472. [PubMed: 14984023]
178. Savarese E, et al. U1 small nuclear ribonucleoprotein immune complexes induce type I interferon in plasmacytoid dendritic cells through TLR7. *Blood.* 2006; 107:3229–3234. [PubMed: 16368889]
179. Fitzgerald-Bocarsly P, Dai J, Singh S. Plasmacytoid dendritic cells and type I IFN: 50 years of convergent history. *Cytokine Growth Factor Rev.* 2008; 19:3–19. [PubMed: 18248767]
180. Gilliet M, Lande R. Antimicrobial peptides and self-DNA in autoimmune skin inflammation. *Curr Opin Immunol.* 2008; 20:401–407. [PubMed: 18611439]
181. Ronnblom L, Alm GV, Eloranta ML. Type I interferon and lupus. *Curr Opin Rheumatol.* 2009; 21:471–477. [PubMed: 19525849]
182. Crow MK, Kirou KA, Wohlgemuth J. Microarray analysis of interferon-regulated genes in SLE. *Autoimmunity.* 2003; 36:481–490. [PubMed: 14984025]
183. Han GM, Chen SL, Shen N, Ye S, Bao CD, Gu YY. Analysis of gene expression profiles in human systemic lupus erythematosus using oligonucleotide microarray. *Genes Immun.* 2003; 4:177–186. [PubMed: 12700592]
184. Baechler EC, et al. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc Natl Acad Sci U S A.* 2003; 100:2610–2615. [PubMed: 12604793]
185. Bennett L, et al. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J Exp Med.* 2003; 197:711–723. [PubMed: 12642603]
186. Palucka AK, Blanck JP, Bennett L, Pascual V, Banchereau J. Cross-regulation of TNF and IFN-alpha in autoimmune diseases. *Proc Natl Acad Sci U S A.* 2005; 102:3372–3377. [PubMed: 15728381]
187. Nestle FO, et al. Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. *J Exp Med.* 2005; 202:135–143. [PubMed: 15998792]
188. Lande R, et al. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature.* 2007; 449:564–569. [PubMed: 17873860]
189. Li Q, Xu B, Michie SA, Rubins KH, Schreiber RD, McDevitt HO. Interferon-alpha initiates type 1 diabetes in nonobese diabetic mice. *Proc Natl Acad Sci U S A.* 2008; 105:12439–12444. [PubMed: 18716002]

190. Isaksson M, et al. Plasmacytoid dendritic cells promote priming of autoimmune Th17 cells and experimental autoimmune encephalomyelitis. *Eur J Immunol.* 2009
191. Bailey-Bucktrout SL, Caulkins SC, Goings G, Fischer JA, Dzionek A, Miller SD. Cutting edge: central nervous system plasmacytoid dendritic cells regulate the severity of relapsing experimental autoimmune encephalomyelitis. *J Immunol.* 2008; 180:6457–6461. [PubMed: 18453561]
192. Guo B, Chang EY, Cheng G. The type I IFN induction pathway constrains Th17-mediated autoimmune inflammation in mice. *J Clin Invest.* 2008; 118:1680–1690. [PubMed: 18382764]
193. Prinz M, et al. Distinct and nonredundant in vivo functions of IFNAR on myeloid cells limit autoimmunity in the central nervous system. *Immunity.* 2008; 28:675–686. [PubMed: 18424188]
194. Auerbuch V, Brockstedt DG, Meyer-Morse N, O'Riordan M, Portnoy DA. Mice lacking the type I interferon receptor are resistant to *Listeria monocytogenes*. *J Exp Med.* 2004; 200:527–533. [PubMed: 15302899]
195. Carrero JA, Calderon B, Unanue ER. Type I interferon sensitizes lymphocytes to apoptosis and reduces resistance to *Listeria* infection. *J Exp Med.* 2004; 200:535–540. [PubMed: 15302900]
196. O'Connell RM, et al. Type I interferon production enhances susceptibility to *Listeria monocytogenes* infection. *J Exp Med.* 2004; 200:437–545. [PubMed: 15302901]
197. Stockinger S, et al. Characterization of the interferon-producing cell in mice infected with *Listeria monocytogenes*. *PLoS Pathog.* 2009; 5:e1000355. [PubMed: 19325882]
198. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. *Annu Rev Immunol.* 2003; 21:685–711. [PubMed: 12615891]
199. Zou W, et al. Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells. *Nat Med.* 2001; 7:1339–1346. [PubMed: 11726975]
200. de Heer HJ, et al. Essential role of lung plasmacytoid dendritic cells in preventing asthmatic reactions to harmless inhaled antigen. *J Exp Med.* 2004; 200:89–98. [PubMed: 15238608]
201. Goubier A, et al. Plasmacytoid dendritic cells mediate oral tolerance. *Immunity.* 2008; 29:464–475. [PubMed: 18789731]
202. Contractor N, Louten J, Kim L, Biron CA, Kelsall BL. Cutting edge: Peyer's patch plasmacytoid dendritic cells (pDCs) produce low levels of type I interferons: possible role for IL-10, TGFβ₂, and prostaglandin E₂ in conditioning a unique mucosal pDCs phenotype. *J Immunol.* 2007; 179:2690–2694. [PubMed: 17709480]
203. Ochando JC, et al. Alloantigen-presenting plasmacytoid dendritic cells mediate tolerance to vascularized grafts. *Nat Immunol.* 2006; 7:652–662. [PubMed: 16633346]
204. Mellor AL, et al. Cutting edge: induced indoleamine 2,3 dioxygenase expression in dendritic cell subsets suppresses T cell clonal expansion. *J Immunol.* 2003; 171:1652–1655. [PubMed: 12902462]
205. Fallarino F, et al. Murine plasmacytoid dendritic cells initiate the immunosuppressive pathway of tryptophan catabolism in response to CD200 receptor engagement. *J Immunol.* 2004; 173:3748–3754. [PubMed: 15356121]
206. Munn DH, et al. Expression of indoleamine 2,3-dioxygenase by plasmacytoid dendritic cells in tumor-draining lymph nodes. *J Clin Invest.* 2004; 114:280–290. [PubMed: 15254595]
207. Sharma MD, et al. Plasmacytoid dendritic cells from mouse tumor-draining lymph nodes directly activate mature Tregs via indoleamine 2,3-dioxygenase. *J Clin Invest.* 2007; 117:2570–2582. [PubMed: 17710230]
208. Sharma MD, et al. Indoleamine 2,3-dioxygenase controls conversion of Foxp3⁺ Tregs to TH17-like cells in tumor-draining lymph nodes. *Blood.* 2009; 113:6102–6111. [PubMed: 19366986]
209. Baban B, et al. IDO activates regulatory T cells and blocks their conversion into Th17-like T cells. *J Immunol.* 2009; 183:2475–2483. [PubMed: 19635913]
210. Ito T, et al. Plasmacytoid dendritic cells prime IL-10-producing T regulatory cells by inducible costimulator ligand. *J Exp Med.* 2007; 204:105–115. [PubMed: 17200410]
211. Levings MK, Sangregorio R, Galbiati F, Squadrone S, de Waal Malefyt R, Roncarolo MG. IFN-α and IL-10 induce the differentiation of human type 1 T regulatory cells. *J Immunol.* 2001; 166:5530–5539. [PubMed: 11313392]

212. Jahrsdorfer, B., et al., editors. Plasmacytoid dendritic cells suppress T cell expansion by a mechanism involving active granzyme B secretion. Berlin, Germany: 2009.
213. Ito T, et al. Plasmacytoid dendritic cells regulate Th cell responses through OX40 ligand and type I IFNs. *J Immunol.* 2004; 172:4253–4259. [PubMed: 15034038]
214. Kumagai Y, et al. Alveolar macrophages are the primary interferon-alpha producer in pulmonary infection with RNA viruses. *Immunity.* 2007; 27:240–252. [PubMed: 17723216]
215. Allman D, et al. Ikaros is required for plasmacytoid dendritic cell differentiation. *Blood.* 2006; 108:4025–4034. [PubMed: 16912230]
216. Kaplan DH, Jenison MC, Saeland S, Shlomchik WD, Shlomchik MJ. Epidermal langerhans cell-deficient mice develop enhanced contact hypersensitivity. *Immunity.* 2005; 23:611–620. [PubMed: 16356859]
217. Kaplan DH, Kissenpfennig A, Clausen BE. Insights into Langerhans cell function from Langerhans cell ablation models. *Eur J Immunol.* 2008; 38:2369–2376. [PubMed: 18792030]
218. Ohnmacht C, et al. Constitutive ablation of dendritic cells breaks self-tolerance of CD4 T cells and results in spontaneous fatal autoimmunity. *J Exp Med.* 2009; 206:549–559. [PubMed: 19237601]
219. Birnberg T, et al. Lack of conventional dendritic cells is compatible with normal development and T cell homeostasis, but causes myeloid proliferative syndrome. *Immunity.* 2008; 29:986–997. [PubMed: 19062318]
220. Smit JJ, Rudd BD, Lukacs NW. Plasmacytoid dendritic cells inhibit pulmonary immunopathology and promote clearance of respiratory syncytial virus. *J Exp Med.* 2006; 203:1153–1159. [PubMed: 16682497]
221. Wang H, Peters N, Schwarze J. Plasmacytoid dendritic cells limit viral replication, pulmonary inflammation, and airway hyperresponsiveness in respiratory syncytial virus infection. *J Immunol.* 2006; 177:6263–6270. [PubMed: 17056556]
222. Cervantes-Barragan L, et al. Control of coronavirus infection through plasmacytoid dendritic-cell-derived type I interferon. *Blood.* 2007; 109:1131–1137. [PubMed: 16985170]
223. Diana J, et al. NKT cell-plasmacytoid dendritic cell cooperation via OX40 controls viral infection in a tissue-specific manner. *Immunity.* 2009; 30:289–299. [PubMed: 19217323]
224. GeurtsvanKessel CH, et al. Clearance of influenza virus from the lung depends on migratory langerin+CD11b- but not plasmacytoid dendritic cells. *J Exp Med.* 2008; 205:1621–1634. [PubMed: 18591406]

Fig. 1. DT administration systemically depletes pDCs in BDCA-2-DTR mice
 (A) Spleen, blood, lymph node (LN), and liver were analyzed for the expression of B220 and Siglec-H by flow cytometry 24 h after i.p. injection of PBS or DT. (B) pDCs-depleted mice have reduced type I IFN responses to CpG. BDCA-2-DTR mice were injected with CpG 2216 and DOTAP i.v. 24 h after i.p. injection of PBS or DT. Serum was collected 6 h later, and IFN- α levels were measured by ELISA.

Fig. 2. Innate and adaptive responses to VSV-OVA in pDC-depleted mice

Mice were injected with PBS or DT on days -1 and 3 and infected with VSV-OVA (5×10^6 pfu, i.v.). (A) Total cells per spleen on day 7 post-infection in control and pDC-depleted mice. (B) pDCs-depleted mice have a reduction in OVA-specific CD8⁺ T cells. Splenocytes from infected mice were stained with H-2K^b/OVA₂₅₇₋₂₆₄ peptide tetramers and anti-CD8 and the frequencies and total numbers of antigen-specific CD8⁺ T cells were determined by flow cytometry. (C) Control and pDC-depleted mice produce similar amounts of systemic type I IFN and IL-12p40 following challenge with VSV-OVA. Serum was collected at various timepoints post-infection, and IFN- α and IL-12p40 levels were measured by ELISA and cytometric bead array, respectively. (D and E) pDC depletion does not affect DC

maturation or antigen presentation in VSV-OVA-infected mice. (D) The expression of molecules on CD11c^{hi} DCs from spleens of naïve mice or mice infected for 24 h with VSV-OVA were assessed by flow cytometry using antibodies to CD11c, CD80, CD86, MHC II, and CD40 (red histograms, DT; blue histograms, PBS; and black histograms, naïve mice). (E) CD11c⁺ cells from spleens of VSV-OVA-infected mice were enriched with MACS beads 24 h post-infection. CD11c⁺ cells were stained with anti-CD11c, -B220, -Siglec-H, and -CD8α antibodies and sorted into 3 populations: CD11c⁺CD8⁺ (CD8α⁺ DC), CD11c⁺CD8⁻ (CD8α⁻ DCs), and Siglec-H⁺B220⁺ (pDCs). DC subsets were co-cultured at different ratios for 72 h with CD8⁺ T cells purified from OT-1 transgenic mice by negative selection. IFN-γ levels in culture supernatants were determined by cytometric bead array. (F) pDC-depleted mice infected with VSV-OVA produce less MIP-1β (CCL4) compared to control mice. Serum levels of MIP-1β were calculated by cytometric bead array. (G and H) Antibody responses to VSV-OVA in pDC-depleted mice. (G) Serum IL-6 levels were determined at 24 and 72 h post-infection by cytometric bead array. (H) Top panel: levels of OVA-specific IgM in serum on days 3 and 7 post-infection were assessed by ELISA. Bottom panel: Serum was collected on day 7 and used in standard virus neutralization assays with Vero cell monolayers. Briefly, VSV-OVA was incubated for 90 min with heat-inactivated, diluted mouse serum then added to Vero cells for 1 h. Wells were overlaid with methyl cellulose and plates were incubated for an additional 24 h. Cells were fixed with Carnoy's fixative and stained with crystal violet. Virus incubated with medium or serum from naïve mice served as controls. (I) Virus titers in the brains of control or pDC-depleted mice on day 7. Confluent Vero cell monolayers were infected with brain homogenates for 1 h then overlaid with 1% methyl cellulose in complete medium. The overlay was flicked off 24 h later and cells were fixed with Carnoy's fixative and stained with crystal violet. Data are combined from 2–3 separate experiments. Results depicted in bar graphs are the means ± SEM. Student's t test was used to calculate p values.

Fig. 3. Innate and adaptive responses to LCMV in pDC-depleted mice

Mice were injected with PBS or DT on days -1 and 3 and infected i.p. with either 2×10^5 or 1×10^6 pfu of LCMV-Armstrong. (A) Control and pDC-depleted mice produce comparable levels of IFN- α , MCP-1, IL-6, and TNF- α during LCMV infection. Serum was collected at 24 and 72 h post-infection and analyzed by ELISA or cytometric bead array. (B) pDCs are not required for robust anti-LCMV CTL responses. Splenocytes were isolated on days 10 or 11 post-infection and stained with H-2D b /LCMVgp33–41 tetramers and anti-CD8 antibody, then analyzed by flow cytometry. The frequencies (top panels) and total numbers (middle panels) of antigen-specific T cells are shown. Splenocytes from day 10 infected mice (2×10^5 pfu) were incubated *in vitro* for 6 h with LCMV peptides gp33–41 or gp276–286 and Brefeldin A (BFA). Cells were stained with antibodies to CD4, CD8, and IFN- γ and analyzed by flow cytometry. The frequencies and total numbers of cells producing IFN- γ are shown in the lower panels. Antigen-specific cytotoxicity was assessed using splenocytes from day 10 infected mice as effector cells and EL-4 cells pulsed or not pulsed with LCMV peptides gp33–41 or gp276–286 as targets in a standard 4-h ^{51}Cr release assay. Results depicted in bar graphs are the means $\pm$ SEM ($n = 3$ mice per dose/group).

Fig. 4. Potential role of pDCs in the induction of antiviral CTL responses

pDCs sense viral nucleic acids via TLR7/9 in endosomal compartments. Upon activation, pDCs produce type I IFNs, IL-12, and chemokines such as MIP-1 α and MIP-1 β that attract naive CD8⁺ T cells to T-cell areas of secondary lymphoid tissues where they become virus-specific CTLs. Virus-specific CTLs proliferate and migrate to the periphery where they produce IFN- γ and kill virus-infected cells via perforin (Per) and granzyme (Gr). In our experimental settings, pDCs promote CD8⁺ T-cell responses mostly by secreting chemoattractants. However, pDCs may help CD8⁺ T-cell responses by secreting type I IFN and IL-12, which may act directly on T cells, or indirectly, by inducing the maturation of DCs.

Fig. 5. The emergent roles of pDCs during HIV infection

It has been proposed that HIV-activated pDCs have beneficial and detrimental effects on HIV pathogenesis. Beneficial effects (green lines): pDCs secretion of type I IFNs and TNF- α promote the bystander maturation of DC which cross-prime and present antigens to T cells. Type I IFNs also limit viral replication in CD4⁺ T cells. Detrimental effects (red lines): HIV-activated pDCs express IDO, which induces Tregs that suppress CD4⁺ T-cell proliferation and DC maturation. Chronic pDC activation and type I IFN secretion has also been associated with the upregulation of activation markers on CD8⁺ T cells and the progressive depletion of infected and uninfected CD4⁺ T cells through apoptotic mechanisms such as TRAIL/DR5 or Fas/Fas ligand.

Fig. 6. pDC depletion does not affect the replication of *Listeria monocytogenes* (LM) in organs of infected mice

BDCA-2-DTR mice were injected with PBS or DT on days –1 and 2 post-infection and infected with 1×10^5 cfu of the EGD strain of LM i.p. Spleens and livers were collected on day 4 post-infection, homogenized and plated on BHI agar. Homogenates were serially diluted and plated in duplicate. Colony-forming units per organ were determined 24 h later. Bar graphs show the means $\pm$ SEM (4 mice/group).

Table 1

PDC cell surface receptors that regulate type I IFN secretion

Receptors	Effect on type I IFN production	Adapter/cytoplasmic ITIM	References
Mouse			
Siglec-H	Inhibitory	DAP12 (ITAM)	(20)
PDC-TREM	Activating	DAP12 (ITAM)	(77)
Ly49Q	Activating	ITIM	(78)
Human			
BDCA-2	Inhibitory	FcR γ (ITAM)	(80, 81)
ILT7	Inhibitory	FcR γ (ITAM)	(82)
NKp44	Inhibitory	DAP12 (ITAM)	(84)
DCIR	Inhibitory	ITIM	(85)
Fc γ RIIa (CD32)	Activating	FcR γ (ITAM)	(86)
CD300a/c	Activating	CD300a (ITIM) CD300c (unclear)	(89)

Ligation of mouse and human BST-2 also inhibits pDC type I IFN responses to TLR ligands. BST-2 does not contain any known signaling motifs in its cytoplasmic domain. In humans, BST-2 associates with ILT7 to suppress type I IFN; however, the mechanism in mice is undefined.

Table 2

Models of viral infections and tolerance studied by antibody-mediated depletion of pDC

	Antibody	Effect	References
Virus			
MCMV	mPDCA-1	↓ systemic type I IFN, ↓ DC and NK cell activation	(95)
	RB6-8C5	↓ systemic type I IFN, no major effect on IL-12, ↑ viral titers	(11, 92)
RSV	120G8	↓ type I IFN in lung, ↑ airway hyperreactivity, ↑ viral titers	(200)
HSV	mPDCA-1, RB6-8C5	↓ CTL responses, ↑ viral titers	(102)
MHV	mPDCA-1	↓ systemic type I IFN, ↑ viral titers, ↑ liver damage	(201)
LCMV	120G8	↑ viral titers in pancreas, ↑ hyperglycemia	(202)
	RB6-8C5	No effect on systemic type I IFN	(92)
Influenza	120G8	No effects on type I IFN in BALF or lung, viral titers or CTL responses, ↓ virus-specific Ab titers	(203)
Tolerance			
Asthma	120G8, RB6-8C5	↑ sensitization, ↑ effector cells, ↓ Tregs	(181)
Oral tolerance	120G8, RB6-8C5	↓ tolerance, ↑ oral priming	(182)
Transplantation	120G8	↓ graft survival	(184)
EAE	mPDCA-1	↓ systemic type I IFN, ↓ T _H 17 cells, ↓ disease severity	(171)

RB6-8C5 (anti-Gr-1 Ab) cross-reacts with both Ly6C on pDC and plasma cells and Ly6G on granulocytes. 120G8, mouse plasmacytoid dendritic cell Ag-1 (mPDCA-1) and 927 Abs react with bone marrow stromal Ag-2 (BST-2) expressed by pDC, plasma cells and other cell types following exposure to IFNs. BALF, bronchoalveolar lavage fluid.

Table 3

Tolerogenic mechanisms of pDC

Model	Mechanism	Outcome	References
Tumor (ovarian)	IL-10-producing T regs	Poor anti-tumor T cell responses	(180)
Asthma	Tregs	Prevention of sensitization and T _H 2 responses	(181)
Oral tolerance induced by Ag feeding	T cell anergy/deletion	Prevention of CD8 ⁺ T cell-mediated DTH responses	(182)
Transplantation	Tregs	Prolonged graft survival	(184)
Tumor (melanoma)	IDO-induced Tregs	Poor anti-tumor T cell responses	(187–189)
HIV	IDO-induced Tregs	Impairment of DC and HIV-specific T cells	(138, 139)
GVHD	Tregs	Prolonged graft survival and reduced IL-17 production	(28)
<i>In vitro</i> exposure to IL-3/CD40L or TLR ligands	IL-10-producing Tregs via ICOS-L	Impairment of T cell proliferation	(191)
<i>In vitro</i> exposure to IL-3 and IL-10	Granzyme B secretion	Impairment of T cell proliferation	(193)
<i>In vitro</i> exposure to IL-3	OX40L- OX40 interactions	T _H 2 polarization	(194)

DTH, delayed-type hypersensitivity; GVHD, graft-versus-host disease