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Ralph M. Steinman

Margit D. Witmer

Michel C. Nussenzweig

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Dendritic Cells of the Mouse: Identification and Characterization

RALPH M. STEINMAN, M.D., MARGARET D. WITMER, B.Sc., MICHEL C. NUSSENZWEIG, B.Sc., LEI L. CHEN, PH.D., SALLY SCHLESINGER, AND ZANVIL A. COHN, M.D.

Department of Cellular Immunology and Physiology, The Rockefeller University, New York, New York, U.S.A.

We have identified and characterized a distinctive population of dendritic cells (DCs) in mouse spleen, lymph nodes, thymus, and liver. Dendritic cells can adhere to tissue culture surfaces but otherwise differ considerably from macrophages, the other major class of adherent cell. Morphological differences are evident by phase contrast and electron microscopy, and by cytochemistry. Dendritic cells exhibit little or no binding and phagocytosis of opsonized particles. During culture, they retain their unusual morphological features and surface markers, but lose the capacity to adhere. All DCs express and synthesize Ia antigens for several days *in vitro*, whereas only a subpopulation of mouse macrophages expresses Ia in all organs we have studied. Thus, DCs can be distinguished from macrophages in several independent and stable traits. Highly enriched preparations of the 2 cell types have been obtained.

Spleen DCs are derived from bone marrow and are present in nude mice. Dendritic cells do not proliferate, but exhibit a rapid turnover. Other features in their life history are not known.

We are studying the contribution of DCs to several immune responses. In all organs we have studied, they are powerful stimulators of the primary mixed leukocyte reaction. B cells, T cells, and macrophages from these organs are weak or inactive. Dendritic cells are potent accessory cells in T cell proliferative responses to mitogens and tuberculin antigens.

These dendritic cells and Langerhans cells may belong to a similar lineage, but to date, Birbeck granules, surface ATPase, and binding of opsonized erythrocytes have not been demonstrated in spleen dendritic cells. However, in functional assays, both DCs and Langerhans cells synthesize Ia antigens and contribute to transplantation reactions, accessory cell function, and the development of contact sensitivity.

Our laboratory has long been interested in the contribution of macrophages to the induction, or afferent limb, of the immune response [1, 2]. Our views differ from long-standing dogma because we do not believe that macrophages act as critical accessory cells in several models. There are several reasons for our position, the most compelling of which is summarized here. Specifically, the macrophage-enriched adherent cell preparations used to study accessory cell function contain another population of irregularly shaped dendritic cells (DCs). First we will summarize the properties of DCs from various tissues and then we will attempt to compare DCs with epidermal Langerhans cells (LCs).

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Reprint requests to: Ralph M. Steinman, M.D., Department of Cellular Immunology and Physiology, The Rockefeller University, New York, New York 10021.

Abbreviations:
DC: dendritic cell
EA: antibody-coated erythrocyte
HRP: horseradish peroxidase
LC: Langerhans cell
MLR: mixed leukocyte reaction

MATERIALS AND METHODS

Identification of Dendritic Cells and Macrophages

These 2 cell types are routinely distinguished by phase contrast microscopy of glutaraldehyde-fixed specimens. Adherent DCs are flat cells with many processes, abundant phase-dense granules (mitochondria), and refractile irregularly shaped nuclei [3]. Immediately after explanation *in vitro*, adherent macrophages tend to spread circumferentially. They exhibit surface ruffles, endocytic vacuoles, many lysosomes, and oval or kidney-shaped nuclei. One can distinguish the 2 cell types further by administering endocytic markers. Dendritic cells bind opsonized erythrocytes (antibody-coated erythrocytes [EAs]) weakly, if at all, and do not phagocytose a number of test particulates, including EAs, immune complexes of horseradish peroxidase and anti-horseradish-peroxidase (HRP-anti-HRP), and latex [4]. When DCs and macrophages are present in nonadherent populations, the cells are examined after centrifugation onto poly-L-lysine-coated coverslips. The DCs exhibit an array of bulbous protrusions, large thin flaps of cytoplasm, or both [5], while macrophages exhibit the same cytologic features evident in adherent preparations. Binding of EAs can also be checked on cells attached to poly-L-lysine.

Purification of Dendritic Cells and Macrophages

Currently we use 2 techniques to separate DCs and macrophages from adherent spleen populations. In both we culture the mixture of adherent DCs and macrophages overnight, and then dislodge the cells by pipetting. In 1 procedure [5], EA rosettes are formed on ice, and the suspension is centrifuged on dense albumin columns. Most DCs float and most macrophages pellet. Alternatively [6], the DC-macrophage mixture can be recultured on glass or plastic for 1 or 2 hr; most macrophages readhere firmly, and most DCs do not.

Purification of B Cells and T Cells

B lymphocytes can be obtained in high purity and yield by "panning" on anti-Ig-coated plates [7]. T cells are enriched by panning (Ig-negative population) or by passage over nylon wool. These T cell preparations differ in DC content, i.e., DCs are Ig-negative, but many adhere to nylon wool.

Mixed Leukocyte Reactions

4 to 5 × 10^6 unprimed responder spleen cells, or nylon-wool-nonadherent spleen cells, are maintained in 16-mm (diameter) tissue culture wells in 1 ml of RPMI-1640 medium supplemented with 5 × 10^{-5} M 2-mercaptoethanol and 1% heat-inactivated responder mouse serum. Stimulator cells in suspension, or adherent to 15-mm glass coverslips, are added after treatment with mitomycin C or ionizing irradiation. Proliferative activity is assayed on day 3 or 4 by replating aliquots of the cultures in fresh medium for 2 hr in ^{3}H-thymidine.

RESULTS

Distinguishing Dendritic Cells from Macrophages

Dendritic cells and macrophages have been studied and distinguished in 4 organs of mice: the spleen, lymph nodes, thymus, and liver. In all organs, they account for less than 1% of the nucleated cells in single-cell suspensions, whereas macrophages are more abundant. Although DCs and macrophages both adhere to tissue culture surfaces, the 2 cell types differ significantly.

Morphology: Dendritic cells are irregularly shaped; they

constantly form and retract tiny cytoplasmic processes in the living state [3]. Their shape and topography are best appreciated by phase contrast and scanning microscopy [3,5]. When adherent to glass or plastic, DCs are smooth-surfaced and have 1 or more large pseudopods and a variable number of smaller processes (Fig 1 and Fig 2). The predominant cytoplasmic organelles are large, phase-dense spherical mitochondria. Adherent macrophages generally spread circumferentially and exhibit surface ruffles, microvilli, and ridges (Fig 2). The cytoplasm contains variable numbers of endocytic vacuoles and lysosomes, and the mitochondria are usually rod-shaped (Fig 1). In suspension, DCs have a large number of bulbous cell processes, large thin flaps of cytoplasm or both, whereas macrophages are covered with surface ridges [5]. Cytochemistry reveals clear-cut differences between DCs and macrophages, e.g., DCs lack abundant acid phosphatase, divalent-cation-dependent ATPase, and, in spleen, hemosiderin granules [3].

Endocytic activity [4, 8]: Both *in vitro* and *in vivo,* DCs do not actively endocytose a variety of tracers, including soluble proteins; small particulates; latex; heat-killed microorganisms; and antigens coated with antibody, fresh serum as a source of complement or both. Two useful opsonized tracers are EAs and HRP-anti-HRP immune complexes.

Behavior in vitro: During culture, DCs lose the capacity to adhere to glass, whereas macrophages become more adherent [5]. Dendritic cells retain their distinctive morphological and surface properties over several days in culture [5]. Macrophages develop into larger, more actively phagocytic, and more tightly adherent cells *in vitro,* especially when the starting macrophage population is immature, e.g., blood, resident peritoneal cells, and spleen disrupted by manual rather than enzymatic means.

Ia antigens: All DCs express Ia alloantigens [5]. Expression persists for at least 4 days *in vitro,* and synthesis of I-A and I-E region products has now been demonstrated. Most Ia reagents fail to detect Ia on the majority of macrophages in all mouse tissues we have studied. We have developed several potent monoclonal rat, anti-mouse I-A reagents that usually kill less than 10 to 20% of the phagocytes in the spleen, liver, thymus, and peritoneal cavity. The expression of macrophage Ia diminishes quickly *in vitro.* The latter observation may be related to the fact that synthesis of Ia by mouse macrophages depleted of DCs has been difficult to detect.

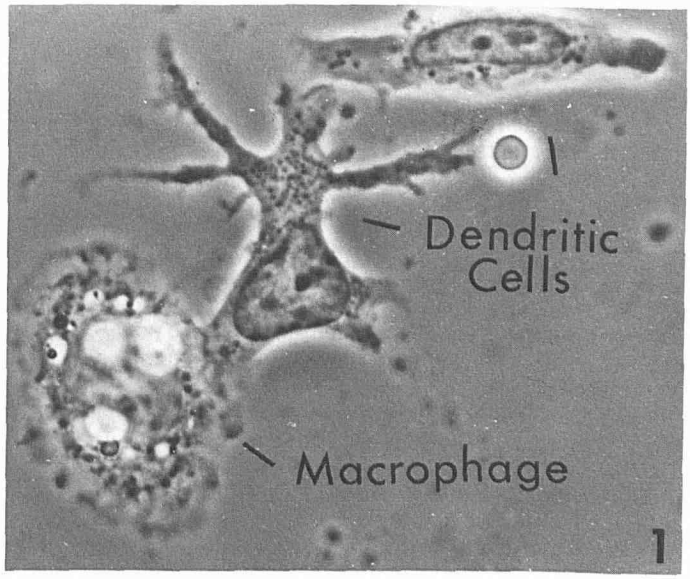

FIG 1. Phase contrast micrograph of glutaraldehyde-fixed spleen adherent cells. Dendritic cells have refractile nuclei and many spherical mitochondria. Macrophages contain endocytic vacuoles, lysosomes, and peripheral ruffles (× 3,800).

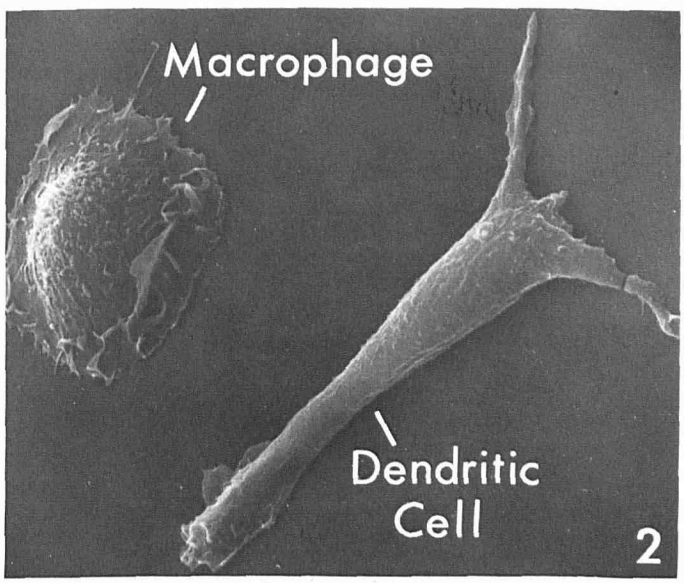

FIG 2. Scanning micrograph of a typical smooth, flat dendritic cell and a ruffled macrophage (× 4,000).

Purification of Dendritic Cells and Macrophages

The differences outlined above between DCs and macrophages have enabled us to prepare highly enriched preparations of the 2 cell types, and to monitor purification procedures [5, 6]. Recently these principles have been applied to thymus and liver as well, but our data are unpublished. In spleen, most DCs and some phagocytic cells can be dislodged from tissue culture surfaces after overnight culture. The 2 cell types can then be separated by either of the 2 procedures outlined in the Materials and Methods section. In one, DCs fail to rosette EAs, and in the other, they do not readhere firmly to glass or plastic. Both of these single-step procedures are not 100% efficient in separating the 2 cell types; consequently, the enrichment must be monitored by phase contrast microscopy, EA rosetting, and phagocytosis assays.

Life History of Dendritic Cells [9]

Dendritic cells are bone-marrow-derived, and are thymus-independent in that they are present in normal numbers in the spleens of nude mice. The bone marrow precursor has not been visualized, but is likely to be a nonadherent cell. Dendritic cells do not appear to proliferate *in situ* or *in vitro.* Their numbers remain stable in the spleen, even when active immune responses are induced. However, continuous labeling with ^{3}H-thymidine indicates that the population of splenic DCs undergoes an active turnover, i.e., at least 10% of the DC pool is replaced by labeled DCs each day. This high turnover differs considerably from that of tissue macrophages.

Dendritic Cells and Stimulation of the Primary Mixed Leukocyte Reaction

The contribution of DCs to the induction of transplantation reactions has been studied *in vitro* with the 1° mixed leukocyte reaction (MLR). Small numbers of purified DCs (3 to 10 × 10^{4}) induce maximal proliferative and cytotoxic responses when cocultured for 3 to 4 days with 5 × 10^{6} responder cells [6, 10]. There are several lines of evidence that DCs may be unique MLR stimulators in primary leukocyte populations.

First, using a quantitative assay for stimulatory capacity, we noted that DCs were 100 to 300 times more potent than unfractionated spleen cells [10]. Our current estimates, by several criteria, are that DCs represent 0.3 to 1.0% of unfractionated spleen cells.

Second, subpopulations of mouse spleen cells were obtained

by physical techniques, i.e., floatation on dense albumin columns and glass adherence. The stimulatory capacity varied with the content of DCs rather than that of B cells, T cells, and macrophages [10].

Third, enriched preparations of B and T cells from the spleen, lymph nodes, and thymus, and of macrophages from the spleen, peritoneal cavity, liver, and thymus, stimulate MLRs weakly if at all [e.g., 6, 10].

Fourth, selective depletion of B cells, T cells, and macrophages by cytotoxicity or by panning with specific antisera, does not reduce the absolute level of the stimulatory capacity in the remaining spleen cells [6, 10]. In contrast, removal of DCs with different monoclonal antibodies that recognize DCs and some other leukocytes diminishes stimulatory capacity (unpublished data).

Because of the potent stimulative capacity of DCs in lymphoid organs, we have proposed that they may be present in other organs and may be responsible for initiating transplantation reactions [10]. For example, DCs may be the critical cells that can be flushed from organs by perfusion or prolonged culture [11], either because they are passenger leukocytes or because they are intrinsic elements of nonlymphoid organs. The 1st organ in which we have looked for DCs is adult (8- to 20-wk) mouse liver. After dissociation with collagenase and a variety of enrichment steps, we have been able to identify small numbers (2 to 5 $\times$ 10^5) of DCs per mouse liver. They are powerful 1° MLR stimulators, whereas Kupffer's cells, hepatocytes, and other mononuclear cells are inactive.

Dendritic Cells as Accessory Cells

We are now looking at the contribution of DCs to the development of several antigen-dependent T cell responses *in vitro*. These include proliferation, development of killer cells, and lymphokine production. In each case, DCs can act as potent accessory cells. We still need to establish whether other types of leukocyte can function as accessory cells in these assays.

Dendritic Cells and Langerhans Cells

We have not worked directly with LCs, but the data presented in this issue provide considerable material for comparison. Dendritic cells and LCs seem similar in shape and other cytologic features. However, we have yet to identify membrane ATPase and Birbeck granules in DCs; admittedly, we have used different preparative procedures and have never had LCs as "positive controls." Dendritic cells and LCs seem to be weakly phagocytic or nonphagocytic, and it is unreasonable to refer to either as a macrophage in the absence of endocytic activity. Langerhans cells do bind EAs, whereas DCs do not in our rosetting assay. It remains possible that Fc receptors are present on DCs either in small amounts, in a masked state, with particular subclasses of antibody, or after some sort of activation step. Again we have never attempted to rosette DCs and LCs under the same experimental conditions. It is not yet possible to compare the behavior or DCs and LCs *in vitro*. Our impression is that LCs are difficult to obtain free from microbial contamination. Dendritic cells and LCs are all Ia-positive, and both cell types can synthesize Ia.

The functional similarities between LCs and DCs are especially noteworthy. *In vitro,* DCs are powerful MLR stimulators. The *in situ* data from Streilein's lab (this issue) indicates that skin LCs may be unique stimulators of major transplantation reactions across I-region differences. Both LCs and DCs act as accessory cells for T cell responses *in vitro*. Recently we have collaborated with Ptak on another functional property that may be unique to LCs and DCs. When cells are derivatized with a hapten, intravenous injection induces specific tolerance to that hapten. But Ptak et al noted that hapten-conjugated epidermal cells induced immunity rather than tolerance, and that the active cells appeared to be LCs [12]. Likewise, when purified DCs are hapten-conjugated and injected intravenously, contact sensitivity is induced. Thus, DCs are the only other cell type capable of inducing immunity rather than tolerance.

DISCUSSION

Dendritic cells are readily distinguished from other cell types that have previously been studied in lymphoid organs. We have followed DCs in tissue culture for up to 4 days, and they continue to express their repertory of unusual characteristics, including: structure, surface markers, and potent ability to stimulate the 1° MLR. We emphasize that the many differences between DCs and macrophages involve apparently independent criteria that persist for several days in culture. It is therefore difficult to argue that DCs represent some morphological or other variant of the mononuclear phagocyte. The known differences between DCs and macrophages have enabled us to separate the 2 cell types in several organs, and distinctive functional properties have become apparent.

Dendritic cells and LCs may indeed be part of an important new cell lineage with a much wider tissue distribution than skin and lymphoid organs. Obviously we need to know more about the life history of these cells and whether they move in and out of different organs in a recirculating fashion. Specific antibodies or lectins for either cell type would be useful in further characterizing the lineage and establishing its functional properties. From a functional point of view, it is obvious that DCs and LCs are both being considered in the same light, i.e., they are both thought to be critical in the induction or sensitization phase of immune responses. In contrast, the mononuclear phagocyte lineage is specialized to exhibit a variety of unique "effector" capacities critical for the final expression of immune responses. Macrophages often await "instructions" from both humoral and cellular limbs of the immune system, whereas DCs may be critical for the afferent limb.

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