Commentary

Will the Making of Plasmacytoid Dendritic Cells In Vitro Help Unravel Their Mysteries?

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In this issue, two papers describe the in vitro generation of an intriguing subset of human dendritic cells (DCs), the plasmacytoid DCs (pDCs [1, 2]). For nearly 20 years after their discovery (3), DCs had to be painstakingly isolated from tissues (4). Progress was rather slow, as DCs represent a minor cell population in all tissues, and relatively few investigators appreciated their importance in antigen presentation and the control of immunity. In 1992, in vitro culture systems were identified to generate large numbers of mouse (5) and human DCs (6). These culture systems considerably accelerated the study of DCs, with many groups joining the search to unravel their mysteries (7–9). Currently, DCs are being developed in vitro from (a) bone marrow progenitors cultured in GM-CSF, with TNF being essential for human cells (6), and (b) human blood monocytes cultured with GM-CSF and IL-4 (10–12). Recently, Flt3 ligand (Flt3-L), a stromal cell product, was found to induce a massive expansion of DCs in vivo in mice (13, 14) and in humans (15, 16).

Subsets of DCs. The concept of distinct DC subsets in humans came from several routes including analyses of (a) skin DCs (17), (b) DCs generated in vitro by culture of $CD34^+$ hematopoietic progenitors (HPCs [18]), and (c) blood DC precursors (19). Human skin contains two distinct subsets: Langerhans cells (LCs) within the epidermis, characterized by the expression of CD1a and Birbeck granules, and interstitial (dermal) DCs, lacking Birbeck granules but expressing coagulation factor XIIIa. These two subsets also emerge in cultures of $CD34^+$ HPCs driven by the addition of GM-CSF plus TNF-α (18). These subsets have common as well as unique functions (20). In particular, interstitial DCs, but not LCs, are able to induce the differentiation of naive B cells into immunoglobulin-secreting plasma cells. Although no unique function has yet been formally attributed to LCs, there are hints they may be particularly efficient activators of cytotoxic CD8 T cells (21, 22). Two subsets of DCs were identified in the blood, each representing a small fraction (~0.3%) of the mononuclear cells (19). One subset, $CD11c^+$ population, differentiated into mature DCs in response to inflammatory stimuli, whereas a second $CD11c^-$ subset was prone to prompt apoptosis in culture, and was later identified as "pDCs."

pDCs and "DC2." Plasmacytoid cells were first observed by experts in the histopathology of lymphoid organs (23–26) who saw cells within the T cell areas that looked like plasma cells. Because plasma cells are normally localized outside the standard B cell follicles and T cell areas, it was concluded that these "plasmacytoid" cells were different. Indeed, due to the expression of CD68 (27) and CD4, these cells were sometimes called either plasmacytoid monocytes or plasmacytoid T cells. Their isolation from tonsils by Grouard et al. revealed a unique phenotype (28). Transmission electron microscopy showed an abundant rough endoplasmic reticulum and an eccentric nucleus, quite similar to plasma cells. Flow cytometry demonstrated the expression of IL-3 receptor, conferring responsiveness to IL-3 but sparse expression of GM-CSF receptor, consistent with the lack of responsiveness to GM-CSF (29). In addition, many other myeloid markers (such as CD13, CD14, and CD33) were missing. pDCs can be further distinguished from $CD11c^+$ DCs by differential expression of immunoglobulin-like transcripts (ILTs), with pDCs being $ILT1^-ILT3^+$ and $CD11c^+$ DCs being $ILT1^+ILT3^+$ (30). The latter report emphasized that pDCs home to the T cell areas of lymphoid tissues, e.g., via L-selectin. At the International Workshop on DCs held in Pittsburgh in September 1998, Liu and co-workers presented two novel exciting properties of these cells: their ability to produce large amounts of IFN-α and their ability to polarize a fraction of T cells towards IL-4 and IL-5 production (type 2 cells [31]).

This rare cell type has been given many names, including pDCs, $CD11c^-$ DCs, DC2, and IFN-producing cells (IPCs). For the sake of simplicity, and by reference to the terminology used in the two papers from the current issue, we shall call these cells "DC2." It is anticipated that two recent findings will accelerate research on human DC2. First, systemic administration of both Flt3-L and G-CSF increase the number of DC2 in blood (15, 16, 32), and second, the new studies presented in this issue provide culture systems to generate DC2 in vitro. The group of Blom et al.

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(1) demonstrates the existence of $CD34^{low}$ cells with the phenotype and function of DC2, as well as a simple culture system in which early $CD34^+$ progenitors yield DC2 (1). The group of Spits et al. (2) provides another culture system in which early $CD34^+$ progenitors yield DC2, and demonstrates that their differentiation can be blocked by overexpression of inhibitor of DNA binding (Id)2 and Id3 proteins (2).

$CD34^+$ Cells with Features of DC2. Several features permit the identification of DC2s: the expression of CD4, CD45RA, and CD123, the production of high levels of IFN-α upon viral triggering or CD40 ligation (30, 33, 34), and the morphology of plasma cells. Blom et al. analyzed hematopoietic tissues and looked for the presence of $CD34^+$ cells with the features of DC2 (1). Accordingly, a minor subset of $CD34^{low}CD45RA^+CD4^+CD123^+$ cells was identified in cord blood, adult blood, fetal liver, and most abundantly, fetal bone marrow. These cells produce large amounts of IFN-α in response to viruses and can differentiate into mature DCs in response to IL-3 and CD40-L.

In Vitro Generation of DC2 from Early $CD34^+$ Hematopoietic Progenitors. Culturing early HPCs ($CD34^+$ $CD45RA^-$) with Flt3-L gives rise to cells (a) able to secrete IFN-α upon viral exposure and (b) displaying the typical DC2 phenotype ($CD4^+CD123^+CD45RA^+$). The generation of DC2 under these conditions is slow, as they are first observed after 10 d and reach a maximal frequency (~10%) after 3 wk of culture. The identification of Flt3-L as the DC2 hematopoietic differentiation factor does not come as a surprise inasmuch as Flt3-L increases circulating DC2. More surprising is the fact that G-CSF, which also increases DC2 in vivo, does not permit the generation of these cells in vitro. Thus, G-CSF acts in vivo as a mobilizing agent for DC2, as it does for $CD34^+$ HPCs (35).

Using a different approach, Spits et al. were able to generate DC2 by culturing early uncommitted $CD34^+CD38^-$ fetal liver HPCs on the murine stromal cell line S17 (2). This cell line is unusual in that it supports the development of human B cell and myeloid progenitors (36–38). At variance with the above system, it takes only a few days to see the emergence of DC2, but then the cells quickly disappear. The newly generated cells express pre-TCR-α transcripts, unlike the starting $CD34^+$ HPCs. They also display phenotypic and functional features of DC2: they secrete IFN-α upon viral challenge and differentiate into mature DCs after exposure to IL-3 and CD40-L. Cells with the same properties can be obtained from thymic $CD34^+$ progenitors, but from the $CD34^+CD1a^-$ subset only. Thus, the cells generated in Spit et al. culture system (2) appear similar to those obtained in Blom et al. (1) system.

Because the kinetics of DC2 growth and survival are so different in the two culture systems, we surmise that the S17 system may reveal a DC2 growth factor different from Flt3-L.

Arguments for the Lymphoid Origin of DC2. The term "lymphoid DC" was coined by Shortman and colleagues to describe a mouse DC subset in the thymus that developed from a population of thymic lymphoid progenitor cells in vivo (39). In these studies, transfer of purified thymic lymphoid precursors into irradiated hosts resulted in the development of T cells, B cells, NK cells, and DCs expressing $CD8\alpha^+$, but not cells of the myeloid lineage (39, 40). These were striking findings because previous systems for developing DCs also led to the production of macrophages and granulocytes. Based on these observations, CD8α was postulated to be a characteristic marker for lymphoid DCs in mice. Particularly intriguing was the postulate that lymphoid DCs play a role in the induction of central tolerance (41, 42). $CD8\alpha^+$ DCs with a similar phenotype to thymic DCs were also observed in the spleens, lymph nodes, and Peyer's patch, and these DCs have been presumed to be of lymphoid origin as well (43). In this context, the role of DCs in the establishment and maintenance of peripheral tolerance has recently been discussed (44, 45).

It should be noted that there is no clonal analysis to support the claim that $CD8\alpha^+$ DCs and lymphoid cells arise from the same precursor cell. Furthermore, recent studies suggest that CD8α can also be expressed on activated DCs and LCs (46, 47), a cell type that has hitherto been considered to be of myeloid origin. Therefore, at present the lineage origins of DC subsets in mice are a matter of considerable debate.

The concept of a lymphoid DC subset in mice sparked great interest in the search for lymphoid DCs in humans. Unlike in mice, human thymic DCs do not express CD8, but do express CD4 (48), rendering the phenotypic discrimination between human thymic DCs and myeloid DCs difficult. However, the identification of DC2 and some of their features has offered new clues. Despite their lack of expression of CD11c and CD8a (two markers expressed by murine lymphoid DCs), several features of human DC2 are consistent with a lymphoid origin: (a) DC2 lack expression of the myeloid antigens CD11c, CD13, and CD33; (b) DC2 isolated from the thymus, express the lymphoid markers CD2, CD5, and CD7; (c) DC2 do not differentiate into macrophages after culture with GM-CSF and M-CSF; and (d) the thymic DC precursors as well as the tonsillar DC2 expressed significant levels of pre-TCR-α transcripts (49). Spits et al. now provide another element to this claim using the S17 system described above (2). Transduction of early CD34 progenitors with Id2 and Id3 (proteins that prevent the binding of basic helix-loop-helix transcription factors to DNA) inhibits their differentiation into DC2 as well as T cells. In contrast, generation of $CD4^+CD14^+$ cells, as well as the development of DCs in a standard culture system of $CD34^+$ HPCs with GM-CSF and TNF, are not affected. Furthermore, Id3 inhibits the development of primitive HPCs into T cells and B cells while stimulating that of NK cells (50). These observations therefore support the notion of shared molecular cues for the development of DC2 precursors, T and B cells (Fig. 1).

DCs: Regulators of Innate and Adaptive Immunity. Although DCs have long been recognized to initiate the immune response, their roles in regulating both the innate and adaptive arms of immunity are beginning to be elucidated. DCs have a role in innate immunity in that they

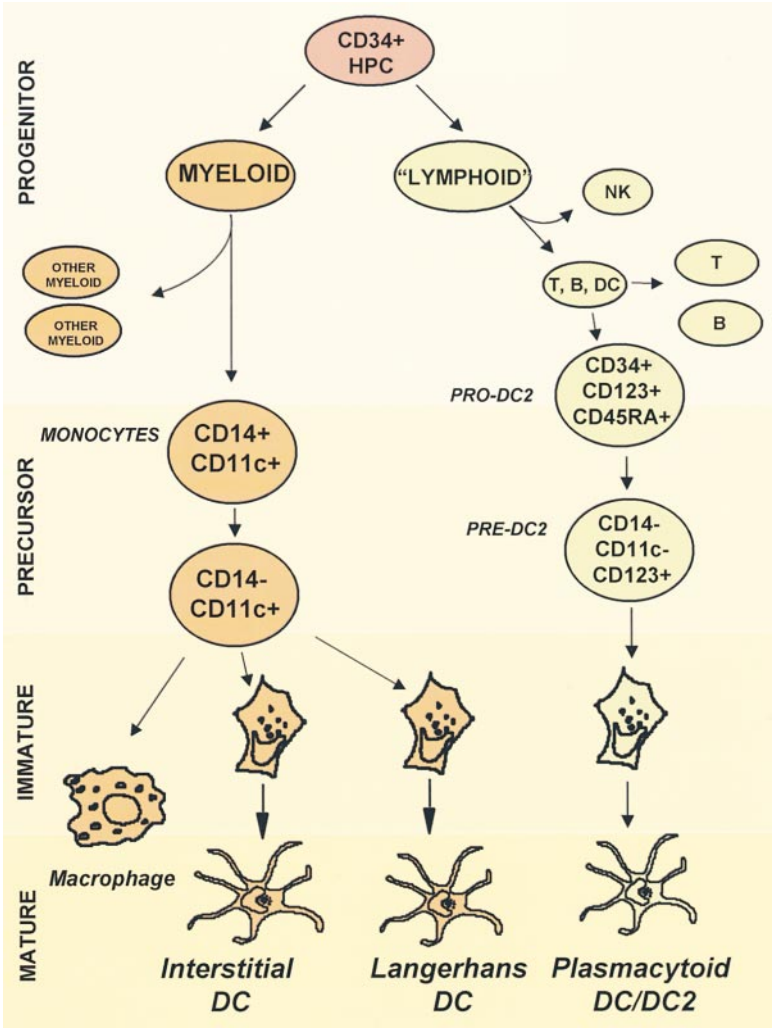

Figure 1. A summary of potential differentiation pathways of human DCs. $CD34^+$ myeloid progenitors give rise to monocytes, a large reservoir of antigen presenting cell precursors that yield circulating $CD11c^+$ precursors. $CD11c^+$ cells can differentiate into either macrophages or interstitial DCs or LCs depending on the cytokine microenvironment (reference 64). A distinct precursor population may give rise to plasmacytoid DCs. In some circumstances, this population also contains cells that can yield lymphocytes, hence the current term "lymphoid." $CD34^+$ cells contain a subset with the phenotypic and functional characteristics of DC2, pro-DC2, with a limited proliferative capacity. Thus, human blood contains two DC precursors, $CD11c^+$ (myeloid) and $CD11c^-$ (potentially lymphoid). Two main questions need to be resolved regarding these putative lymphoid DCs. There is no clonal system to produce plasmacytoid DCs and T cells or B cells. The classical immature (antigen capturing) and mature (antigen presenting) DC differentiation stages have not yet been characterized, in contrast to myeloid DCs derived from skin, blood, and bone marrow in culture.

have nonclonotypic receptors that permit a prompt response to microbial antigens, leading to production of cytokines that mobilize NK cells and have antimicrobial activity. For example, human monocyte–derived DCs certainly make abundant amounts of IL-12 (51, 52), whereas DC2 make IFN-α (34, 53).

DCs also have a powerful role in regulating the adaptive immune responses, as distinct DC subsets induce different Th responses. In mice, there is a consensus that $CD8\alpha^+$ (putative lymphoid) DCs prime Th1 responses, whereas $CD8\alpha^-$ (putative myeloid) DCs prime Th2 responses (54, 55). In humans, the picture is less clear. Monocyte-derived DCs activated with CD40-L have been shown to prime Th1 responses through an IL-12–dependent mechanism, whereas $IL\text{-}3^+$ CD40-L–activated DC2s have been shown to secrete negligible amounts of IL-12 and can prime Th2 responses. However, later it was observed that the polarizing effects of DC subsets was not immutable, as different environmental cues could instruct a given DC subset to elicit different Th responses. Thus, monocyte-derived DCs can induce T cells to make IL-4 if (a) the DCs are used at low numbers (56), (b) after exposure to factors such as prostaglandin E2 (PGE2), corticosteroids, or IL-10, or (c) upon prolonged activation with CD40-L (>3 d in vitro culture; references 57–60). Furthermore, DC2s, when stimulated by virus (rather than $IL\text{-}3^+$ CD40-L), secrete IFN-α that drives Th1 responses in humans (61, 62), and mature into DCs that can induce T cells to produce IFN-γ and IL-10 (34, 53). Thus, both the type of DC subset as well as microenvironmental cues are important for Th polarization. Another recently proposed parameter is the stage (early or late) of DCs activation. According to this "DC exhaustion model," monocyte-derived DCs produce IL-12 shortly after stimulation with lipopolysaccharide, thereby inducing Th1 differentiation. After the burst of IL-12 production, DCs no longer polarize T cells into the Th1 pathway but into the Th2 pathway (9, 63).

To summarize the current literature, there are three models through which DCs may control T cell polarization: (a) subset of DCs; (b) the type of DC maturation or activation signals; and (c) the duration of DC activation. It would not surprise us if each operates under different circumstances of the immune response.

Conclusions. DC2 have several striking features: their distinctive morphology and surface markers, their localization in the T cell areas in vivo, their responsiveness to IL-3

and not to GM-CSF, their capacity to induce T cells to make either IFN-γ or IL-4 under different conditions, and their prodigious production of IFN-α upon challenge with many enveloped viruses. Two papers in this issue provide methodology to make these cells in vitro. This will help investigators learn more about their biology and to exploit them for therapeutic purposes. The demonstration of the critical role for Flt3-L and stromal cells in the generation of these cells, and their unique ability to secrete large amounts of IFN-α may permit the identification of the mouse equivalent. This should lead to identification of mouse models for preclinical studies aimed at establishing DC2 as targets and vectors for therapy.

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