Production of type I interferons: plasmacytoid dendritic cells and beyond

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Plasmacytoid dendritic cells (pDCs) are specialized producers of type I interferons (IFNs) that respond to most viruses. Because of their antiviral activity and regulatory functions in innate and adaptive immunity, type I IFNs are important not only for antiviral resistance but also in other types of infections and in immune pathology. Here we discuss recent data that begin to reveal the unique molecular mechanisms underlying the remarkably rapid and efficient type I IFN production by pDCs.

Plasmacytoid DCs in mice and men

The first evidence for the existence of a cell type specialized for the production of type I IFNs (IFN-α, IFN-β) came from human studies showing that a non-T, non-B, nonmonocytic cell type was required for NK cell–mediated killing of virus-infected cells or tumor cell lines. These cells mediated activation of NK cells through secretion of type I IFNs (1) and were defined as natural interferon-producing cells (NIPCs). NIPCs were characterized by several groups in the 1980s and found to be a rare HLA class II–positive cell type distinct from conventional dendritic cells (cDCs) (2). NIPCs were recently found to correspond to plasmacytoid cells that had previously been identified in the T cell zone of human lymphoid tissue and in peripheral blood, now known as pDCs (2, 3).

pDCs have a round morphology with an eccentric nucleus and abundant endoplasmic reticulum (thus "plasmacytoid"). When activated in vitro with interleukin (IL)-3 and CD40 ligand (CD40L) they acquire a dendritic cell morphology and many DC functions (Fig. 1) (2). Importantly, pDCs express a different profile of the microbial pattern recognition Toll-like receptors (TLRs) than cDCs (4). cDCs preferentially express TLR3, TLR8, and low levels of TLR2 and TLR4, whereas pDCs preferentially express TLR7 and TLR9. TLR7 and TLR9 are intracellular endosomal receptors that allow pDCs to respond to single stranded RNA and DNA viruses, respectively, by triggering signal transduction through the adaptor protein MyD88.

Early in vitro studies in humans showed that pDCs are the only cell type within peripheral blood that efficiently produces type I IFNs in response to certain viruses, including inactivated ones. Thus, they were also hypothesized to produce most type I IFN during in vivo virus infection. However, it is well known that most cells, including cDCs (5, 6) and other cells, hematopoietic or not, produce type I IFN when exposed to viruses. One key difference between pDCs and other cell types is that TLR7 or TLR9 ligation induces efficient production of type I IFNs in pDCs (Fig. 2), but not in other cells expressing these receptors (such as B lymphocytes). Type I IFNs are produced by other cell types in response to triggering of TLR3 and TLR4 by their ligands double stranded RNA (dsRNA) and lipopolysaccharide, respectively. TLR3 and TLR4 signal through the adaptor molecule TRIF that associates with the kinase TBK1 and induces phosphorylation and nuclear translocation of the transcription factor interferon regulatory factor (IRF)-3.

Type I IFNs are also induced by activation of cytoplasmic receptors, including those that recognize dsRNA. Although the dsRNA-binding protein kinase R (PKR) was initially described to play an important role in type I IFN production (6), gene silencing studies have indicated that the RNA helicases encoded by retinoic *acid-inducible gene I* (*RIG-I*) and, likely, *melanoma differentiation-associated gene 5* (*MDA-5*) are the prevalent cytoplasmic receptors responsible for triggering type I IFN secretion (7). TLR3 and TLR4, which are expressed in cDCs and other cells types, are minimally expressed by pDCs, whereas the cytoplasmic dsRNA receptors are expressed by most cells. The production of type I IFNs is further regulated by a positive feedback loop that is based on the ability of IFN-β (in mice and humans) and IFN-α4 (in mice)—produced in response to activated IRF-3 and other transcriptional factors—to induce the transcription of other signaling proteins, such as IRF-7 (8) and likely IRF-8 (Tailor, P., and K. Ozato, personal communication). Once phosphorylated, these transcription factors help drive the expression of all the genes of the type I IFN family, thereby amplifying type I IFN production. Thus, the unique pattern of TLR expression and the molecular mechanisms responsible for type I IFN production do not alone explain the exquisite ability of pDCs to produce type I IFNs.

NIPCs and pDCs were not identified in mice until 2001, years after their human counterparts (2). The characterization of mouse pDCs finally allowed the investigators to test whether these cells play a predominant role in type I IFN production during virus infection. Upon infection of mice with many DNA viruses, such as murine cytomegalovirus (MCMV) or vesicular stomatitis virus (VSV), pDCs were shown to be responsible for the majority of the type I IFN production in vivo, confirming the specialized role of these cells (2, 9–11).

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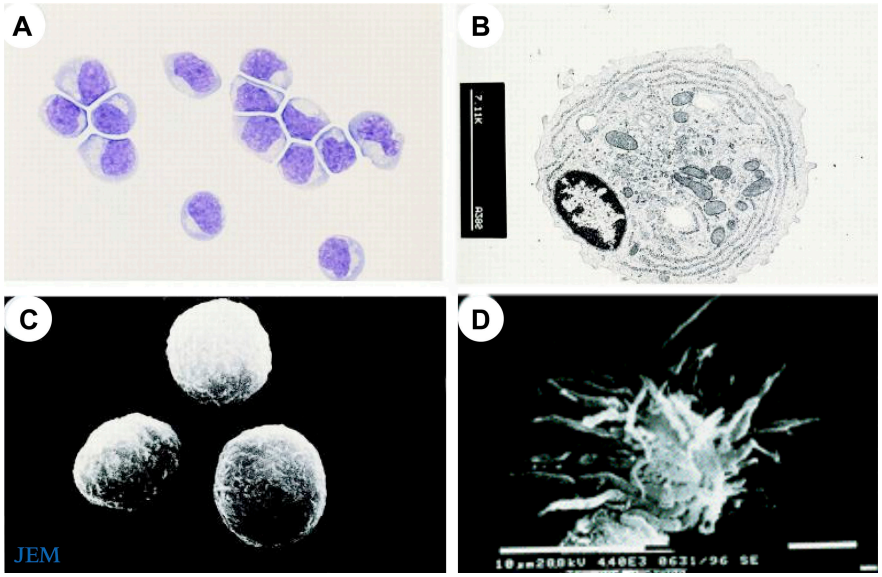

Figure 1. Human plasmacytoid DCs. Optical microscopy (A), transmission electron microscopy (B), and scanning electron microscopy (C) images showing the typical morphology of human pDCs. (D) Scanning electron microscopy image showing the dramatic morphological changes of activated pDCs after culture in the presence of IL-3 and CD40 ligand. All images are reproduced from Grouard et al. (3).

However, this was not the case with other viruses, such as lymphocytic choriomeningitis virus (LCMV) and West Nile virus (2, 10). Although the peak of type I IFN production at 48 h after LCMV infection was not decreased by depleting the mice of pDCs (10), these cells were shown to produce some type I IFN at earlier times after infection (12). Similarly, although the peak production of type I IFNs at 36 h after MCMV infection was MyD88 dependent and was almost completely abolished if pDCs were depleted, a minor peak of type I IFN production occurred a few hours later and was MyD88, pDC, and, most likely, TLR independent (13). cDCs contributed to the second peak of type I IFN production and were fully capable of activating the antiviral activity of NK cells in the spleen of MCMV-infected animals, indicating that pDCs are not essential for this function in vivo (13). However, the exact identification of the cell type(s) other than pDCs that are responsible for producing type I IFNs in vivo has proven difficult, and many different cell types are likely to be involved, including cDCs and macrophages as well as nonhematopoietic cells.

Type I IFNs in DC maturation and function

Mice lacking functional type I IFN receptors (*IFNAR*$^{-/-}$) have provided a useful model for analyzing the role of type I IFNs in DC maturation and function. cDCs are only partially dependent on type I IFNs for their maturation (5) and do not require these cytokines for activation-induced migration to the central T cell zones in the spleen (14). Unlike cDCs, the maturation of pDCs in response to TLR7 or TLR9 agonists is drastically reduced in the absence of type I IFN signaling, as is their migration to and clustering in the marginal zone of the spleen (14), suggesting an important role for positive feedback in plasmacytoid pDC migration and function in vivo.

Type I IFNs produced during virus infection inhibit the production of the Th1-promoting cytokine IL-12 by cDCs. IL-12 production is recovered if pDCs are depleted or type I IFN signaling is prevented (10). However, the effect of type I IFNs on IL-12 production is somewhat paradoxical: high levels of type I IFNs, such as those produced during virus infection, inhibit IL-12 production mostly by decreasing transcription of the IL-12p40 chain, whereas lower levels of type I IFNs are actually required for the efficient transcription of the *IL-12p35 gene* and thus for the production of the biologically active IL-12 heterodimer (IL-12 p70) (15). The type I IFN required for IL-12 p70 production is produced in an autocrine manner by cDCs rather than by pDCs. This cDC-derived type I IFN is partly responsible for the synergy of IL-12 p70 production that is observed when TRIF-dependent and MyD88-dependent TLRs are triggered simultaneously (15).

Mechanisms of type I IFN production by pDCs

Mouse studies confirmed that pDCs were potent producers of type I IFNs in vivo, but the unique molecular mechanisms that endowed them with this ability remained to be determined. IRF-3, which is required for type I IFN production in response to TLR3 and TLR4, is neither required for nor activated by TLR7/9 signaling in pDCs (16). Instead, IRF-7 is required for type I IFN production in response to TLR7/9 (16). Ligation of TLR7 or TLR9 in pDCs activates a signaling complex composed of MyD88, IRAK-1, and TRAF6, which physically associates with IRF-7 and is required for the production of type I IFNs (17, 18). Unlike IRF-7, IRF-5, which also forms a complex with MyD88 and TRAF6 downstream of TLR7/9, is required for the transcription of proinflammatory genes such as *IL-6*, *TNF*, and *IL-12 p40* (16, 19). Contrasting results have been reported regarding whether IRF-5 can substitute for IRF-7 to stimulate type I IFN induction (16, 19).

Both human and mouse pDCs constitutively express somewhat higher levels of IRF-7 than other cell types and this could account for the ability of pDCs to produce type I IFNs in the absence of positive feedback driven by de novo IRF-7 induction (20, 21). pDCs also constitutively express IRF-8 that, similarly to IRF-7, appears to be involved in positive feedback (22). Indeed, type I IFN production by pDCs is in part independent of this positive

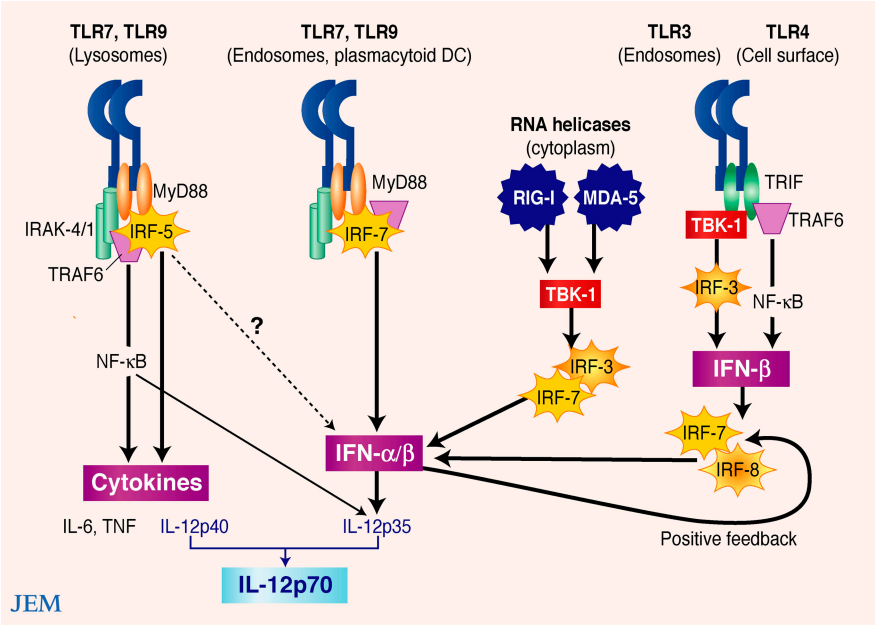

Figure 2. Major signaling pathways for type I IFN gene transcription. TLR7 and TLR9 are highly expressed on pDCs and, depending on the chemical properties and formulation of the ligands used and on the cell type, activate signaling pathways involving either IRF-7 or IRF-5 and NF-κB. The IRF-7 pathway preferentially induces transcription of the type I IFN gene family and it is activated when the TLR–MyD88–IRAK–IRF-7 complex is retained in the endosomal compartment, as observed in pDCs treated with type A CpG ODNs. Other receptors able to induce production of type I IFN are TLR3 and TLR4 signaling through the adaptor molecule TRIF and the kinase TBK-1. TLR4, in addition to TRIF, also utilizes the adaptors TRAM, MyD88, and TIRAP (not shown in the figure). The cytoplasmic dsRNA receptor RIG-I also utilizes TBK-1. IRF-7 is required for optimal production of type I IFN induced by all these receptors, whereas IRF-3 is essential only for the response to TLR3 or TLR4. A positive feedback mechanism for IFN production involves the de novo synthesis of IRF-7 and IRF-8 in response to type I IFNs that amplify the transcription of all the genes of the type I IFN family.

feedback. In *IFNAR*$^{-/-}$ mice type I IFN production is reduced but not absent in response to MCMV and VSV infection or to the TLR7 ligand R848 (10, 14). However, *IFNAR*$^{-/-}$ pDCs produce only minimal levels of type I IFNs in vivo in response to the TLR9 ligand CpG oligodeoxynucleotides (ODNs), indicating that in some conditions pDCs require positive feedback for type I IFN production (14).

The constitutive expression of IRF-7 is not sufficient to explain why only pDCs, and not other TLR7- or TLR9-expressing cells, efficiently produce type I IFNs. Indeed, IRF-7 is rapidly induced, through both type I IFN-dependent and -independent mechanisms, in all cells at the site of virus infection. In fact, the level of IRF-7 induction in these cells is much higher than those constitutively present in the pDCs, yet the participation of other cells in type I IFN production in response to viruses or other TLR7 and TLR9 ligands is minimal.

In a recent study, a mechanism that likely accounts for the preferential ability of pDCs to produce type I IFNs was described. The study showed that the intracellular translocation of CpG ODNs bound to TLR9, as well as the ability of TLR9 to induce IRF-7 activation, are differentially regulated in pDCs and in other TLR9-expressing cell types (23). In pDCs, the TLR9-associated MyD88–IRF-7 complex was retained in the endosomal compartment for over 90 min after treatment with CpG ODNs. In other cell types, the complex rapidly translocated to lysosomes. This was observed only with type A CpG ODNs, which are efficient inducers of type I IFN production. However, when type A CpG ODNs were formulated within cationic liposomes (DOTAP), they caused the TLR9–MyD88–IRF-7 complex to be retained in the endosomal compartment and induced type I IFN production in cDCs and other cells that normally do not produce these cytokines. Type B CpG ODNs, which preferentially induce proinflammatory cytokines other than type I IFNs, induced rapid translocation of the MyD88–IRF-7 complex to lysosomes in both pDCs and cDCs. However, type B CpG ODNs formulated in DOTAP retained the TLR9 signaling complex in the endosomal compartment and induced type I IFN secretion in pDCs.

The results above on the effect of formulation of CpG ODNs in liposomes are compatible with data indicating that type A CpG ODNs, unlike type B CpG ODNs, have the ability to spontaneously form multimolecular complexes assembled in nanoparticles (24). Type B CpG ODNs loaded on polysterene nanoparticles acquired the ability to induce type I IFNs (24).

Thus, type I IFN production in response to TLR9 ligation requires a spatiotemporal regulation of the MyD88–IRF-7 complex such that it is retained in the endosomal compartment. This is observed only in pDCs unless the CpG ODNs are formulated in cationic liposomes or nanoparticles and are thus trapped in the endosomes. This provides an explanation for the unique ability of these cells to produce type I IFN in response to TLR9 and probably TLR7 ligands (23). The complex of nucleic acids and cationic liposomes may mimic TLR9-activating viruses that accumulate in endosomes (23). TLR9 and probably TLR7 bind their ligands at low pH (5.0–6.5); thus, the interaction between receptor and ligand could take place in early endosomes (pH 6.3–6.5), in the endosomal carrier vesicles (pH 5.0–5.5), or in the late endosomes/lysosomes (pH ~5.0). In nonstimulated cells, TLR9 is localized in the endoplasmic reticulum (ER) (25), but after the cells are exposed to CpG ODNs it associates either with endosomes or with lysosomes, depending on the type and formulation of CpG ODNs and the re-

sponding cell type. The lack of fully glycosylated forms of TLR9 in CpG-stimulated cells suggests that the ER might directly communicate with the endosomal compartment under these conditions, although a more canonical pathway through the Golgi and into endosomal carrier vesicles cannot be completely excluded (25). The mechanisms that determine the delayed movement from endosomes to lysosomes in CpG-stimulated pDCs or in other cells exposed to CpG ODNs formulated in cationic liposomes remain unknown. It is also unclear why activation of the TLR9–IRF-7 signaling pathway takes place in endosomes, whereas the TLR9–NF-κB–IRF-5 pathway is activated in lysosomes. It is possible that retention in the endosomal compartment allows the receptor to interact with its ligand for a longer period of time and that this prolonged interaction may be required for IRF-7 activation but not for IRF-5 and NF-κB activation. Alternatively, prolonged signaling through TLR9 may phosphorylate de novo–produced IRF-7, thus allowing the activation of the positive feedback and optimal production of type I IFNs (26).

Why do we need a specialized type I IFN–producing cell?

Although type I IFNs are mostly produced by pDCs during several virus infections, depending on the virus, a variable proportion of type I IFN is also produced by other cell types, including cDCs (5, 6, 13). Type I IFNs produced by cell types other than pDCs have important direct antiviral effects and immunomodulatory effects such as the activation of NK cells and the efficient and early production of the IL-12 p70 heterodimer (13, 15). Yet, one may speculate why it is useful for the organism to have a specialized IFN-producing cell type. Not only are pDCs wired for a rapid production of high levels of type I IFNs, but the TLR7 and TLR9 used by these cells—unlike other receptors leading to type I IFN production—respond to viral nucleic acid from inactivated or noninfectious viruses and do not require infection of the cells. This is beneficial, as many viruses have developed mechanisms to block the production of type I IFNs early after infection (27). In general the viral strategies used to block type I IFN production are not effective in pDCs because either the cells are not infected or the virus targets signaling pathways for type I IFN induction other than those used by TLR7 and TLR9 (6). Exceptions are measles virus and respiratory syncytial virus, which specifically inhibit type I IFN production in pDCs (27), and human herpes virus 8, which specifically blocks IRF-7 with its ORF45 protein (28).

Despite the importance of type I IFN production in vivo, it should be noted that a unique and essential function for the type I IFNs that are produced by pDCs has not yet been identified and that much work remains to be done to understand the precise biologic role of pDCs both as type I IFN producers and as accessory cells in innate resistance and adaptive immunity (2).

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