Natural Interferon- α Producing Cells: The Plasmacytoid Dendritic Cells

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INTRODUCTION

The type I interferons (IFNs), which include IFN- α , β , and ω have a long history that involves many firsts: they were the first cytokines to be described, the first to enter human clinical trials and the first to be approved for human use. IFN was described by Isaacs and Lindenmann in 1957 as a soluble substance produced by influenza virus-infected chick chorioallantoic membrane cell cultures that was able to “interfere” (hence the name “interferon”) with subsequent infection of previously uninfected cells (57). Although originally described as substances that were able to confer antiviral resistance, type I IFNs are now appreciated for their immunomodulatory, antitumor, and growth-inhibitory properties as well (42).

Rather than being encoded by a single gene, the type I IFNs are a family of highly related proteins that signal through a common cell-surface receptor (86). Included within this family are the alpha IFNs, which in the human are encoded by a family of 13 highly related genes plus several pseudogenes located on the short arm of chromosome 9, a single IFNB gene that shares approximately a 50% homology with the IFNA genes, and a single gene for IFN- ω , which shares approximately 70% homology with the IFNA family. A fourth type I IFN, IFN- τ , has been described in ruminants and is believed to be important for the establishment of pregnancy in these species. IFN- γ , although having some overlapping functions with the type I IFNs, is genetically unrelated to these IFNs and signals through its own receptor.

IFN can be produced by a wide variety of cells and in response to a wide variety of substances including not only viruses, but also double-stranded RNA, LPS, some bacteria, bacterial DNA and CpG-containing DNA sequences, as well as small-molecular-weight compounds such as imiquimod. In humans, much of the work has focused on the ability of peripheral blood leukocytes to produce type I IFN in response to virus stimulation. Indeed, the first large-scale production of type I IFN was “human leukocyte IFN,” which was derived by culturing peripheral blood mononuclear cells with Sendai virus, and the first cloned IFN- α gene was cloned from leukocytes cultured with Sendai virus (86).

In subsequent studies, it became apparent that there was a unique population of cells within peripheral blood that are potent producers of IFN- α in response to stimulation with a wide range of enveloped viruses (36). These cells, which lacked cell

surface markers typical of cells within the T cell, B cell, natural killer (NK) cell, or monocyte lineage were termed natural interferon-alpha producing cells or NIPCs to indicate that they belonged to the “natural” immune system, which is now more commonly referred to as “innate” immune system (92). A second population of peripheral blood cells, the monocytes, can also produce IFN- α in response to some enveloped viruses including Sendai virus but are poor producers of type I IFN in response to herpes simplex virus (HSV). The history and characterization of NIPCs was the topic of an extensive review article in 1993 (41). Since then, and, in particular, since 1999, there has been enormous progress in the characterization of both the phenotype and function of NIPCs. It is the purpose of the current review to bring up to date the description and characterization of these important cells.

CHARACTERIZATION OF NIPCs

Phenotype of NIPCs

The NIPCs were initially characterized based on their lack of expression of cell surface markers typical of lineages found in the peripheral blood. They were found to lack cell surface markers typical of T cells (such as CD3), B cells (such as surface immunoglobulin, CD19, and CD20), monocytes (CD14), or NK cells (CD16 and CD56) but were found to express high levels of the major histocompatibility complex (MHC) class II determinant, HLA-DR (1,44,85). Limiting dilution assays and enzyme-linked immunospot (ELISPOT) studies utilizing antibodies specific for IFN- α demonstrated that NIPCs are rare cells in the peripheral blood, representing about 0.1% of peripheral blood mononuclear cells (PBMCs) (14,55). However, although rare, each functional NIPC was calculated to produce between 1 and 2 international units of IFN- α , which represents between 3 and 10 pg of IFN, an astronomical amount of secreted cytokine. Although HSV-induced NIPCs express both IFNA and IFNB message, the secreted product of these cells is almost all IFN- α , with IFN- β accounting for only a small portion of the IFN (16,36).

Although the NIPCs lacked a unique definable phenotype, their low frequency along with the HLA-DR⁺, lineage-negative phenotype was reminiscent of a population of cells initially described by Ralph Steinman and his colleagues, the dendritic cell

(DC) (107). Indeed, in studies in our laboratory, NIPCs were found to co-localize with DCs when separated on metrizamide or Percoll gradients, further suggesting that these cells might belong to the DC lineage (33). This idea was further supported by the several other studies (39,40,72,110). However, the lack of specific cell surface determinants to define dendritic cells made more specific isolation and characterization of the NIPC impossible. Moreover, a study by Chemihi et al. (23) in which dendritic cells were isolated after overnight culture demonstrated that these more mature dendritic cells were not functional NIPCs. However, since IFN- α producing capacity of NIPCs is also sensitive to overnight culture, this study did not rule out a DC origin for the NIPCs.

Although a unique phenotype was not yet identified for the NIPCs, the ability to measure their function by measuring their secreted product, IFN- α , allowed for significant characterization of the NIPCs (see Figure 1). Svensson et al. utilized intracellular flow cytometry for IFN- α combined with cell surface markers to further define the phenotype of the virus-responsive NIPC (110). The phenotype provided by this study further implicated a lineage-negative, dendritic-like cell as the NIPC. Moreover, the ability to measure the functional frequency of the NIPCs by ELISPOT assay as well as the total capacity of PBMCs to produce IFN in response to virus stimulation (as measured either by enzyme-linked immunosorbent assay [ELISA] or functional bioassay) allowed for following their function in healthy donors and in patients with various disease states such as HIV-1 infection (15,37,55) (the latter to be discussed below). However, it was not possible to define the nature of functional deficiencies in peripheral blood NIPCs—i.e., were these a result of numerical depletion of the cell or did they represent dysfunction at the individual cell level?

Microscopically, the NIPCs that were actively producing IFN- α in response to virus stimulation were found to be large cells with abundant cytoplasm (33,52). When enriched, they tended to cluster, and indeed, when present in mixed populations of PBMCs, allowed for the clustering with other cell types (34). Ferbas et al. sorted the NIPCs and viewed them microscopically. The NIPCs were found to have large amounts of endoplasmic reticulum to the extent that they resembled antibody producing plasma cells (40,47).

Parallel to the developing understanding of NIPCs was the description of a population of cells that were called plasmacytoid T cells or plasmacytoid monocytes. These cells were first described by pathologists in the late 1950s and later defined as a population of cells located in the T cell zones of human lymphoid tissues (53). Although they resembled immunoglobulin-secreting plasma cells, they did not have markers typical of these cells and were rather described as plasmacytoid T cells due to their localization in T-rich zones. These cells were found in both normal reactive lymph nodes as well as in large numbers in lymphoid tissues in certain pathological conditions including Castleman's disease, Hodgkin's disease, lymphadenitis, and epithelioid cell granulomas (reviewed in Reference 24). Further phenotypic analysis of the so-called plasmacytoid T cells by Facchetti et al. (30) indicated that these cells expressed some markers in common with cells of the myeloid lineage such as CD68, leading these investigators to suggest that the cells belonged to the myeloid lineage, and to refer to them as plasmacytoid monocytes. A blood counterpart of the tis-

sue plasmacytoid cells was described by Liu and colleagues in 1997 (53). They cultured isolated plasmacytoid T cells/monocytes with IL-3 and CD-40-ligand and determined that they developed into immunostimulatory DCs, thus defining these cells as belonging within the DC lineage.

Although DCs were initially assumed to be of one origin, studies in the human as well as the mouse were indicating that there was more than one lineage. O'Doherty et al. described the existence of at least two distinct subsets of peripheral blood DCs (75). In 1997, Olweus et al. defined subsets of blood DCs and described the plasmacytoid cell as one of these subsets and as being of myeloid lineage (76). This second population of DC was found to be similar to Langerhans cells and a cell with phenotypic and functional similarity could be derived in large numbers by culturing peripheral blood monocytes with granulocyte macrophage-colony stimulating factor (GM-CSF) and IL-4. The ease with which these so-called monocyte-derived DCs or MDDCs could be obtained led to extensive study of this subpopulation of DCs. The MDDCs were found to be extremely efficient at capturing antigen, and when induced to mature with LPS or TNF- α , were found to be efficient antigen presenting cells (APCs) (108).

In retrospect, it is obvious that the phenotypes of the plasmacytoid T cell/monocyte and the NIPC were overlapping. The convergence of these two phenotypes was directly tested by Siegal et al. (102) who demonstrated that highly purified plasmacytoid cells (defined by their phenotype of being lineage negative, CD11C⁻, HLA-DR⁺ cells) produced large quantities of IFN- α upon stimulation with HSV. In contrast, the CD11C⁺ population of DCs did not produce IFN- α in response to HSV. This phenotypic characterization has been confirmed in other studies (18) and the NIPC/plasmacytoid dendritic cells have

Figure 1. Phenotype of the NIPCs/PDCs. Products and inhibitors of the HSV-stimulated NIPCs/PDCs are shown as a partial cell surface phenotype.

been found to be CD123^{bright}, (corresponding to one of the DC populations described by Olweus et al. (76)) and blood dendritic cell antigens (BDCA) 2 and 4 (27). BDCA2 is a type II C type lectin that appears to be fairly specific for the plasmacytoid dendritic cells (PDCs) whereas BDCA4 is found on peripheral blood PDCs but not on the CD11c⁺ myeloid dendritic cells (MDCs). However, upon culture and maturation the myeloid DCs also up-regulate expression of BDCA-4, making it a less specific marker for PDCs than BDCA-2. Intracellular flow cytometry studies indicate that while only the CD123^{bright} PDCs produce IFN in response to HSV, not all of the PDCs respond to the stimulus, with the IFN- α expressing PDCs ranging from 20% to 60% of the total CD123^{bright} PDC population (35).

The extraordinary amount of IFN production by NIPCs/PDCs along with the wide range of pathogens to which they are responsive, has led to the suggestion that these cells are “professional IFN-producing cells” (102).

Other Type I IFN-Producing Cells

Among PBMCs, PDCs are by far the most potent interferon-producing cells (IPCs) and can respond to a wide range of enveloped viruses as well as some bacteria, bacterial DNA, and other inducers such as the imidazaquinones. However, monocytes can also produce IFN- α in response to certain viruses such as Sendai virus and HIV-1 (41), with PDCs also responding to these viruses as well. Indeed, the first large-scale preparations of IFNs for clinical use were prepared using Sendai virus-induced mononuclear cells from units of blood under conditions that would not be favorable to PDC function and survival, leading to the description of monocytes as the major IFN- α producing cells in peripheral blood. Several companies still utilize the Sendai-monocyte induction system for commercial preparations of “natural” type I IFN, which has been reported to be less immunogenic and better tolerated in patients over long periods of administration than recombinant IFNs. A number of features distinguish the production of IFN- α by monocytes from that of PDCs: most striking of the differences is the magnitude of the IFN- α response: although the Sendai-responsive monocytic IPCs are more numerous than the PDCs, each monocyte makes far less IFN- α (as much as 10- to 20-fold less) than the PDC counterparts (36,52).

In addition to the PDCs and monocytes, B cells and CD11c⁺ DCs can also produce IFN- α under some conditions as can macrophages, fibroblasts, and a variety of other cell types. The transformed B cell line, NAMALWA, is used for the commercial production of virus-induced natural IFN- α . Interestingly, the range of type I IFNs expressed in different cell types varies depending upon the type of cell and the nature of the stimulation, with, for example, fibroblasts expressing primarily IFN- β , monocytes expressing predominantly IFNA1 along with IFNB, and PDC expressing a wide-range of IFNA subtypes as well as IFNB (7).

Description of NIPCs in Mice

Despite the long-standing observations of a unique population of highly potent IPCs in human peripheral blood, the iden-

Table 1: Phenotype of Unstimulated Peripheral Blood NIPCs/PDCs

Lineage (CD3,14,16,19,20,56) ^{negative}
HLA-DR ⁺
CD4 ⁺
CD123 ^{bright}
BDCA-2 ⁺
BDCA-4 ⁺
Mannose receptor ^{lo}
DC-SIGN ⁺ (subset)
CD62-L ⁺
CD45RA ⁺
Pre-TCR- α ⁺
TLR-7 ⁺
TLR-9 ⁺
CXCR3 ⁺
CXCR4 ⁺
CCR5 ⁺
CCR7 ⁺
IRF-3 ⁺
IRF-7 ^{high}
IRF-1,2,4,5,8 ⁺

The phenotype of the NIPCs/PDCs was determined by flow cytometry except for IRF-1, 2, 4, 5, and 8 and pre-TCR- α , which were analyzed by RT-PCR in enriched PDC populations.

tification of a comparable cell type in mouse was elusive. In fact, studies by Siegal et al. (9) and from Alm's group failed to identify a cell with a DC-like phenotype in murine models. Rather, Eloranta and Alm (29) reported metallophilic macrophages as the major IFN producers. However, most recently several groups have identified mouse IPCs as plasmacytoid cells found in the spleen that are GR-1⁺, CD11c⁺, B220⁺, but CD11b⁻(4,50,73). These murine IPCs are located in the paracortical areas of spleen and other secondary lymphoid organs, and like their human counterparts, produce IFN- α and can be mobilized by in vivo treatment of mice with Flt3 ligand (10).

The identification of the murine equivalent of human NIPCs opens up possibilities for genetic studies of in vivo regulation of NIPCs. For example, L-selectin deficient mice were found to have decreased numbers of IPCs in their lymph nodes, underscoring the importance of L-selectin in migration of PDCs and supporting the observation in humans that in the periphery, PDCs express high levels of L-selectin, which allows for homing and adhering to high endothelial venules, but that this molecule becomes down-regulated in the lymph node (73). Genetic knock-outs have also allowed for the identification of Toll-like receptors (TLRs) as the receptor molecules on PDCs that are responsive to imidazoquinolins and CpG sequences (54).

The utility of the mouse as a model for understanding the role of PDC IFN-producing cells is limited, however, by the failure of IFN- α in the mouse to signal for the development of Th1 responses in contrast to the clear role for IFN- α in induction of Th1 responses in humans. This signaling in humans is dependent upon the IFN-induced recruitment of STAT4 to the

carboxy terminus of STAT2. However, the Stat2 gene in mouse is inactive due to a minisatellite insertion, thus explaining the differences in the roles of type 1 IFN in mouse and human (32).

ORIGIN AND DISTRIBUTION OF PDCs

NIPCs/PDCs were first described as rare cells in the peripheral blood. Additionally, we (unpublished data) and others (18,53) have found that NIPCs/PDCs are present in healthy bone marrow and spleen as well as in tonsillar tissue and lymph nodes of patients undergoing tonsillectomy or lymph node dissection. Cella et al. have proposed that PDCs migrate to inflamed lymph nodes where they are found near high endothelial venules (suggesting recent emigration from the blood) or in the T cell zones, where they produce IFN- α (18); however, we have observed that PDCs isolated from tonsil or lymph node resemble those found in peripheral blood in their lack of constitutive expression of IFN- α but have the ability to produce IFN- α upon viral stimulation (Amrute et al., in preparation). PDCs have also been found in normal thymus (98), in the naso-pharynx of patients with allergy (60), in cerebro-spinal fluid (80) and in suction blisters of individuals injected subcutaneously with *Treponema pallidum* but not in control suction blisters (99). In one report, PDCs were described as cells in the peri-tumor area (113). Interestingly, MDCs were not found in the same tumors.

Most recently, we have found cells that phenotypically resemble PDCs in the bronchioalveolar lavage fluids from certain patients with lung carcinoma or mycobacterial infections (Ryan et al, unpublished observation). Additionally, the cells are believed to be the same as those found in lymph nodes in a variety of conditions such as Castleman's and the others, but this has not been directly phenotypically confirmed, nor is it known whether these cells function as IPCs. Treatment of patients with FLT3 ligand is able to mobilize and/or induce the production of both the PDCs and MDCs, thus giving higher yields of these cells from the peripheral blood (12). It is unclear whether this represents de novo differentiation of PDCs or a mobilization of these cells from the bone marrow into the blood.

The cellular origin of NIPCs/PDCs remains controversial. PDCs have certain markers and properties that make them similar to the classical, so-called MDCs: both are lineage-negative cells that express CD4 and high levels of MHC class II. Both can mature into APCs, although of the two, the MDC is a more potent APC (reviewed in Reference 100). When mature, both MDCs and PDCs express co-stimulatory molecules (CD80 and CD86) as well as CD83. PDCs were originally described as either plasmacytoid T cells or plasmacytoid monocytes by different investigators, and the expression of particular surface markers has led investigators to propose either myeloid (76) or lymphoid origin for PDCs (106). For example, the expression of CD31, CD36, CD68, and CD123 (the IL-3 receptor) led to the suggestion that these cells belong to a myeloid lineage. However, PDCs lack other typical myeloid markers, are CD45RA⁺ and have also been found to express pre-TCR α and CD3 ϵ , transcripts associated with the T cell lineage, as well as the lymphocyte-specific transcription factor, Spi-B. Further suggestion of a lymphoid origin of PDCs/NIPCs comes from the observations of Spits et al. that ectopic expression of Id2 or Id3 inhibits gen-

eration of CD123^{bright} PDCs as well as T cells and B cells (106).

The existence of a leukemic counterpart of PDCs has been suggested (19). These leukemic cells produce IFN- α in response to influenza virus stimulation, can mature into antigen presenting cells, and express typical PDC surface markers (CD4, CD45RA, HLA-DR, IL-3R, and pre-TCR- α) as well as some lymphoid markers, and also express CD56 and granzyme B, a typical component of lytic granules. Unfortunately, although these leukemic cells can be kept alive in culture for some time using cytokine support, the cells have not been successfully cultured and expanded in vitro (Reference 19 and our unpublished findings). The in vitro development of PDCs from CD34 precursor populations has been reported, albeit with low efficiency (12). The lack of a clear cell-line model or a means to generate large quantities of PDCs has hampered some of the biochemical characterization that requires large quantities of cells.

Although some have ascribed the PDC to a lymphoid or independent lineage, others have proposed a plasticity in DC lineage, with Galibert et al. proposing that PDCs represent a composite group of early-committed cells that can differentiate in vitro into DCs. These authors further propose that PDCs might alternatively represent a population of lymphoid origin that are undergoing an in vivo "cell fate conversion" from a lymphoid to a myeloid cell type, thus accounting for their mixed phenotype (25).

MECHANISMS OF TYPE I IFN INDUCTION IN NIPCs/PDCs

Inducers of IFN Production in NIPCs/PDCs

Viruses. The induction of type I IFNs has best been studied in fibroblast or monocyte cultures with infectious virus as the stimulating agent. In these cells, double-stranded RNA, which is an intermediate form during the replication of many viruses, has been identified as a primary inducer of the IFN- α . Indeed, polyI:polyC, a synthetic dsRNA, is a potent inducer of IFN in CD11c⁺ MDCs (63,64). However, the mechanisms involved in the induction of IFN- α in PDCs appear to be very different than those in other cell types. Whereas induction of IFN- α in monocytes by Sendai virus requires live, replication-competent virus, PDCs respond with vigorous IFN production in response to a variety of replication-deficient or UV-inactivated enveloped viruses (Reference 36 and Olshalsky et al., in preparation). In some instances, recombinant viral glycoproteins such as HIV gp120 and gD from HSV have been reported to induce IFN- α production by PDCs (2,3). In contrast, PDCs fail to respond to polyI:polyC with IFN production (63). Further, we have observed no expression of green fluorescent protein (GFP) in PDCs infected with a HSV that expresses GFP under an immediate early promoter despite efficient induction of IFN- α by this virus. In contrast, monocyte derived dendritic cells can be productively infected by this virus but fail to produce IFN- α in response to it (unpublished data).

Bacteria. While the best studied inducers of IFN in PDCs have been the enveloped viruses, some bacteria have also been observed to stimulate IFN- α in these cells. Included among these inducers of IFN- α is *S. aureus* (46,109) and the production of IFN- α by PDCs in *Mycobacterium tuberculosis* (Mtb)-in-

fected lymph nodes has been reported (48). In addition, PDCs were recently reported to produce IFN in response to Mtb (88).

Imiquimod and CpG. The imidazoquinones have been known for a number of years as potent inducers of IFN- α as well as other cytokines (49). The imidazoquinolines are low-molecular-weight molecules that have broad antiviral and antitumor effects. One of the family members, imiquimod (trade name AldaraTM; 3M, Minneapolis, MN, USA) is currently available for the treatment of genital and perianal warts caused by papillomavirus, and another, resiquimod, is in clinical trials for the treatment of genital herpes virus infections. Recently, the imidazoquinones have been described as agonists for both murine and human TLR7 receptors (54). TLR7 is expressed on both human PDCs and MDCs and both DC subsets can respond to the imidazoquinolines with increased survival and up-regulation of costimulatory molecules. However, while PDCs produce IFN- α in response to the TLR7 agonists, the MDCs produce IL-12 (59), thus suggesting cell-type specific differences in response mediated through the same receptor/ligand pair.

Bacterial DNA containing unmethylated CpG motifs (CpG DNA) is another potent inducer of cytokines in a variety of cell types that has been reported to induce IFN- α production in PDCs (59,61,63,64-66). The receptor for CpG DNA is TLR9, which along with TLR7, is expressed on PDCs. Interestingly, immature MDCs (but not PDCs) express TLR 1,2, and 3 and respond to poly I:poly C with the production of IFN- α . It is unclear which, if any of these, is the receptor for the poly I:poly C (63,64). Together, these data indicate that different TLRs confer IFN- α responsiveness in both cell type-dependent and ligand-dependent manners.

Role of Endocytic Receptors and Endocytosis in Virally-Induced IFN- α Production

Mechanisms involved in antigen or pathogen uptake by dendritic cells has been the subject of study over the past several years. Monocyte-derived dendritic cells (MDDCs) are well-known for their endocytic capacity, and demonstrate rapid uptake and concentration of ligands. One receptor that has been implicated in the uptake of ligands by MDDC is the mannose receptor, a C-type lectin (93). This receptor is expressed at high levels on MDDC but not monocytes or peripheral blood CD11c⁺ MDCs. Studies from our laboratory have also demonstrated that it is expressed at low levels on NIPCs as well, with HSV-stimulated PDCs that express intracellular IFN- α also expressing low levels of the mannose receptor (72). In addition, polyclonal antibody to the mannose receptor or ligands for the mannose receptor such as mannan as well as certain monosaccharides blocked IFN production by NIPCs in response to HSV or HIV-1 but not by monocytes to Sendai virus or HIV-1 (72). While other investigators have reported that PDCs do not express the mannose receptor, we have further confirmed its expression on PDCs purified to 99%. Similarly, PDCs have been reported to express low levels of another mannose receptor family member, DC-SIGN, in contrast to high expression of this molecule by MDDCs (104). In addition to the MR and DC-SIGN, immature PDCs also express BDCA-2, another C-type lectin in the mannose receptor family and which has demonstrated endocytic activity (28). BDCA-2 expression is profoundly

down-regulated as the PDC mature to antigen presenting cells, concomitant with down-regulation of their ability to produce IFN- α . The relative roles of these different C-type lectins in virally induced IFN induction has not been fully elucidated.

Glycosylation of envelope proteins is clearly involved in induction of IFN- α in PDCs: Charley and colleagues (20,21,68) demonstrated that mutation of the single glycosylation site on the major surface glycoprotein on transmissible gastroenteritis virus, a swine pathogen, rendered the virus non-interferogenic in swine cells. Such glycosylation could be important in interaction of the envelope proteins with cell-surface receptors on the PDC such as the C-type lectins. Further evidence for a role for glycoproteins is the observation that blocking of gD from HSV inhibits its ability to induce IFN- α (69) and recombinant gD from HSV and gp120 from HIV are able to induce IFN production (2,3).

The role of endocytosis in induction of IFN- α in PDCs is unclear. While PDCs are clearly not as highly endocytic as MDCs, they are capable of taking up FITC-dextran in a time and temperature-dependent manner (Olshalsky et al, in preparation). Moreover, IFN- α induction in PDCs but not monocytes is sensitive to inhibitors of endocytosis or endosomal acidification such as chloroquine and bafilomycin A (36,69). Many viruses require endosomal acidification to activate fusion proteins that allow escape of the virion from the endosome into the cytoplasm. Whether this is happening in the viral induction of IFN- α production by PDCs is unknown but is currently under investigation. Indeed, it has not been clearly established whether the live or UV-inactivated virus needs to be taken up by the PDCs or whether binding to and signaling through a cell-surface receptor is sufficient. However, it is clear that unlike the case with viral stimulation of monocyte IFN by Sendai virus, where virus must be alive, UV-inactivated enveloped viruses are competent to induce IFN production in PDC. Additionally, the potential role of TLRs in induction of IFN in response to viruses or viral envelope glycoproteins has yet to be addressed.

In contrast to the enveloped viruses, non-enveloped viruses such as adenovirus, polio virus, and simian virus 40 (SV40) are poor inducers of IFN in PDCs (36,79). However, when polio virus is mixed with immune serum, these immune complexes become far more interferogenic than the virus by itself, with PDC being the responding population (79). It is not known how these virus/immunoglobulin complexes induce IFN, but it is possible that the immune complexes allow the virus to be delivered to the endosomal compartments of the PDCs, possibly via interaction with Fc receptors such as CD32 on the PDCs (79).

Regulation of Type I IFN Production by NIPCs/PDCs

IRF expression in PDCs. Induction of the type I IFN gene expression is transcriptionally regulated, with the interferon regulatory factor (IRF) family playing important roles in the induction of both IFNA and IFNB gene expression in infected cells (74,111). The 9 cellular IRFs share an amino-terminus homology encompassing a highly conserved DNA binding domain, but have diverse carboxyl-termini, thus contributing to the differential activities of each individual IRF. Several of the IRFs have been implicated in the regulation of type I IFN gene expression, and expression of some of the IRFs, notably IRF-4 and IRF-8, are restricted to lymphoid or myeloid cells (84). Three other

IRFs (3, 5, and 7) have been shown to have critical roles in the expression of type I IFN genes (7,8,97). IRF-3 is sufficient for the induction of IFN- β in infected cells, but IRF-5 or 7 is required for stimulation of IFNA gene expression, with different IFNA genes induced by each of these IRFs.

While IRF-3 is constitutively expressed in most cells, IRF-7 expression is detected primarily in cells of the lymphoid system and its induction is stimulated by the expression of early IFN- α production, with subsequent activation of a broader range of IFN subtypes (96). In collaboration with the laboratory of Dr. Paula Pitha, we have examined both the range of IRFs expressed in DC subsets and monocytes as well as the range of IFNA genes expressed in the different cell populations upon viral stimulation (Barnes et al., submitted for publication). We observed that while the other cell populations express many of the IRFs, the PDCs expressed all of the IRFs constitutively, with high levels of expression of IRF-4 and IRF-7, the latter of which was confirmed both at the message and the protein. We hypothesize that the high constitutive levels of these regulatory factors by PDC accounts, at least in part, for their extraordinary ability to produce IFN- α rapidly and at high levels without the autocrine IFN feedback required for efficient IFN- α expression in other cell types.

In a murine study, Barchet et al. demonstrated that regulation of IFN- α production by vesicular stomatitis virus (VSV) infected mouse embryo fibroblasts was dependent upon feedback regulation of IFN- α through the type I IFN receptor (IFNAR) (6). In contrast, the murine PDC counterpart produced high levels of IFN- α in response to VSV whether they came from IFNAR +/- or IFNAR knockout mice. Thus, as in the human, the potent IFN-producing capacity of PDCs may result from unique transcriptional regulation.

IFN subtypes expressed by NIPCs. It is interesting that there are a large number of type I IFN genes maintained within the human genome, with the 13 highly related IFNA genes and the single IFNB gene all clustered on human chromosome 9p22. Although these type I IFNs all signal through a common receptor, the IFN- α / β receptor, different biological activities have been attributed to different IFN- α species (112). For example, IFN- α 8 has the most potent antiviral activity and antitumor activity while IFN- α 1 is a weak antiviral agent (45). Further, while many subtypes stimulate NK cell activity, IFN α 7 was found to have little effect on NK activity (77). In addition to their expression of a unique pattern of IRFs, we also investigated the expression of IFNA genes in subpopulations of virus-stimulated DCs and monocytes (Barnes et al., submitted for publication). We observed that whereas monocytes and MDDCs expressed a range of IFNA genes, IFNA1 was by far the predominant gene expressed. In contrast, the PDCs expressed a wider range of subtypes of IFN, with a strong, but less dominant expression of IFNA1. Thus, it is likely that the IFNs produced by the PDCs are qualitatively as well as quantitatively distinct from the IFNs produced by other IFN-producing cells. It remains to be determined how differential expression of IFN- α genes by the PDCs affects their functional activity.

Regulation of IFN- α production. Induction of IFNA in PDCs appears to be under tight regulation. In vitro, the optimal viral conditions for stimulation of IFN production are very tight, with too little or too much virus resulting in suboptimal

generation of IFN measured either as total IFN produced or as frequency of NIPCs as measured by ELISpot (36). In addition, production of IFN by PDCs is highly dependent upon the maturational state of the PDCs; as PDCs mature, they acquire the ability to present antigen but lose the ability to produce IFN- α . Additionally, several cytokines are known to positively or negatively affect the expression of IFN- α in PDCs. IFN pretreatment of PDCs results in both the up-regulation of subsequent IFN production by the cells, with an increase in the frequency of responding cells as well as more IFN produced on a per cell basis. At least some of this “priming” effect by IFN- α may be explained by positive feedback regulation of type I IFN genes through up-regulation of IRF-7 (95) or other transcription factors. However, although the PDCs can be primed for up-regulation of IFN production by IFN- α exposure, murine studies using IFNAR knockouts indicate that this is not required for high levels of virus-induced IFN production (6).

A negative regulator of IFN- α expression by PDCs is IL-10. We observed that exogenous IL-10 is able to inhibit the expression of HSV or Sendai-induced IFN- α in PDCs and monocytes (82). Moreover, neutralization of endogenous IL-10 by the inclusion of neutralizing antibodies in the virus-stimulated cultures led to an up-regulation of the virally induced expression of IFN- α at both the message and protein levels in PDCs. Interestingly, while IL-4 was able to inhibit the Sendai-induced monocyte-derived expression of IFN- α , it had no effect on HSV-induced IFN production in PDCs (82). The mechanism of IL-10 inhibition of IFN- α expression is not known, but IL-10 is known to induce apoptosis of some DC populations. The cellular source of the IL-10 is not the PDC itself, but rather is virally stimulated monocytes, thus suggesting a paracrine regulation of the IFN α expression.

Ligation of BDCA-2, a type II C-type lectin on PDCs has been found to result in tyrosine phosphorylation of cellular substrates and down-regulation of the ability of these cells to produce virally stimulated IFN- α (28). Whether such a phenomenon occurs naturally and may account for the decreased ability of the PDC to produce IFN- α upon challenge with high titers of virus (36) remains to be determined.

OTHER PRODUCTS OF PDCs

Although characterized by their ability to produce large quantities of type I IFNs, the PDCs also express other cytokines as well as chemokines upon stimulation. For example, PDCs produce both TNF- α and IL-6 upon viral stimulation (47). Whether PDCs can produce IL-12 has been controversial. However, in recent studies, murine plasmacytoid IPCs have been shown to secrete IL-12 during murine cytomegalovirus infection, and IL-12 is released by human PDCs in response to CD40L and CpG (65,66).

In collaboration with Dr. Howard Young, we have observed that PDCs produce chemokines including MIP-1 α and IP-10 as well as TNF- α in response to stimulation with HSV (Megjorac, submitted for publication). While the populations of chemokine- and IFN-producing cells were overlapping among the PDCs, with some cells producing more than one cytokine or chemokine, the populations were not identical, raising the pos-

sibility of subsets of PDCs with differential responses. A functional role for the chemokines expression by PDCs was suggested by our observation that supernatants from the HSV-stimulated PDCs attracted NK cells as well as activated T cells in a transwell system. This production of chemokines by PDCs suggests that they might contribute further to immune responses by attracting cells involved in cell-mediated response to virus. PDC have also been shown to express a number of chemokine receptors (83,94), thus indicating that they both produce and respond to chemokines during the developing immune response.

FUNCTIONS OF NIPCs: LINKING OF INNATE AND ADAPTIVE IMMUNITY

NIPCs/PDCs are the most potent producers of IFN- α and, as such, have been proposed to be “professional IFN-producing cells.” However, IFN-producing ability is a relatively transient property of these cells; once they begin to mature, they no longer retain the capacity to produce IFN but rather become antigen presenting cells, which, depending on the nature of the maturation stimulus received by the cell, induce either Th1 or Th2 responses, or in some instances, regulatory T cells (17,51, 62,70,90). IFN- α is a pleiotropic cytokine, having not only classical antiviral activity, but also growth inhibitory activity, anti-apoptotic activity and it modulates a variety of immune responses, including up-regulating of cell-mediated immunity. In humans, IFN- α is an effective inducer of Th1 responses (13). Thus, IFN- α production by NIPCs in response to virus would be expected to not only induce an antiviral effect in cells in the vicinity of the NIPCs, but also affect the developing immune response. Thus, like MDCs, NIPCs/PDCs are ideally suited for linking innate and adaptive immunity.

Survival and Maturation of NIPCs/PDCs

A property of NIPCs/PDCs in both humans and mice is their extreme sensitivity to culture, with the cells rapidly undergoing apoptosis and/or down-regulating their ability to produce IFN- α . Survival of human NIPCs in culture can be enhanced by IL-3, and IFN- α is itself a survival factor for these cells, as are imiquimod and CpG (58,59,62,64).

Induction of Th1 Versus Th2 Responses by NIPCs/PDCs

Depending upon the stimulus received, PDCs have been demonstrated to induce either a Th1 or Th2 type of response. Virus stimulation of PDCs not only induces large quantities of IFN- α production by these cells, but also induces them to differentiate into mature DCs that stimulate naive T cells to produce IFN- γ and IL-10 (17,62). In contrast, IL-3 and CD40 ligand induced PDCs have been shown to induce T cells to produce the Th2 cytokines IL-4, IL-5, and IL-10 (53,89). As described above, whether the PDC themselves produce IL-12 in response to virus stimulation has been controversial; however, data presented by Dalod et al. demonstrate that type I IFN production by PDCs controls the production of IL-12 by myeloid DCs (26). The expression of C-type lectins on PDCs such as BDCA-2 and, at lower levels, the mannose receptor, suggest that

the cells may function as antigen presenting cells as well as IPCs (27,28,72). In fact, as PDCs mature beyond the stage where they produce IFN- α , they down-regulate their expression of BDCA-2 and up-regulate costimulatory molecules and CD83, markers typical of antigen presenting myeloid dendritic cells.

Tolerance Induction by PDCs

The presence of PDCs in the thymus has led to the suggestion that this DC subset is involved in the induction of central tolerance. Further, studies by Kuwana et al. have demonstrated that PDCs isolated from human peripheral blood have a capacity to induce anergy in tetanus toxoid specific CD4⁺ cell lines. These anergic T cells failed to proliferate in secondary cultures following primary culture with tetanus toxoid-pulsed PDCs and lost the capacity to produce IL-2, but produced IFN- γ and IL-10 upon antigen stimulation. The authors concluded that PDCs are involved in maintenance of peripheral T tolerance *in vivo* (67).

Further evidence of a regulatory role for PDCs comes from the study of Gilliet and Liu who demonstrated that CD40L-activated PDCs induced naive CD8⁺ T cells to differentiate into CD8 T cells with poor secondary proliferative and cytolytic responses and which expressed high levels of IL-10 but not IFN- γ (51). These data support a role for PDCs in immunological tolerance through the induction of CD8⁺ regulatory cells.

Interaction of PDCs with Other Cell Types

In addition to their production of IFN- α and other cytokines and chemokines and their ability to interact with CD4 and CD8 T cells, PDCs have also been demonstrated to interact, either directly or indirectly, with other cell types.

NK cell interaction with PDCs. Some of the earliest observations of the functions of NIPCs were their participation in the lysis of HSV-infected fibroblasts by human NK cells (5,43). The observation was made that highly purified NK cells or NK cells depleted of HLA-DR⁺ cells could not lyse the virally infected targets. This lysis was restored, however, by adding back the HLA-DR⁺ population. The HLA-DR⁺ "accessory cells" were found to be identical to the IFN- α producing NIPCs, and cytokines with known ability to activate NK cells such as IFN- α , IL-2, and IL-12 could substitute for the requirement for accessory cells. However, the production of IFN by NIPCs was only partially responsible for the activation of the NK cells; rather, some cell-contact dependent mechanisms were also required for the optimal induction of lysis, and cell mixtures containing the NK cells, target cells, and PDCs were found to cluster, thus facilitating the interactions of these populations (34). Most recently, we have observed that supernatants from HSV-stimulated PDCs produce chemokines that can selectively recruit both NK cells and activated T cells (Megjugorac et al., submitted for publication).

DC:DC interactions. As well as interacting with effector cells of adaptive immunity and NK cells, IFN- α production by PDCs has been demonstrated in a murine system to regulate the production of IL-12 by MDCs, perhaps preventing overexpression of the IL-12 (26). We have observed that MDCs and PDCs may directly communicate, with virus-pulsed MDDCs being able to transmit an interferogenic signal to PDCs (Izaguirre et al., in preparation). This communication might allow for cap-

ture of virus at sites of pathogen entrance (e.g., mucosal surfaces) by MDC, with subsequent signaling of the PDC, perhaps within the draining lymph node.

FUNCTION OF NIPCs IN HUMAN DISEASE

PDCs in HIV Infection

Evidence for dysfunction of the IFN system in HIV infection stems from studies in the mid-1980s, shortly after the dawn of the AIDS epidemic. We observed that PBMCs from patients with AIDS had a decreased capacity to produce IFN- α in response to HSV and that this deficiency preceded and was predictive of development of opportunistic infections (71,103). The decreased IFN- α was initially detected by measuring bulk IFN in culture supernatants, which was further reflected in a decreased frequency of functional NIPCs within HSV-stimulated PBMCs (55). Further, we calculated that on average, each NIPC produced less IFN- α than cells from healthy donors. This observation of decreased IFN production by NIPCs was in contrast to the response to Sendai virus, which remained relatively intact in the patients until much later in the disease (37,38). However, the lack of a unique phenotype attributed to the NIPCs hampered further definition of the deficiency in that it was not possible to determine whether the functional depletion represented a numerical depletion of the cells from the periphery or the presence of non-responsive cells in the blood. We and others have recently addressed this issue using the newly described phenotype of PDCs. We observed that there is a decrease in the numbers of circulating CD123^{bright}, HLA-DR⁺, lineage⁻ cells in the blood of patients with HIV infection and that the deficiency is most profound in patients with more advanced HIV disease, a finding in agreement with several concurrent reports (22,35, 78,105). In addition to the numerical depletion of PDCs, the patients also demonstrated a decreased frequency of the CD123^{dim}, CD11c⁺ MDC population (35,78). Strikingly, we also observed using intracellular flow cytometry for IFN- α that among the population of PDCs remaining within the peripheral blood of patients with advanced HIV, there was a lower percent of cells capable of responding as compared to healthy controls or patients with less advanced disease (35). There has been some suggestion that within PDCs, there are subsets of cells. It remains to be determined whether there is selective depletion of some PDCs in HIV infection. In functional studies, Siegal et al. have studied reconstitution of the NIPC function (as measured by IFN production) in patients receiving highly active antiretroviral therapy. The NIPC function was found to recover functionally with highly active antiretroviral therapy (HAART) and at a faster rate than CD4⁺ T cells in the peripheral blood (101), although Chehimi et al. have reported that the cells do not numerically return to the full levels despite effective HAART therapy (22). The functional recovery, although perhaps not complete, appears to be protective in the patients (101).

The mechanism(s) accounting for the functional and numerical depletion of NIPCs in HIV infection remains to be determined. These deficiencies might be accounted for by infection of PDCs, leading to their death or dysfunction or possibly to their trafficking to other areas of the body. It is known that iso-

lated PDCs can be productively infected with HIV-1 (81), and that this infection is more efficient than that of MDCs. The plausibility of altered trafficking is supported by the observation that in patients with lupus, there is a peripheral depletion of NIPC function but an accumulation of the PDC in skin lesions (15,31,60). Moreover, in late HIV infection as well as in patients with lupus, there is a substantial level of serum IFN- α , the source of which is unknown but is not the circulating PBMCs. It is possible that infection and or destruction of PDCs as well as altered trafficking might all contribute to the functional and numerical deficiencies that are observed.

PDCs in Cancer

Although there has been considerable interest in the trafficking of DCs to tumors in both human and mouse models, and in the utilization of DCs as APCs for the induction of antitumor immunity, the vast majority of this work has been carried out with MDC models. It is known that MDC migration and function can be inhibited by tumors but that when in vitro derived MDDCs are fed tumor antigens or apoptotic tumor cells, then reinfused in the autologous donors, these cells can sometimes elicit a potent antitumor response. Little is known, however, about the role and functions of PDCs in tumors. Zou et al. isolated tumor-associated PDCs from the malignant ascites of patients with ovarian epithelial carcinomas (113). They observed that the tumor cells express high levels of stromal-derived factor-1 (SDF-1), the ligand for CXCR4, which was shown to lead to the recruitment and activation of the PDCs. The PDCs from the ascites fluid could function as NIPCs in response to viral stimulation and expressed MxA, an IFN- α inducible protein thus indicating exposure to local IFN- α production. Interestingly, MDCs were found only rarely in the ascites fluids that contained high levels of PDC. The SDF-1 was also found to inhibit the apoptosis of PDC induced by tumor macrophage IL-10. However, despite the ingress and survival of PDCs in the tumor environment, these PDCs were found to be inefficient in inducing protective T cell immunity, with their activity dampened by the tumor-induced secretion of IL-10. Further study will be required to determine whether PDC have an important in vivo role in tumor immunity.

The potential usefulness of PDCs in antitumor therapy, and in particular, with respect to their ability to produce IFN- α , which has both anti-proliferative and Th1 inducing effects, remains to be studied.

PDCs in Lupus

Like individuals with chronic HIV infection or acute viral infection, patients with systemic lupus erythematosus (SLE) have increased serum levels of IFN- α that were proposed to have a significant role in the disease process (87). As in HIV infection, patients with SLE have been found to have decreased numbers of functional NIPCs in circulation, and activated (IFN-producing) NIPCs/PDCs have been found in the skin of SLE patients (15,31,60). Moreover, patients with SLE have been shown to have an IFN-inducing factor in their serum that selectively induces IFN production in NIPCs (91). This IFN- α inducing serum factor has been shown to be immune complexes consist-

ing of anti-double-stranded DNA antibodies with DNA derived from apoptotic cells, and involvement of Fc γ RII (CD32) has been implicated in the process. The immune activation by the PDC-derived IFN- α is hypothesized to contribute to the immune activation leading to autoimmunity in SLE, with, for example IFN- α involved in the maturation of antigen-presenting MDC (11). A role for IFN- α in autoimmunity is further supported by the observations of increased autoantibodies and autoimmune syndromes in patients receiving long-term IFN- α therapy and overexpression of type I IFN in insulin dependent diabetes mellitus and a variety of other Th1-mediated autoimmune diseases (56).

PDCs in Allergy

As described above, depending on the maturational stimulus received by PDCs, they can mature into cells capable of stimulating either Th1 or Th2 responses. A potential role for PDCs in nasal allergies was suggested by Jahnsen et al. who observed that PDCs were infrequent in normal nasal mucosa but were increased dramatically during experimentally induced nasal allergy (60). The recruitment of PDCs was hypothesized to occur via L selectin (CD62L) binding to peripheral lymph node addressin expressed on the nasal vascular endothelium.

PDCs in Other Diseases

Although little is known about the presence and role of DCs in the human central nervous system, Pashenkov et al. have recently reported the presence of both PDCs and MDCs in the cerebral spinal fluids in patients with inflammatory and non-inflammatory neurological diseases (80). PDCs were found to be elevated in all neuroinflammatory conditions studied, with especially high numbers found in the cerebral spinal fluid (CSF) in neuroborreliosis. The presence of PDCs in the CSF and the capacity of these cells to produce IFN- α in response to virus suggests both their potential role in inducing protective Th1 immunity as well as the possibility of their induction of autoimmunity. Interestingly, among the diseases with increased PDCs in the CSF was multiple sclerosis, another disease in which serum IFN is observed and where IPCs may contribute to the pathology of the disease.

SUMMARY AND CONCLUSIONS

NIPCs have had a long history of study, with the first observations of peripheral blood cells with IFN-producing capacity being made approximately 20 years ago. Over the past three years with the identification of NIPCs as plasmacytoid dendritic cells, an enormous amount has been learned about their function in both innate and adaptive immunity and their dysregulation in certain viral infections such as HIV infection and in autoimmunity. Further studies of their mechanisms of IFN- α induction, of their distribution and in vivo function are required. It is anticipated that a further understanding of the PDC will lead to the ability to specifically manipulate these cells in human disease.

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