Commentary

New Insights into The Mobilization and Phagocytic Activity of Dendritic Cells

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A study published in this issue of *The Journal of Experimental Medicine*, sheds new light on the behavior and functions of dendritic cells (DC) which play a pivotal role in initiation of T and T-dependent immune responses (1). The study is important in at least three respects: first, it provides information on recruitment of DC progenitors to a non-lymphoid tissue, the liver; second, it provides evidence that DC have transient phagocytic activity for particulates in vivo, a prerequisite for induction of immune responses against bacteria for example; and third, it defines a migration pathway for DC, that have acquired particulates from the blood, from the liver to draining nodes.

To put this paper (1) into context it is necessary to summarize the general consensus of understanding about the DC lineage that has emerged over the past decade or so. DC progenitors that originate from the bone marrow of adult mammals enter the blood and seed non-lymphoid tissues, where they develop into a stage of DC (sometimes referred to as immature DC) with optimal capabilities for antigen uptake and processing, MHC production, and the formation of foreign peptide-MHC complexes ("processing" DC). These cells are localized in epithelia, such as skin epidermis, gut, genito-urinary tract, and the lung and airways, and in the interstitial spaces of many solid organs such as heart and kidney. Inflammatory mediators (cytokines and other agents) then promote their maturation and migration out of non-lymphoid tissues into blood and/or afferent lymph. These migratory cells enter secondary lymphoid tissues where they express newly acquired capabilities for the initiation of primary T and T-dependent immune responses, at least in part due to their expression of costimulatory molecules and synthesis of certain cytokines such as IL-12 ("presenting" DC, sometimes referred to as mature DC). Migratory DC, travelling between non-lymphoid and secondary lymphoid tissues, are generally considered to be undergoing "maturation" from the processing to presenting stages, although maturation is likely to be initiated within non-lymphoid tissues and to continue within secondary lymphoid tissues.

At the outset, it is therefore convenient to define four stages of the DC lineage in distinct anatomical compartments: DC progenitors in bone marrow and blood; immature DC at the processing stage in peripheral non-lymphoid organs; migratory DC in the process of maturation in afferent lymph and blood; and mature DC at the presenting stage in secondary lymphoid tissues (2–4). Arguably this view is an over-simplification. For example, DC progenitors may enter secondary lymphoid tissues directly; some DC in non-lymphoid tissues may possess a degree of costimulatory activity; some DC in lymphoid tissues may be capable of antigen uptake and processing to some extent; and DC in thymus may play a role in the induction of T cell tolerance to self peptide-MHC complexes. Nevertheless, it provides a useful framework for understanding how, where, and when DC regulate the induction of primary immune responses. It is also important to add that once antigen-specific T cells have been activated by DC, the activated T cells appear not to require the specialized costimulatory signals that are delivered by DC, and may respond to other types of antigen-presenting cell expressing the peptide-MHC complexes for which they are specific (2, 3).

DC Progenitors. A major advance over the past few years has been the ability to grow DC from bone marrow and blood progenitors and to control production of different stages of the lineage in vitro. For example, development of immature human DC is promoted by culture of bone marrow or blood progenitors in the presence of GM-CSF and IL-4 (5, 6), and further maturation can be induced by subsequent exposure to TNF-α or CD40 ligand, or to other agents such as bacterial lipopolysaccharide (LPS) (7). Inclusion of stem cell factor (*c-kit* ligand) increases cell yields and permits discrete colonies of DC to be grown in semi-solid culture systems (8). Nevertheless the identification of bona fide DC progenitors remains difficult: DC may share a committed stem cell with monocytes and neutrophils (9); or with T cells, B cells and NK cells (10); or indeed they may differentiate from monocytes. However, it is possible to generate mouse DC from presumptive liver progenitors cultured in the presence of GM-CSF, further maturation being promoted by the presence of type-1 collagen in vitro (11); these progenitors may be capable of seeding secondary lymphoid tissues, and possibly some non-lymphoid tissues, following liver allografting in vivo (12).

What stimuli recruit DC progenitors into the tissues? Regarding epithelia, there is evidence that intradermal administration of GM-CSF leads to an increase in numbers of DC within the dermis of human skin, which may be the precursors to epidermal Langerhans cells (LC, the DC of skin epidermis) (13). In addition, GM-CSF produced by normal and inflamed tissues, and some carcinomas, within

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human lung appears to recruit CD1a+ LC to local sites (14). Additional information comes from studies showing that DC are recruited into the epidermis of airways of rats in response to LPS delivered in aerosol form to the lungs, and that the kinetics of the transient influx of DC mirror those of the neutrophil component of the acute inflammatory response (15). Furthermore, DC progenitors are rapidly recruited into the airway epithelium of rats after inhalation of *Moraxella catarrhalis* organisms (which induce an inflammatory tracheitis in humans); remarkably, in this system the initial wave of DC arrives in advance of the neutrophil influx to the tissues (16). In the case of solid organs, systemic administration of LPS to mice promotes the recruitment of DC progenitors into heart and kidney and probably into other non-lymphoid tissues, presumably due to the cytokines elicited by this agent (17).

The paper of Matsuno et al. (1) now presents data indicating that DC are recruited into the liver (and possibly spleen) in response to a bolus of particulates delivered intravenously. However, in contrast to the agents studied above (cytokines, LPS, and bacteria; 13–17), the particles used in the present study (1; carbon, latex microspheres) are immunologically inert (although colloidal carbon fixes C3). One interpretation is that DC are recruited to the liver in response to cytokines synthesized by Kupffer cells that have phagocytosed particles. If so, this adds a further level of complexity to macrophage regulation of DC migration and function since, for example, it has also been shown that macrophages in lung may maintain DC at an immature, processing stage in situ (18).

Non-lymphoid Dendritic Cells. Non-lymphoid DC resident within epithelia and the interstitial spaces of solid organs are ideally placed to perform a "sentinel" function for the immune system, with the capacity to internalize and process foreign antigens that gain access to these sites before migration into secondary lymphoid tissues for the induction of immune responses. Important insights into the properties of non-lymphoid DC came from studies of Langerhans cells (LC), the DC of skin which are normally resident within the epidermis (2). It was shown that freshly-isolated LC develop into cells resembling DC isolated from lymphoid tissues during culture in the presence of GM-CSF, normally produced by keratinocytes within cultures of epidermal cell suspensions. During this process of "maturation," which can be further modulated by TNF-α for example, the cells undergo phenotypic remodelling accompanied by profound changes in function.

Freshly isolated LC express plasma membrane molecules such as Fc receptors, complement receptors, and mannose receptors, some of which appear to target antigens into the cellular antigen processing pathways. The cells are capable of pinocytosis and processing of soluble antigens which are subsequently expressed as peptide-MHC complexes at the cell surface. (Cells grown from human blood progenitors and presumably DC in tissues are also capable of macropinocytosis, which provides an additional route for uptake of soluble molecules; 7, 19.) However, the freshly isolated cells have little capacity to initiate primary in vitro immune responses. During maturation in culture, Fc receptors and probably mannose receptors are downregulated together with the capacity to process soluble native antigens. These events are accompanied by the disappearance of acidic intracellular compartments that are associated with processing of exogenous antigens and a cessation of biosynthesis of invariant chain and MHC class II molecules, which are synthesized at a high rate in freshly isolated LC presumably for loading with antigenic peptides. The maturing cells become capable of triggering T cell activation, in part due to new or increased expression of costimulatory molecules including CD40, CD80 (B7-1) and CD86 (B7-2), the ligands for CD40 ligand, and CD28 / CTLA-4 on T cells (2, 3). But this is only part of the story.

For DC to initiate responses against pathogens such as bacteria and yeasts, it would seem essential for them to have phagocytic activity. And yet the general consensus is that DC in lymphoid tissues are, by and large, non-phagocytic. A resolution to this problem was offered when it was shown that freshly isolated LC can phagocytose a variety of particles, at least in vitro, and that this capacity is lost when the cells mature in culture (20). Particles that can be phagocytosed by freshly isolated mouse LC include latex microspheres (0.5 to 3 micron in diameter), zymosan (probably via mannose receptors), and *Saccharomyces cerevisiae, Staphylococcus aureus,* and *Corynebacterium parvis* organisms. However, there are marked quantitative and qualitative differences between the phagocytic capabilities of freshly isolated LC and macrophages. For example, LC phagocytose relatively few particles whereas macrophages internalize many, and mouse LC fail to ingest antibody- and complement-opsonized erythrocytes whereas these cells are readily phagocytosed by macrophages (20). Presumably, these differences reflect the specialized capacities of these cell types for antigen presentation and initiation of immune responses (DC) versus scavenging and clearance of particulates (macrophages).

LC also appear to phagocytose *Leishmania* organisms and can present *Leishmania* antigens to antigen-specific, primed T cells (21). Further, it was demonstrated that DC cultured from mouse bone marrow in the presence of GM-CSF possess transient phagocytic activity for mycobacteria, and that such antigen-pulsed DC can present mycobacterial antigens to primed T cells (22). Further evidence that DC have phagocytic activity comes from studies showing that LC within vaginal epithelium of mice contain apoptotic epithelial cells (23), and that following intravenous administration of allogeneic lymphocytes to rats these (possibly apoptotic) lymphocytes are detectable within interdigitating cells (IDC) of T areas of spleen (24), although it seems likely that phagocytosis occurred elsewhere before translocation of DC to these areas.

Studies of DC isolated from mouse heart and kidney have revealed similarities between interstitial DC and LC of skin (25). For example, freshly isolated cells are unable to initiate primary in vitro responses, but this activity is acquired after overnight culture of bulk parenchymal cell suspensions, which presumably elaborate the cytokines necessary for maturation. Freshly isolated DC from both organs

are able to phagocytose latex microspheres, although this capacity is not lost after overnight culture which may reflect a paucity of cytokines necessary for complete maturation in this time period. The study of Matsuno et al. (1) now provides direct evidence that intravenously-administered particulates can be phagocytosed in vivo by DC within the liver (and/or spleen). This activity is transiently expressed by the cells, most likely between the time of recruitment of DC progenitors into the liver (and/or spleen) and migration of the more mature cells, which exhibit costimulatory activity in vitro, into hepatic lymph.

Migratory Dendritic Cells. As a general rule, DC migrate from epithelia perhaps exclusively via afferent lymph into regional nodes, and from solid organs via both afferent lymph and blood into regional nodes and spleen, respectively (see below). Within afferent lymph, DC are often referred to as "veiled cells" because of their distinctive cytology, namely the presence of sheet-like lamellipodiae. Early experiments (reviewed in reference 26) demonstrated a flux of DC in afferent lymphatics draining normal skin that was considerably enhanced after the application of contact sensitizing agents. Subsequent studies of the behavior of Langerhans cells in mouse skin transplants and explants revealed that epidermal Langerhans cells migrate into dermal lymphatics and thence to regional nodes, and that they begin to mature before or during their exit from the skin (reference 27 and Liddington, M.I., and J.M. Austyn, manuscript in preparation); similar studies have been performed with explants of human skin (28). Experiments using DC isolated from afferent lymph draining skin of larger animal species such as sheep (29) have also demonstrated that the migrating cells can present antigens derived from local sites to primed T cells and can initiate primary immune responses in vitro.

Studies have also been performed of veiled cells draining from gut epithelium into afferent lymphatics. In small animals, such as rats, an important approach has been to surgically excise the mesenteric lymph nodes (mesenteric lymphadenectomy), permit re-anastomosis of lymphatics, and cannulate the thoracic duct. The DC collected represent cells that are normally trafficking from gut to mesenteric nodes, and their output in pseudo-afferent lymph is considerably increased following systemic administration of LPS (30). The cells are non-phagocytic in vitro, but often contain Feulgen-positive inclusions, indicative of endocytosis of DNA-containing material at some point in the past. Following administration of soluble antigens by oral feeding or intraintestinal injection, these DC can stimulate T cells that have been primed to the corresponding antigen in vitro, and can prime T cells in vivo after footpad injection into naive recipients (31). By autoradiography it was shown that, following injection into the rat footpad, such DC migrate to popliteal nodes and home to paracortical (T) areas (32). From these and other studies, it seems clear that DC in epithelia ultimately migrate into regional lymph nodes to initiate immune responses.

In the case of interstitial DC of solid organs, there is an additional migratory route for the cells via the blood to the spleen. This was revealed in studies tracing the migration of DC that were isolated from mouse spleens and injected intravenously (33, 34). The cells, tagged with a radiolabel or fluorochromes, were found to enter the spleen and to home to specific regions. Using a fully vascularized cardiac allograft model in mice, DC were observed to leave the organ and also to enter recipient spleens, where they again localized in particular compartments (35). Presumably, interstitial DC that acquire antigens in solid organs can also migrate via blood (as well as via afferent lymph) for initiation of responses. In addition to migration of DC from blood to spleen, the cells were found to enter livers of euthymic mice (33). In nude mice, however, homing to the spleen did not occur unless the mice were reconstituted with T cells; instead, cells that would normally home to the spleen quantitatively entered the liver. Furthermore, veiled cells obtained from central lymph of mesenteric lymphadenectomized rats were shown to migrate into the liver and thence to celiac nodes after intravenous injection (32). The significance of these observations was not fully appreciated in these earlier studies but becomes apparent from the study of Matsuno et al. (1) (see below).

What stimuli promote migration of DC from non-lymphoid tissues? It seems likely that cytokines generated during inflammatory responses promote the maturation and migration of DC to regional lymph nodes. For example, intradermal injection of murine IL-1β (but not IL-1α) or of TNF-α into mouse skin leads to a reduction in numbers of epidermal LC, and both cytokines are elicited during exposure to contact allergens (36, 37). In addition, systemic administration of LPS induces a profound loss of MHC class II+ cells from mouse heart and kidney, most likely due to migration, and qualitatively similar effects are observed following administration of human TNF-α or IL-1α (38). The increased output of cells from rat gut in response to systemic LPS, but not the loss of interstitial DC from mouse heart and kidney, can be ablated by pretreatment with neutralizing anti-TNF antibodies (30, 38). Hence these cytokines may induce, directly or indirectly, the migration of DC from solid organs, although LPS could conceivably act directly on the cells (e.g., reference 7). The capacity of LPS to promote migration of immature DC (presumably to secondary lymphoid tissues) and to recruit a wave of DC progenitors to the same tissues (17) may explain the adjuvant properties of this agent.

Physiologically, the migratory routes noted above are likely required for initiation of immune responses to soluble or particulate antigens that have gained access to the epithelia or interstitial spaces and been internalized by DC. The paper of Matsuno et al. (1) now provides information on a probable specialized role of DC in the liver for initiation of responses to antigens acquired directly from the blood circulation. In particular, evidence is presented that intravenously administered particulates can be phagocytosed by DC within the liver before migration of these cells via hepatic lymph to regional nodes, and most likely via blood to spleen.

Lymphoid Dendritic Cells. It seems possible that DC are

localized within distinct compartments of lymphoid tissues during different stages of immune response induction. For example, DC migrating from allografts appear to be retarded in B areas (peripheral white pulp) before moving to T areas (central white pulp) of spleen (35, 17). In contrast, following intravenous administration, syngeneic DC appear to bind within marginal zone of spleen, and then to home, respectively, to red pulp and T areas by 3 and 24 h later (34). In an analogous manner, if syngeneic DC are injected into the footpad of mice, they home from subcapsular sinus to interfollicular areas and ultimately into paracortical (T) areas (32, 34). The data of Matsuno et al. (1) reinforce these observations, in that they demonstrate homing of particle-laden DC from liver to discrete compartments of the celiac nodes and probably spleen.

New Insights. An important marker of rat DC that is central to the present study (1) is the OX62 antigen. Within secondary lymphoid tissues, OX62+ cells are present in red pulp and T areas of the central white pulp of spleen, and in the subcapsular sinus, medulla, and T areas of the paracortex of peripheral lymph nodes; OX62+ cells are also present in some non-lymphoid tissues including the liver (39). It seems reasonable at the present time to assume that OX62 is stably expressed by DC and is not expressed by other non-lymphoid cells.

The system used (1) is analogous to mesenteric lymphadenectomy of rats for studies of DC migrating from the gut to the mesenteric lymph nodes. Namely, celiac nodes are excised, lymphatics are allowed to re-anastomose, and the thoracic duct in cannulated (40). Following intravenous administration of carbon and latex microspheres, many of the OX62+ DC that would normally migrate in afferent lymph are found to be laden with particles. Importantly, particle-laden OX62+ cells are not evident following mesenteric lymphadenectomy, indicating a likely specialized role for at least some DC in liver in the acquisition of particles from the blood circulation, and in the initiation of responses to blood-borne antigens at least in part within the celiac nodes.

It seems probable that, following intravenous administration of particles, DC progenitors are recruited to the liver where they may internalize particulates. In normal rat liver, OX62+ cells are localized predominantly in portal areas, very few being present in sinusoids or around the central vein. Within 1 h of administration of particles, particle-laden OX62+ cells accumulate in the sinusoids and their numbers increase and plateau between 6 h and 2 d later. A close association between the OX62+ cells and Kupffer cells is often observed, raising the possibility that Kupffer cells that have phagocytosed particles elaborate cytokines that recruit DC progenitors from the blood and possibly promote their development and further maturation; alternatively, the DC could originate from preexisting progenitors within the liver (11). Certainly, many of the antigen-laden OX62+ cells that enter the hepatic lymph have recently divided, their cytology is relatively immature compared to that of DC in normal lymph, and the output of these cells into the lymph is over and above that of DC lacking particles (the latter possibly originating from distinct sites such as biliary epithelium; see below). During or after the phagocytic event, the OX62+ cells appear to undergo a maturation step before leaving the liver, since cells isolated from the lymph cannot phagocytose additional particles but can initiate primary immune responses in vitro.

In normal mouse liver, many cells around the bile duct in portal areas can be labeled with N418, a DC-restricted antigen which, like OX62 (39) appears to be a member of the integrin family of adhesins (41). These cells do not internalize carbon that is administered intravenously (41) suggesting that DC in the biliary epithelial system, which subsequently enter hepatic lymph, may be specialized to take up antigens within the epithelium itself. This pool of DC may thus be distinct from the DC that appear to be recruited to the sinusoids to internalize particles from the blood (1).

Migration of particle-laden DC from the liver occurs in hepatic lymph, and probably the blood. Within the liver, particle-laden OX62+ cells are present in portal areas at 6 h, and in central (hepatic vein) areas at 18 h after administration of particles, and can be detected in these sites for at least 2 d. This suggests that the cells translocate from sinusoids into these areas before entry to the descending lymphatics and migration to celiac nodes, and possibly also into ascending lymphatics. Particle-laden OX62+ cells first appear in the marginal sinus and interfollicular areas of celiac lymph nodes at 6 h, and appear to home to paracortical (T) areas by 18 h, consistent with earlier reports (32, 34).

Particle-laden OX62+ cells probably also migrate from the liver to the spleen via the blood. The cells are detected in splenic marginal zone and red pulp from 1 h to 2 d, and appear to home to white pulp 18 h after administration of particles, reminiscent of earlier observations (34). An alternative explanation for particle-laden OX62+ cells in spleen is that DC progenitors are recruited to the spleen before internalization of particles; for example, splenic red pulp macrophages, which avidly ingest intravenously administered carbon particles (41) could conceivably elaborate cytokines to recruit DC to the red pulp. An additional, perhaps less likely, possibility is that particle-laden cells may migrate from the spleen to the liver and thence to celiac nodes. Shaving with Occum's razor, however, the former hypothesis is perhaps to be preferred at the present time.

In summary, we now appear to have three routes by which DC can initiate responses to antigens from different anatomical compartments: (*a*) DC in epithelia such as skin and gut acquire local antigens and migrate via lymph to nodes; (*b*) DC in interstitial spaces of organs such as heart and kidney acquire local antigens and migrate via lymph to nodes, and via blood to spleen; and (*c*) a subset of DC in liver acquires antigens from the blood and migrates via lymph to regional nodes, and probably via blood to spleen. The present study (1), by approaching the liver and by demonstrating the recruitment and phagocytic capacity of DC following particle administration, has helped to close significant gaps in our understanding of the behavior and functions of the DC lineage.

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