THE DENDRITIC CELL SYSTEM
AND ITS ROLE IN
IMMUNOGENICITY

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Abstract

Dendritic cells are a system of antigen presenting cells that function to initiate several immune responses such as the sensitization of MHC-restricted T cells, the rejection of organ transplants, and the formation of T-dependent antibodies. Dendritic cells are found in many nonlymphoid tissues but can migrate via the afferent lymph or the blood stream to the T-dependent areas of lymphoid organs. In skin, the immunostimulatory function of dendritic cells is enhanced by cytokines, especially GM-CSF. After foreign proteins are administered in situ, dendritic cells are a principal reservoir of immunogen. In vitro studies indicate that dendritic cells only process proteins for a short period of time, when the rate of synthesis of MHC products and content of acidic endocytic vesicles are high. Antigen processing is selectively dampened after a day in culture, but the capacity to stimulate responses to surface bound peptides and mitogens remains strong. Dendritic cells are motile, and efficiently cluster and activate T cells that are specific for stimuli on the cell surface. High levels of MHC class-I and -II products and several adhesins, such as ICAM-1 and LFA-3, likely contribute to these functions. Therefore dendritic cells are specialized to mediate several physiologic components of immunogenicity.

1 Abbreviations: APC, antigen-presenting cell; LC, Langerhans cells; MLR, mixed leukocyte reaction; MHC, major histocompatibility complex.
such as the acquisition of antigens in tissues, the migration to lymphoid organs, and the identification and activation of antigen-specific T cells. The function of these presenting cells in immunologic tolerance is just beginning to be studied.

INTRODUCTION

Many tissues contain a trace population of cells with an unusual dendritic shape, high levels of MHC class-II products, and strong accessory function for the stimulation of T lymphocytes. A clearer picture of the physiology of these cells is emerging and is reviewed here. Dendritic cells comprise a system that occupies discrete portions of nonlymphoid and lymphoid organs and is interconnected by defined pathways of movement (Table 1). In each site, dendritic cells share features of structure and function, the most notable being the ability to capture antigens and initiate T cell-mediated immunity. The mechanisms of dendritic cell action are important for understanding how T cells are primed and how one might begin to manipulate the immune response at the early sensitization phase of immunity. Three areas of function are considered here, each exhibiting differences from other antigen-presenting cells (APC): a sentinel role in which antigens are captured and presented, a migratory function in which dendritic cells move to the T-dependent areas of lymphoid organs and bind antigen-specific T cells, and an adjuvant or activation role in which T-cell growth and effector function are induced. Recent progress on dendritic cells is reviewed here. More detailed reports on many topics are in Research in Immunology Volume 140, International Reviews in Immunology Volume 6, Advances in Immunology Volume 47, and Epidermal Langerhans Cells, ed. G. Schuler.

DENDRITIC CELLS: A WIDELY DISTRIBUTED AND CONNECTED SYSTEM OF POTENT ANTIGEN-PRESENTING CELLS

The characterization of dendritic cells has required the isolation of enriched populations of what is a trace cell type in all tissues. Mouse spleen remains

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the organ most readily analyzed, but many other tissues have been utilized successfully. Typically, dendritic cells do not adhere to tissue culture surfaces, especially after a day in culture, and they have low levels of Fc receptors (FcR) but high levels of MHC class-II products. Macrophages in contrast are adherent, with abundant FcR but variable levels of class II. We review the isolation and function of dendritic cells in different sites and include new evidence that these antigen-presenting cells capture antigens in situ and move from nonlymphoid to lymphoid organs.

**Nonlymphoid organs**

**Epidermis** Epidermal Langerhans cells are the best characterized nonlymphoid dendritic cells. It is feasible to prepare partially enriched (30–60%) populations in mice, since keratinocytes can be depleted with α-thy-1 and complement plus adherence (1, 2). Partially enriched Langerhans cells suffice in most studies of function and are a better starting material for purification by sorting and panning methods than are unfractionated cells (2, 3). For human epidermis there is no mAb like α-thy-1 which binds keratinocytes, but anti-CD1 mAb selectively identify Langerhans cells (4).

Mouse epidermal Langerhans cells, in spite of high levels of MHC class-II products and detectable FcR are not active antigen-presenting cells for the mixed lymphocyte reaction (MLR) or α-CD3 response. Activity develops after a 1–3 day period of culture in which the cytokine GM-CSF plays a key role (2, 3, 5). GM-CSF does not act simply to maintain Langerhans cell viability, since another cytokine, TNF-cachectin, maintains viability without inducing accessory function (6). Structure and phenotype also change in culture. The Langerhans cells enlarge, express more MHC class-II and adhesion molecules (below), and lose Fc receptors and Birbeck granules. Romani et al (7) and Teunissen et al (8) noted entirely analogous changes during the culture of human Langerhans cells. As a result, cultured Langerhans cells fully resemble blood and lymphoid dendritic cells (1, 7, 9, 10).

There is evidence that Langerhans cells can leave the skin and move via the afferent lymph to draining lymphoid organs. Cells with Birbeck granules have been noted in lymph (11, 12) and lymph node (13). When contact allergens are applied, allergen is found 8–24 hr later on lymphoid dendritic cells (14). Larsen (15) directly monitored the movement of Langerhans cells out of the epidermis. Grafting itself leads to movement in either syngeneic or allogeneic hosts. Within a day, the Langerhans cells enlarge and express high levels of MHC class II. Epidermal Langerhans cells numbers fall to about one third steady state, and the antigen-presenting cells appear in the dermis in what appear to be lymphatics. Similar events occur in organ cultures of skin fragments. Langerhans cells move...
into the dermis and then the culture medium (15). Kaplan et al suggest that dermal Langerhans cells also arise from the blood in delayed type hypersensitivity reactions (16).

**Heart** Fabre, Hart, and McKenzie showed that the interstitium of most organs, except brain, contain irregularly shaped cells that stain strongly with mAb to MHC class-II products and the CD45 leukocyte antigen (17–19). Two kinds of resident CD45+ leukocytes have been identified (20) with a new antimacrophage mAb: macrophages that react with the mAb and are radioresistant, and dendritic cells that do not react with the mAb but are strongly MHC class II–positive and are radiosensitive.

Larsen et al, studying mouse cardiac allografts, found that the number of interstitial Ia+ dendritic cells fell during the first four days after grafting (21). Using mAb specific to polymorphisms of the heart donor, they saw donor-derived, MHC class II–rich dendritic cells in the spleen, indicating migration from the heart via the blood stream. These results were confirmed in a rat limb transplant model (22).

**Liver** Except for an initial report (23), there has been little study of isolated dendritic cells. In situ these antigen-presenting cells are in the portal triads, in contrast to sinus-lining phagocytes (Kupffer cells) (18, 20).

**Lung** In rat (24, 25), mouse (26, 27), and human (28, 29), dendritic cells are selected on the basis of their low buoyant density, and lack of plastic adherence and FcR. The enriched cells have abundant MHC class-II and induce strong T cell–mediated immune responses. The parenchyma is the source of lung dendritic cells rather than the bronchoalveolar lavage fluid, a standard source of macrophages. Holt et al (30) localized Ia+ dendritic cells to alveolar septae, as well as within and just beneath airway epithelium. In tangential sections, the network of epithelial dendritic cells is reminiscent of epidermal Langerhans cells. Macrophages are juxtaposed to dendritic cells along the airway.

After aerosolization of proteins, lung dendritic cells present antigen, since the isolated antigen-presenting cells directly stimulate antigen–specific T-cell lines (24). To detect antigen-presenting cells function, suppressive macrophages must be removed.

**Gut** Pavli et al succeeded in isolating dendritic cells from the lamina propria of mouse intestine (31). Again, depletion of suppressive macrophages helped uncover the presence and function of dendritic cells.

**The Circulation**

**Afferent Lymph** Afferent but not efferent lymph contains leukocytes termed “veiled” cells in rabbit (32), pig (33), rat (34, 35), mouse (36),
human (37), and sheep (38). Like dendritic cells, veiled cells have a low buoyant density, low FcR and phagocytic activity, and high levels of MHC class-II and antigen-presenting cell activity. The origin of veiled cells is unclear. They may come from the interstitium and epithelium of many organs, or from a pool of cells that leaves the blood, moves through nonlymphoid tissues, and enters the lymph.

If protein antigens are administered intradermally, afferent lymph dendritic cells carry the antigen in a form that will trigger antigen-specific T-cell lines (38). Therefore, dendritic cells in lymph can carry antigens to lymphoid tissues, as has been proposed for epidermal Langerhans cells.

**BLOOD** In human blood, <0.1% of the white cells are dendritic cells. Prior enrichment methods fell short of substantial purity, but >90% purity has now been achieved (39). Enrichment entails successive depletion of T cells (sheep red cell rosetting), monocytes (adherence to plastic or Ig-coated plates), and B plus NK cells (pelleting in metrizamide). Together, and when performed in the above sequence, these steps yield an E rosette negative, nonadherent, low-density fraction with 30–60% dendritic cells. Further purity is obtained by panning or sorting with mAb, especially to CD45RA, that selectively react with the contaminants. Although this isolation approach is labor-intensive, one simultaneously secures populations of other cell types, each depleted of dendritic cells (39).

The purity of the populations can be assessed by three approaches with comparable results. One approach uses mAb and flow cytometry (39) to phenotype the cell fractions. Markers that dendritic cells lack are CD3 (T cells), CD14 (monocytes), CD19/20 (B cells), and CD56/57 (NK cells). Abundant proteins are class-I and -II MHC products plus a distinct array of receptors and adhesion molecules (see below). The second approach is to observe cell fractions live by video microscopy (39). Dendritic cells continually extend and retract large lamellipodia or “veils.” No other cell in blood shows this motility. The third approach is to test MLR stimulatory function. The dendritic cells are potent, e.g. in cultures of $10^5$ T cells, $1–3 \times 10^3$ allogeneic dendritic cells induce an MLR comparable to that induced by the standard population of $10^5$ bulk mononuclears. Monocytes and B cells have little or no stimulating activity (21).

Dendritic cells in blood may be migrating from nonlymphoid tissues to spleen. Evidence for the latter was recently obtained in a rodent heart transplant model (39). Alternatively, dendritic cells may be en route from the marrow to nonlymphoid tissues.

**Lymphoid Organs**

**TONSIL** Substantial purification of human tonsil dendritic cells also has been obtained (40). The cells are strong MLR stimulators, and their distinct
surface composition has been outlined with a panel of mAb and immunoperoxidase labeling. The results are included in the next section.

T-DEPENDENT REGIONS OF PERIPHERAL LYMPHOID ORGANS In sections of the T-dependent regions of lymphoid organs, there are “interdigitating cells” (41) which resemble dendritic cells in cytology and phenotype (42, 43). If dendritic cells from mouse spleen (44) are administered into the blood or foot pads, Austyn et al noted homing to the T-dependent areas. Fossum injected radiolabeled afferent lymph dendritic cells into rats. By light and electron microscopy, the injected cells assumed the location of interdigitating cells (45). Little is known about the efficiency of this homing. Possibly the only cells that are retained are those that find T cells specific to the presented antigens. The reason is that the flux of dendritic cells in lymph can be $10^7$/hr, yet these antigen-presenting cells do not accumulate in lymph node or efferent lymph. Given the substantial turnover of cells in lymph and in spleen (35, 46), most dendritic cells may not live long upon reaching the lymphoid organ.

In spleen, dendritic cells are more numerous than the interdigitating cells of the periarterial sheaths. When dendritic cells are sorted from fresh spleen suspensions (see below), only a subpopulation label clearly with NLDC145 (47), a mAb to interdigitating cells in the central periarterial sheaths (48). In section, nests of dendritic cells lie in the periphery of the T area interrupting the marginal zone of macrophages (49, 50). Both T cells and antigens leave the arterial tree in the marginal zone, so in effect, dendritic cells are positioned like “doors” through which T cells pass upon entry into the periarterial sheaths. Lymphoid organs may contain two populations: peripheral [?migratory], short-lived dendritic cells, and central, long-lived interdigitating cells.

DENDRITIC CELLS FRESHLY ISOLATED FROM MOUSE SPLEEN As evident above, dendritic cell isolation requires many steps that can take a day or more. Since viability and function can be influenced by cytokines like GM-CSF, IL-1, and TNF (2, 3, 6, 51, 52), it is important to characterize fresh isolates. This recently was done in mouse spleen using a new mAb, N418 to murine CD11c (47, 49). Dendritic cells are the main cell expressing high levels of CD11c in the steady state in spleen. Fresh suspensions contain about 1.5% N418+ cells, while peritoneal washings and blood have few. Two-color immunolabeling shows that splenic N418+ cells have the phenotype of dendritic cells (47). Sorted N418+ cells assume a typical veiled morphology if cultured for a day.

Fresh, sorted N418+ dendritic cells actively stimulate the MLR and present protein antigens to sensitized T cells (47). If proteins are given i.v.
or i.p., the N418 + fraction is the main source of immunogen when spleen fractions are applied to antigen-specific T cells (53).

**THYMUS** Large, bone marrow-derived, class II–rich dendritic cells are found in the medulla (54, 55). These likely are the "interdigitating cells" noted by electron microscopy (56) or the "dendritic cells" in enzyme-digested thymus (57–59). Crowley et al succeeded in enriching mouse thymic dendritic cells to a high degree of purity, using the same approach as was used in spleen (10). Adherent cells with a low buoyant density were cultured overnight so that most dendritic cells dislodged. The dendritic cells were about twice the size as in spleen but similar in phenotype and function. If mg doses of proteins are given i.v., thymic dendritic cells pick up antigen in a form stimulatory for T cells (58).

**Summary: a Dendritic Cell Lineage**

Nonlymphoid organs, blood, afferent lymph, and lymphoid tissues (see Table 1) contain dendritic cells with different names but similar properties. To enrich them, one selects for low buoyant density, nonadherence to plastic especially after a day in culture, and absence of certain markers found on other cell types. These approaches simply deplete other cells but do not positively select for cells that are distinct in shape and have abundant MHC class II plus strong antigen-presenting cell function. This indicates that the negatively selected dendritic cells are an independent cell type.

Evidence is growing that dendritic cells in different tissues, as defined morphologically and by a distinct group of cell surface markers, are part of a system connected by movement and homing. Dendritic cells in nonlymphoid organs, such as epidermal Langerhans cells and heart interstitial cells, can give rise to "veiled cells" in the afferent lymph and blood which migrate to lymphoid tissues where they are isolated as "dendritic" or "interdigitating" cells. Coupled with these migratory abilities is the capacity to capture antigens in an immunogenic form in situ.

It seems appropriate to classify the cells in Table 1 as a separate lineage given their distinct features and tissue distribution. There is no evidence that other white cells convert into dendritic cells or vice versa, even if challenged with a variety of cytokines. For example, macrophage and granulocyte factors, M-CSF and G-CSF, have no known effects on dendritic cells, in contrast to their profound effects on typical phagocytes.

Nonetheless, the progenitor for the putative dendritic cell lineage has not been isolated. Dendritic cells in spleen and lymph originate from a proliferating pool of precursors and undergo rapid turnover (35, 46, 60), but the site for proliferation (3H-thymidine uptake) is not known. A bone
marrow precursor exists (35, 46, 54, 61), but conditions have not been identified that direct its growth in culture.

THE DENDRITIC CELL SURFACE: SPECIALIZATIONS FOR ANTIGEN PRESENTATION

In mouse and human, the fluorescence activated cell sorter (FACS) now has provided detailed descriptions of the dendritic cell surface (10, 39, 47). The results are diagrammed (Figure 1). The surface is distinct among leukocytes in many respects and is consistent with their functional capacities (see below), including antigen presentation (abundant surface MHC products), active clustering with T cells (high levels of several adhesins), and weak phagocytosis (low amounts of Fc and complement receptors). Most antigens are expressed in a homogeneous fashion on dendritic cells from different sites, but deviations can occur. These may represent differences expressed as dendritic cells mature and migrate from peripheral tissues to the T areas.

Products of the MHC

Consistent with their strong antigen-presenting cell function, dendritic cells express all class-II MHC genes at high levels. Both I-A and I-E
DENDRITIC CELLS

are abundant in the mouse, and HLA-DP, DQ, DR likewise in human. Monocytes have low levels of DP and DQ (39). Dendritic cells have surface invariant chain (CD74), as evidence by FACS (39) and precipitation of $^{125}$I-labeled cells with polyclonal antibody (62).

Polymorphic class-I products also are abundant (39, 63–65) and can be used to monitor the repopulation of dendritic cells in bone marrow chimeras (66) or movement during transplantation (22). Nonpolymorphic class I (such as Qa and T1), which may present antigens to the $\gamma/\delta$ subset (68), have not been evaluated on dendritic cells. CD1, which is not encoded in the MHC but is class I-like and may act as a restriction element for $\gamma/\delta$ T cells (69), is abundant on dendritic cells in skin (CD1a) (4, 70) and afferent lymph (CD1b) (38).

**Fc and C3 Receptors (FcR, C3R)**

Dendritic cells are not actively phagocytic. Consistent with this, there is little or no expression of FcγR (CD16, CD32, CD64) or C3R (CD11b, CD21, CD35), as assessed by staining with mAb or binding of opsonized particles. Yet some exceptions are apparent. In skin, CD32 FcγR are noted (1, 71, 72), but their levels fall by 90% when the Langerhans cells are cultured (8, 73, 74). Only freshly isolated Langerhans cells actively process and present native proteins (see below); this fact leads to the idea that FcγR on fresh Langerhans cells are involved in antigen uptake (75). Sheep lymph dendritic cells have cytophilic Ig and specific antibody enhances antigen presentation (76).

FcεR have been studied on epidermal Langerhans cells. Binding of IgE is noted in situ in the skin of atopic patients (77), but the FcεR-mediating binding has not been identified. Low-affinity FcεRII (CD23) are upregulated with IL-4 and IFN-γ (78). Langerhans cells are implicated in the transport of contact allergens as well (12, 79).

C3bi (CD11b) receptors typically are present at trace levels with the exception of mouse Langerhans cells where they are more readily detectable (1).

Other receptors used by phagocytes to scavenge particulates and microorganisms have not been studied. Dendritic cells lack mannose-fucose receptors (35), but no studies have been published on other receptors, e.g. for lipoproteins and scavenging. The lack of phagocytic receptors does not mean that particulate antigens are not processed. It is possible these antigen-presenting cells take up small numbers of particles at defined stages of their natural history, or that particles are processed at the surface. Afferent lymph dendritic cells do not internalize test particles, yet some of the cells have what appear to be phagocytic inclusions (35). The latter perhaps were acquired in the tissues prior to entry into the lymph.
Integrins and Adhesins

These molecules contribute to cell binding and homing, which are both important features of dendritic cell function. Dendritic cells can have high levels of p150/90 or CD11c but low CD11b (39, 49). CD11a or LFA-1 is found on dendritic cells in mouse spleen and human blood (39, 80) but not skin (8, 73). Dendritic cells also have high levels of other adhesins, ICAM-1 (CD54), LFA-3 (CD58), and β1 integrin (CD29) (7, 39).

Differentiation Antigens

Many groups are trying to prepare dendritic-cell specific mAb, but this has proven problematic. This is not for a lack of immunogenicity, since large numbers of mAb to shared markers are obtained following immunization with dendritic cells, e.g. mAb to MHC products and integrins (49). A group of three mAb are useful in the mouse (50). 33D1 reacts with most dendritic cells in spleen and Peyer's patch, but not skin and thymic medulla (10). NLDC145 reacts with dendritic cells in skin and in the T-dependent regions of several lymphoid organs, but also binds to thymic epithelium (48). N418 anti-CD11c reacts strongly with dendritic cells, but CD11c can be upregulated on macrophages.

Many molecules are expressed in a cell-type restricted fashion and are useful for distinguishing dendritic cells. For example, dendritic cells in human blood (39) lack CD3 (T cells), CD19-22 (B cells), CD13-15 and the c-fms receptor for M-CSF (phagocytes), and CD56/57 (NK cells). Dendritic cells can express the B cell-activation marker CD40 (7, 39, 40), and expression of CD4 and CD8 also occurs (10, 47). Some useful markers that mouse dendritic cells lack are CD45RA, thy-1, and certain phagocyte markers that lack a CD designation (SER-4, RB-6) (50).

PRIMARY RESPONSES INDUCED BY DENDRITIC CELLS: DISTINCT ANTIGEN-PRESENTING CELL REQUIREMENTS FOR THE AFFERENT AND EFFERENT LIMBS OF IMMUNITY

The term “antigen-presenting cell” does not fully describe the function of dendritic cells. Their distinctive role is to initiate T-dependent responses from quiescent lymphocytes. Once sensitized, the T lymphoblasts readily interact with other antigen-presenting cells. In effect, efficient T-cell responses both in vitro (81–83) and in vivo (84) seem to occur in two phases. In the afferent limb, dendritic cells are specialized to identify and activate trace clones of antigen-reactive T cells while in the efferent limb, the sensitized blasts find other antigen-presenting cells to induce effector
functions such as macrophage activation and IL-1 production, and B-cell antibody responses. In this section, several primary responses are reviewed, while in the next, aspects of mechanism are considered.

The Primary Antibody Response

The need for spleen adherent cells in the primary antibody response by B and T lymphocytes (85) led to the discovery of dendritic cells which proved to be essential accessories (86). When foreign red blood cells are the antigen, dendritic cells stimulate the production of helper factors bypassing the need for intact T cells (87). For hapten carrier conjugates, the need for dendritic cells is to sensitize carrier-specific T cells which directly interact with hapten-specific B cells as antigen-presenting cells (82). The B cells become responsive to antigen-nonspecific cytokines, and this all occurs in discrete aggregates of dendritic, B, and T cells (88, 89).

Some uncertainty exists concerning the relative roles of dendritic cells and B cells as antigen-presenting cells for sensitizing helper cells in situ (90). The lymph nodes of mice that are treated with anti-μ from birth cannot be primed with proteins in adjuvant (91–94). The nodes presumably are depleted of B cells and not dendritic cells. In contrast, in studies of antigen-presenting cells function for antibody responses, there are in vitro data that mouse spleen responses require dendritic cells (82, 88) and in vivo findings that B cells in chickens are not the initial antigen-presenting cells for responses to heterologous red cells (95). In a study where antigen-pulsed antigen-presenting cells have been used to induce antibody in situ, it has been found that dendritic cells induce the formation of antiviral and antiidiotype antibodies (96).

Primary Responses to Protein Antigens In Situ

If spleen dendritic cells are exposed to protein antigens in vitro and injected into the foot pads of mice, CD4+ T cells in the draining lymph node are sensitized (97). Responsiveness peaks at day 5, wanes by day 9–12, and is rapidly induced by rechallenge with antigen-pulsed dendritic cells. When spleen cells, which are 60% B lymphocytes, are pulsed with antigen and then compared to dendritic cells, the spleen cells are much less efficient as antigen-presenting cells in vivo (97). 6 \times 10^6 spleen cells (the maximum that can be given readily in one dose into a foot pad) induce weak responses whereas 2 \times 10^5 dendritic cells are much more active. As such, spleen B cells lack direct sensitizing function at least for bulk exogenous proteins. Peritoneal macrophages are totally inactive as antigen-presenting cells in this in vivo assay system.

The injected dendritic cells do not simply carry antigens to be presented by host antigen-presenting cells. This can be shown by injecting pulsed,
parental strain dendritic cells into F1 recipients. The T cells that are primed respond to antigen in the context of MHC class-II products of the injected dendritic cells (97). This is the first instance in which antigen-pulsed antigen-presenting cells have primed T cells in an MHC-restricted fashion in vivo.

**Viral Antigens**

The standard assay for studying the generation of antiviral CTL is to prime a mouse by exposure to virus and induce CTL by rechallenge in vitro. Dendritic cells from mice that have been infected with Moloney leukemia virus are active antigen-presenting cells for inducing virus-specific CTL (98). Dendritic cells also present Sendai to virus-primed spleen (98). In each case, dendritic cells are 30–100 times more active than unfractionated spleen.

Macatonia et al found that dendritic cells serve as antigen-presenting cells for primary CTL responses in the influenza system (99). A special culture system was needed. $10^5$ cells were cultured in 20 μl drops that hung from wells of inverted Terasaki plates, a system which may improve gaseous exchange. A puzzling feature is that high levels of CTL activity develop in a primary five-day culture. This implies that the frequency of precursors for flu-specific CTL in naive mice is high. Hengel et al also noted that dendritic cells induce the formation of CTL to herpes simplex in a primary system (100). Limiting dilution assays indicated that herpes-specific CTL precursors represent 1 in 400 of nylon wool passed, spleen T cells.

**The Primary Mixed Leukocyte Reaction**

The strong stimulating activity of dendritic cells in the primary mixed leukocyte reaction (MLR) is established (101). Small B cells and monocytes trigger little or no response from resting CD4$^+$ T cells (39) but do stimulate activated T-cell blasts and T-cell clones (102, 103). Certain B blasts and many B-cell lines are in contrast capable of stimulating the mixed leukocyte reaction (80).

Several lymphokines form when dendritic cells stimulate the mixed leukocyte reaction. IL-2 is noted on day 1 (81, 83, 104, 105) and other factors soon thereafter: IL-4, IFN-γ, and T cell replacing factors for antibody responses (81, 104, 106). Detailed studies have not yet been published that compare dendritic cells with other antigen-presenting cells for defined T$_{H1}$ and T$_{H2}$ subsets.

Dendritic cells also stimulate an MLR from CD8$^+$ T cells, but higher antigen-presenting cell doses are needed (83, 105). Allospecific CTL are detected at day 4–5 using dendritic to T-cell ratios of 1:10 or 1:30. The
need for relatively high doses of dendritic cells could mean that the antigen-presenting cells are killed during the course of the response. The CD8+ mixed leukocyte reaction is sizeable, with 30–40% of the culture at day 5 being T blasts that express CD25 and MHC class-II activation antigens. Monocytes do not stimulate resting CD8+ cells but serve as targets in the efferent limb of the response, i.e. for the CTL that are induced by dendritic cells.

As few as 1 allogeneic dendritic cell per 100 CD4− responders also induces strong NK activity (K562 targets) (105). The precursors to these NK cells can be removed selectively prior to the MLR by panning the CD4− population with α-CD11b mAb (105). That NK and allospecific CD8+ cells are induced in the absence of CD4+ helpers indicates that IL-2 and perhaps other cytokines can be generated in substantial amounts in the cultures.

COMPONENTS OF DENDRITIC CELL FUNCTION
—MECHANISMS OF IMMUNOGENICITY

Because dendritic cells induce primary responses in vitro and in vivo, they are important for studying immunogenicity in a physiologic context. The limitation is that only small numbers of dendritic cells are available from any tissue, and no cell lines exist. Yet dendritic cell numbers are large relative to their specialized task, i.e. to identify and activate the very small numbers of antigen-specific T cells that are present at the onset of an immune response. To describe these specializations, I do not use the "signal 1/signal 2" terminology, since the notion of two signals oversimplifies the many features that contribute to dendritic cell function and does not consider the distinct physiologic properties of different antigen-presenting cells. Dendritic cell function can be considered in three parts.

Sentinel Function—Processing and Presentation of Antigen

SUMMARY OF RECENT ANTIGEN-PULSING EXPERIMENTS If pulsed with relatively low doses of soluble protein (100 μg/ml), dendritic cells stimulate antigen-specific, primed T cells (62, 97). Chloroquine blocks pulsing (62) as it does in other types of antigen-presenting cells (107, 108), implying a need for an acidic intracellular compartment. Several features of antigen-pulsed dendritic cells are apparent:

1. The number of pulsed dendritic cells needed to stimulate T cells is low, about 30–100 times less than for mouse spleen (62, 75, 97), a standard antigen-presenting cell population which contains about 1% dendritic cells.
2. Antigen-pulsed dendritic cells, cultured for 1–2 days without further exposure to antigen, retain activity (62, 97). This could mean that the levels of MHC-peptide complexes are high, the turnover of complexes is slow, or that fewer complexes are needed for presentation by dendritic cells. In contrast, if macrophages are pulsed, the turnover is rapid; the $t_{\frac{1}{2}}$ of presenting activity is a few hours (109).

3. Antigen-pulsed dendritic cells, but not other antigen-presenting cells, can be administered in situ to prime T cells without additional adjuvants (97). Priming is restricted to the draining lymphoid tissue and is direct. If F1 T cells are primed with parental dendritic cells, sensitization is restricted to the MHC of the parental antigen-presenting cells.

4. The processing activity of dendritic cells is regulated. Only fresh isolates from epidermis (62, 75) and spleen (97) present native proteins. After a day in culture, the dendritic cells handle proteins poorly but are the most potent antigen-presenting cells for stimuli that do not require processing, e.g., peptides, alloMHC, and mitogens.

5. It is not yet possible to detect regurgitation of peptides processed by other antigen-presenting cells onto dendritic cells. Such experiments are possible with cultured dendritic cells which can present peptides but not proteins. If MHC-mismatched spleen or peritoneal macrophages (97) or Ia– epidermal cells (62) are mixed with protein plus cultured dendritic cells, antigen-presenting cell function does not occur. If other cells regurgitate immunogenic peptides, one would expect the dendritic cells to have stimulated the T cells.

MECHANISMS OF ANTIGEN PROCESSING IN DENDRITIC CELLS Several variables are being analyzed to explain the differences between fresh and cultured dendritic cells (see above). Both have high levels of surface MHC class-II products, with cultured Langerhans cells expressing at least five times more class II than fresh Langerhans cells (9, 110). When rhodamine-ovalbumin is used to monitor endocytosis, fresh and cultured Langerhans cells internalize protein into intracellular granules (62). However, it is not yet possible to rigorously quantitate endocytic activity in fresh and cultured Langerhans cells, since these antigen-presenting cells are so weak. Stossel et al recently made the fascinating observation that freshly isolated Langerhans cells have more numerous acidic vacuoles, presumably endosomes, whereas cultured mouse and human epidermal Langerhans cells have some acidic lysosomes but few acidic endocytic vesicles (111).

Pure' et al have identified two more striking differences between fresh and cultured Langerhans cells (62). Fresh Langerhans cells actively synthesize class-II products, while cultured Langerhans cells synthesize other proteins but not class II. By blocking synthesis with cycloheximide, the
efficiency with which Langerhans cells can be pulsed with protein antigen is reduced. In addition, fresh but not cultured Langerhans cells express high levels of cytoplasmic invariant chain. The latter enhances presentation by cell lines transfected with MHC class-II genes (112). The data suggest that the efficient processing of native proteins by fresh Langerhans cells is linked to the synthesis of MHC class-II products. Perhaps newly synthesized molecules, associated with invariant chains, are shuttled to an acidic compartment where there is ready access to peptide antigens.

The ability to downregulate the presentation pathway, including a down-regulation of class-II biosynthesis and a loss of invariant chains, is of uncertain consequence at this time. Antigen-pulsed dendritic cells retain antigen for long periods in an immunogenic form (see above). Perhaps acquired peptides would be displaced if antigen processing were to continue in an unregulated fashion. Continued antigen processing, when dendritic cells are migrating from nonlymphoid tissues to lymphoid T areas, also would allow self-peptides to predominate over exogenous ones, and might lead to autoimmunity (see Discussion).

Although dendritic cells can capture protein antigens, it is striking that the absolute amount of protein that accumulates intracellularly is small (97). This suggests that while endocytic activity generates MHC-peptide complexes, the relevant levels of processing and presentation are small especially when compared to the size and activity of the vacuolar system that is used to scavenge and degrade antigens in macrophages. One must distinguish between endocytosis that is primarily directed toward antigen scavenging, the hallmark of the macrophage, and the much lower amounts that are associated with antigen-presenting cells function in dendritic and B cells. It has been known for some time that macrophages internalize and completely degrade at least 10,000 protein molecules per hour at the doses of protein that are used traditionally to pulse antigen-presenting cells. This scavenging function makes endocytosis, but not necessarily antigen processing, easy to visualize in macrophages (113).

**CAPTURE OF ANTIGENS IN SITU** When antigens are administered in situ, the dendritic cells can be isolated and shown to be carrying the antigen in an immunogenic form. This is tested by adding in vivo pulsed dendritic cells to specific T cells. T-cell stimulation occurs with lung dendritic cells after aerosol administration of proteins (24), with afferent lymph sheep dendritic cells after intradermal administration of protein (38), with draining lymph node dendritic cells after application of a contact allergen (14), and with thymic dendritic cells after an i.v. bolus of protein (58). If mice are given foreign proteins i.v. or i.p., dendritic cells seem to be the principal source of immunogen relative to other cell types (53). The latter experi-
ments used selective depletion of in vivo pulsed antigen-presenting cells with 33D1 anti-dendritic cell mAb and complement, as well as positive selection with N418 anti-CD11c mAb and the FACS (47).

THE LEVELS OF ANTIGEN/MHC ON THE DENDRITIC CELL SURFACE  The high levels of class-I and -II MHC molecules on dendritic cells could function to carry many different epitopes, as in the presentation of complex infectious agents or foreign cells, and/or to accumulate large numbers of individual kinds of MHC-peptide complexes to better identify and stimulate resting T cells. It is not yet possible to enumerate specific MHC-peptide complexes on dendritic cells. However, Romani et al (73) studied the number of T cells that bind and respond to presentation of anti-CD3 on defined numbers of Langerhans cells. As few as 250 FcR per Langerhans cell are needed to drive a T cell into cell cycle, and each Langerhans cell on average handles 10–20 T cells.

Migratory Functions—the Binding of Antigen-Specific T Cells

MIGRATION TO T-DEPENDENT AREAS  As detailed above, dendritic cells can migrate via blood or afferent lymph to the T areas of spleen and lymph node respectively. This places the antigen-charged dendritic cell in the path of recirculating T cells, enhancing the chance that relevant T-cell clones can be selected.

The factors that stimulate and direct the movement of dendritic cells in vivo are not clear. Simply grafting skin to isogeneic hosts or placing skin in organ culture induces egress of at least two thirds of the epidermal Langerhans cells (15). This suggests that movement is T cell independent. However, homing may be T dependent since dendritic cells are not retained in the spleens of nude mice (44). Dendritic cells express molecules that are implicated in leukocyte homing, e.g. β2 integrins and CD44.

The migratory properties of dendritic cells do not preclude a role in stimulating immunity locally. This may occur in secondary responses, where dendritic cells are found in association with T cells at delayed type hypersensitivity sites (16). However, in certain primary responses, like sensitization to skin transplants (114) and contact allergens (115), afferent lymphatics need to be intact—a point consistent with the idea that a flux of dendritic cells is critical to immunogenicity.

CLUSTERING OF ANTIGEN-SPECIFIC T CELLS  A feature that helps explain dendritic cell function is the capacity to form stable clusters with antigen-specific T cells during primary responses. Other antigen-presenting cells do not aggregate resting T cells but do bind sensitized T blasts (81, 82, 103, 116).
In the living state, thin sheets or lamellipodia extend from the dendritic cell body in many directions (33, 39, 117). The processes extend, retract, and bend as if the antigen-presenting cells are probing the environment around them. This motility, which is distinct among white cells, likely enables the dendritic cell to survey T cells and select antigen-specific ones.

Two examples indicate that the initial T-cell sampling occurs by an antigen-independent mechanism. First, dendritic cells bind to T blasts that are directed to specificities different from that present on the antigen-presenting cells (116). Second, when cultured in close proximity, dendritic cells bind resting T cells in large numbers, too many for all to be antigen-specific (118). In both instances, bound T cells do not produce IL-2 unless another stimulus like a mitogen is added. Antigen-independent binding could provide a temporary state of antigen-presenting cell–T-cell contact, which may be essential for the appropriate TCR-antigen-MHC interaction to occur.

The molecules that mediate antigen-independent clustering have yet to be identified. As mentioned, adhesins like ICAM-1 and LFA-3 are abundant on dendritic cells (39, 119). To date, mAb to β2 integrins have not blocked heterotypic clustering with T cells in the mixed leukocyte reaction (49, 80, 120), although α-CD18 mAb does block when the stimulus is sodium periodate (121). Other adhesins are under study, including CD2 and CD28.

**Adjuvant Function—T-Cell Activation**

**IL-1 AND DENDRITIC CELL FUNCTION** For years the main hypothesis for the mechanism of T-cell activation has been that macrophage-derived IL-1 induces IL-2 release or responsiveness. Dendritic cells and B cells are active antigen-presenting cells but are not known to make IL-1 (122–125), except for a report of IL-1 in freshly isolated epidermal Langerhans cells (126). It remains to be shown that a response of primary T cells, rather than chronically stimulated cell lines, can be blocked with antibodies to IL-1 or IL-1 receptors. For example, T lymphoblasts can engage macrophages and induce IL-1 production (102, 103), but the T-cell proliferation that occurs in these cultures is insensitive to neutralizing anti-IL-1 (103).

Attempts have been made to identify other activating factors that might be produced under dendritic cell control. These have yet to be found. Inaba et al placed dendritic–T-cell clusters on one side of a millipore filter, and T cells exposed to α-CD3 on the other side. IL-2 was produced by the clusters and crossed the filter, but no activation occurred even though the TCR complex was occupied by α-CD3 (118). This suggests that the signals provided by dendritic cells for T-cell activation are either active over short distances or remain membrane bound.
CYTOKINES AMPLIFY THE FUNCTION OF DENDRITIC CELLS While cytokine production by dendritic cells is not yet well documented, cytokines can enhance T cell–mediated immunity by amplifying the viability and function of these antigen-presenting cells. Interestingly, each active cytokine has distinct effects. Cachectin-TNF maintains Langerhans cell viability but does not enhance sensitizing function (6). IL-1 enhances the function of skin, spleen, and thymic dendritic cells but does not increase viability (3, 51, 127). GM-CSF enhances both viability and function (2, 3, 52, 128).

As yet no cytokine has been identified that upregulates the level of dendritic cell MHC products. Lymphokines like IFN-γ and IL-4 induce class-II products on macrophages and B cells, respectively, but do not similarly increase expression on dendritic cells.

Specific cytokine receptors have not been identified on dendritic cells except for the low affinity, p55, IL-2 receptor chain (CD25), first detected on mouse epidermal Langerhans cells (129). CD25 has since been found on dendritic cells from many sites—mouse spleen (10), rat afferent lymph (52), human blood (39)—but the function on these antigen-presenting cells is not known.

By influencing dendritic cell function, cytokines may play a pivotal role in immunogenicity, e.g. in skin, GM-CSF release by keratinocytes (2) may mobilize stimulatory dendritic cells. The phenotype of dendritic cells in blood, lymph, and lymphoid organs is that of a cell that has been activated by cytokines, since leukocytes commonly upregulate MHC products, adhesins, and IL-2 receptors upon activation. All of these molecules are expressed at high levels on dendritic cells (see above).

SURFACE MOLECULES CONTRIBUTING TO DENDRITIC CELL FUNCTION Many ligand receptor systems, such as LFA-1/ICAM and LFA-3/CD2 may contribute both to cell-cell adhesion and to signalling (130). Studies are under way to test the effects of mAb to these molecules on dendritic cell function, particularly in clusters of antigen-presenting cells and T cells that are the microenvironment for many responses in vitro. Because of the difficulty encountered in identifying secreted lymphocyte activating factors (above), it is important to search for specific dendritic cell surface molecules that contribute to T-cell signalling. However, it is possible that dendritic cells use surface signalling molecules that can be expressed by many cell types, such as LFA-3 and ICAM-1, but that the key feature of these antigen-presenting cells is the capacity to upregulate many adhesion/signalling systems in a T-independent fashion at the site of antigen deposition. Recent studies of human Langerhans cells indicate that dendritic cells rapidly upregulate adhesion molecules in vitro, in the apparent absence of T cells (7, 8). These maturation events, coupled with the other specializations (see...
above) in antigen handling and migration, help explain the immunogenicity of antigen-pulsed dendritic cells.

**CLONAL EXPANSION OF T CELLS** For many mitogens, such as lectins and anti-CD3 mAb, the accessory function of macrophages compares with that of dendritic cells. Mitogens may mediate macrophage–T cell clustering, which is not observed in primary antigen-specific responses. However, the T-cell function being measured is the onset of DNA synthesis, not long-term clonal expansion. When antigen-presenting cell requirements for the latter were addressed (131), dendritic cells proved to be remarkably more efficient than other antigen-presenting cells. A thousand dendritic cells mediate the optimal cloning efficiency of single T cells in the presence of lectin and exogenous IL-2. Some clones develop with just one dendritic cell, while few clones develop with up to $10^4$ monocytes. In effect, dendritic cells are able to induce long-term T-cell responsiveness to growth factors like IL-2.

Using dendritic cells as immunoadsorbents, Pancholi et al cloned T cells that had been bound in clusters (132). Macrophages had to be removed to enhance cluster formation. Antigen was needed to select but not expand the clones. These findings, plus those showing antigen capture in vivo, suggest a use for dendritic cells to select clones that are specific for physiologically relevant antigens on antigen-presenting cells in vivo.

**DISCUSSION**

This review has emphasized dendritic cells and immunogenicity. These antigen-presenting cells are in essence nature's adjuvant and allow one to study responses to specific antigens both in tissue culture and in whole animal models. The data discussed above on distinct antigen handling functions, the lack of IL-1 production, the ability to migrate and form stable contacts with T cells, the afferent and efferent limbs of antigen-presenting cells function, all have become apparent via direct analyses of this trace but specialized subset of antigen-presenting cells.

Given their role in presentation of antigens on self and allogeneic MHC molecules, it will be important to test the contribution of dendritic cells to self-tolerance. Metzinger & Guerder found that dendritic cells tolerated cells in organ cultures of fetal mouse thymus (133). Inaba et al obtained data in an Mls system that neonatal tolerance can be induced with dendritic cells, but it is the result of clonal anergy not deletion (134). Fairchild & Austyn worked out methods for placing dendritic cells within the medulla of thymic organ cultures (135); these methods should be useful for future
studies. The capacity of dendritic cells to induce suppressor cells against self-reactivity has not been tested.

Given the likelihood that many self-reactive T cells are not tolerized (136), one must consider that the avoidance of self can also reflect the extent to which a given protein is captured and presented by dendritic cells, and the extent to which dendritic cells are activated and mobilized by cytokines or other factors. In other words, tolerance in the adult operates not only at the level of the T cell but also at the level of the dendritic cell. For many antigens, the distinction is not between foreign and self, but in the extent to which a given protein, foreign or self, is captured and presented by stimulatory dendritic cells.

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