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IDENTIFICATION OF A NOVEL CELL TYPE IN PERIPHERAL LYMPHOID ORGANS OF MICE*

III. Functional Properties In Vivo

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Previous studies have indicated that peripheral lymphoid organs contain a small population of an apparently novel cell type (13). These cells adhere to glass and plastic surfaces and are called "dendritic" cells, because their most striking morphological feature is the presence of many cell processes of varying size and shape. A number of tests in vitro demonstrated that dendritic cells do not have the functional properties of lymphocytes, macrophages, or non-phagocytic "reticular" cells (14). In this paper, we outline several properties of dendritic cells in situ, features which have been selected because of their demonstrated relevance in understanding the physiology of lymphoid organs. These properties include ontogeny, origin, steroid and radiosensitivity, kinetics and turnover, and response to antigen administration. In addition to their intrinsic importance, these characteristics of dendritic cells in situ further differentiate them from other previously described cell types in peripheral lymphoid organs. A most interesting feature of dendritic cells is that they are not rapidly proliferating but yet are constantly turning over at a surprisingly rapid rate under steady state conditions. The influx of new dendritic cells derives from a proliferating precursor pool located in bone marrow and in the nonglass-adherent cells of spleen.

Materials and Methods

Mice.—Inbred DBA/2, C57BL, and F_1 hybrid (B6DF_1) mice were purchased from the Jackson Laboratories, Bar Harbor, Maine. Outbred, 8-wk old, germ-free mice were obtained from Charles River Breeding Laboratories, Wilmington, Mass, while specific pathogen-free NCS mice were provided by The Rockefeller University colony.

Dendritic Cells—Dendritic cells were harvested from spleen and lymph node suspensions prepared by manually dissociating the organs with fine forceps. Treatment with 0.05% collagenase (wt/vol in medium 199, Fx I, Sigma Chemical Co., St. Louis, Mo.) was used in instances where large numbers of splenic macrophages were required (14). Dendritic cells were cultivated, identified, and quantitated as previously described (13).

Effects of Ionizing Irradiation and Steroids.—Groups of 3–4 mice were examined 2 days

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after exposure to Co^{60} in lucite containers. The surface to skin distance was 60 cm, the field uniformity 95%, and the dosage rate effective at a distance 2 cm from the animal's surface was taken to be 93% of that measured in air. Other groups of mice were exposed to an emulsion of hydrocortisone acetate (Merck & Co., Inc., Rahway, N. J.) administered subcutaneously. After both treatments, the total numbers of trypan blue negative spleen cells, as well as the number of adherent dendritic cells, were determined.

Origin of Dendritic Cells.—Lethally irradiated DBA/2 mice ($H\text{-}2^d$), which lack dendritic cells (v.i.), were intravenously reconstituted with F_1 hybrid (DBA/2 × C57BL; $H\text{-}2^d \times H\text{-}2^b$) bone marrow or spleen cells. The total numbers of viable spleen cells, and adherent dendritic cells were measured at various time points after reconstitution. The origin of the dendritic cells repopulating the recipients' spleens was determined using an anti-$H\text{-}2^b$ antiserum, prepared by injecting $H\text{-}2^b$ cells into $H\text{-}2^d$ mice and kindly supplied by E. A. Boyse and C. Iritani of the Sloan Kettering Institute, New York. The $H\text{-}2^b$ surface component is exhibited only by the donor cells, and binding of the anti-$H\text{-}2^b$ reagent was detected by a mixed hemagglutination assay. Indicator sheep red cells (Colorado Serum Co., Denver, Colo.) were coated successively with mouse, antisheep red cell serum, and pepsin-digested, rabbit antimouse immunoglobulin (courtesy of Dr. S. Gordon, Rockefeller University). The doses of the various reagents were adjusted so that 90–95% of control F_1 dendritic cells were rosetted with three–nine indicator red cells, thus permitting identification of the dendritic cells beneath the rosette. DBA/2 ($H\text{-}2^d$) dendritic cells were entirely negative. In instances where the irradiated mice were reconstituted with nonglass adherent spleen cells, the adherence step was performed on 100 mm, serum-pretreated, glass petri dishes using 6 ml of cells at a cell concentration of 1×10^7/ml.

Kinetics and Turnover.—The kinetics of dendritic cells were followed after nuclear labeling with [^{3}H]thymidine (6.0 Ci/mmol, Schwarz/Mann, Div., Becton, Dickinson & Co., Orangeburg, N. Y.). Several injection schedules were used (see Results). Autoradiograms were prepared from adherent cells fixed 5 min in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate buffer, pH 7.4 at room temperature. The cover slips were air dried, dipped in Ilford L4 emulsion (Ilford Ltd., Ilford, England), and exposed for 3–5 wk at 4°C. Following development, the preparations were examined under phase contrast microscopy to determine the percentage (labeling index) of labeled dendritic cells (three or more grains per nucleus) as well as the grain counts of individual cells. Supranuclear silver grains in dendritic cells can be distinguished from phase-dense mitochondria overlying the nucleus on the basis of their smaller size, visibility with bright field optics and location in the emulsion vs. cell plane (Fig. 7). Depending on the specific study, 500 to several thousand dendritic cells on several cover slip preparations from two or more animals were scored.

Similar studies were also performed on splenic macrophages released by collagenase digestion. Since abundant monocytes and some promonocytes are present in these preparations, cells were classified as macrophages on the basis of size and possession of numerous refractile inclusions, presumably hemosiderin granules. Care was taken to exclude macrophages that exhibited cytoplasmic labeling as a result of the ingestion of labeled cells.

Ontogeny.—Groups of NCS mice (four or more) were sacrificed at ages 1, 2, 3, 4, and 8 wk. The average, total number of nucleated cells per mesenteric lymphnode and spleen was measured, as well as the number of dendritic cells recovered in the glass adherent population.

Immunization.—NCS mice were immunized with 0.2 ml of varying doses of sheep red blood cells, intravenously and intraperitoneally. At day 4 or 5 of these primary responses, the total number of nucleated cells, and the number of adherent dendritic cells, were counted in spleen and mediastinal lymph nodes. The immune response was monitored by Jerne plaque assays (8) on the cell suspensions, using a goat antimouse immunoglobulin reagent (Hyland Div., Travend Laboratories, Inc., Costa Mesa, Calif.) to develop facilitated or indirect plaques.

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RESULTS

Sensitivity to Irradiation and Steroids.—Dendritic cells are recovered in diminished numbers from spleen following exposure of mice to Co^{60} irradiation (Fig. 1). The number of dendritic cells remaining 2 days after irradiation varies exponentially with the dose administered. In accordance with frequently used terminology (10, 19) the D_o, i.e., the dose required to reduce the number of dendritic cells to 37 % of its initial level,[1] is 100 rads. Also, the plot of the number of surviving adherent dendritic cells vs. dose of Co^{60} has no initial "shoulder", i.e., the y-intercept represents the control, unirradiated number of cells (Fig. 1). This implies that dendritic cells may be killed by a single "hit" generated by ionizing irradiation. The total number of spleen cells also diminishes exponentially with the doses of Co^{60} employed (Fig. 1). A D_o of 195 rads was observed, though most likely these data represent effects on a number of subpopulations of cells of varying radiosensitivity. Splenic macrophages were readily identified in adherent populations at all doses of Co^{60} administered, but quantitative data using collagenase treated preparations were not obtained.

It would appear that ionizing irradiation is directly lethal to dendritic cells in spleen. As will be shown below, recovery of dendritic cell numbers produced by adoptive transfer experiments is almost entirely due to cells of donor origin. Also, irradiation seems to be affecting splenic dendritic cells themselves rather than some precursor pool, for the rate of influx from this pool (see below) is too low to account for the dramatic decreases seen just 2 days after irradiation.

The total number of viable spleen cells (Fig. 2 A) and dendritic cells (Fig. 2 B) also both diminished following subcutaneous administration of emulsified hydrocortisone acetate. After a 1-mg dose, the number of dendritic cells actually recovers more quickly than the total number of spleen cells (Fig. 2) so that transiently elevated concentrations of dendritic cells are seen. After higher (2.5 and 5.0 mg) doses, both dendritic cell and total spleen cell numbers recover slowly and do not reach normal levels for 3–4 wk (Fig. 2). Splenic macrophages were never decreased in number at any of the time points studied.

Origin of Dendritic Cells.—Lethally irradiated DBA/2 mice lack dendritic cells, but can be reconstituted with bone marrow or spleen cells from syngeneic or F_1 hybrid (DBA/2 × C57B1) donors. 50×10^6 thymus cells did not reconstitute dendritic cell numbers although the animals died within 5–9 days after transfer; addition of thymocytes to the bone marrow inocula also failed to increase the number of dendritic cells.

With the doses of marrow (10×10^6) and spleen (40×10^6) cells employed, the total number of spleen cells in the recipients returns to normal levels in 1–2 wk following an initial lag period of 4 days (Fig. 3 A). For dendritic cells (Fig. 3 B), the lag period before the onset of recovery was 1 wk using bone

[1] *Abbreviations used in this paper:* D_o, the dose required to reduce the number of dentritic cells to 37% of its initial level; T_{GEN}: average generation time.

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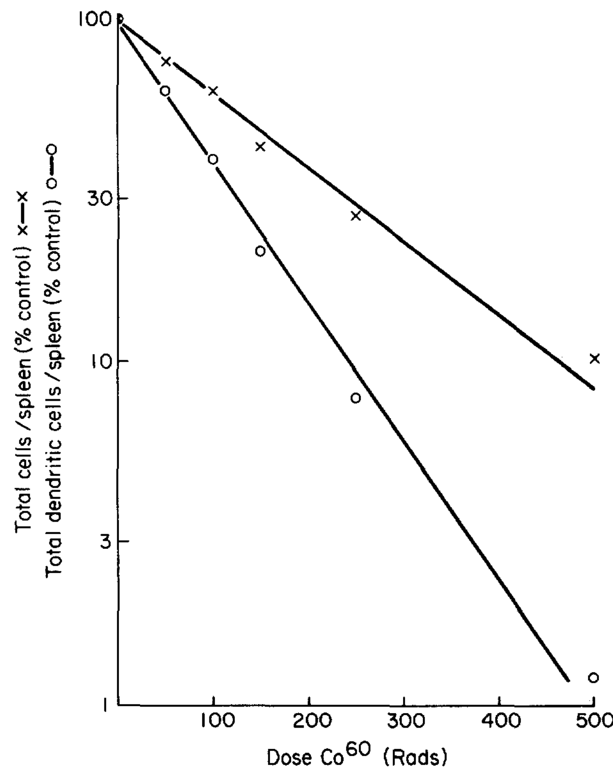

FIG. 1. Radiosensitivity of dendritic cells. DBA/2 or C57BL mice were exposed to varying doses of Co^{60} gamma irradiation. 2 days later, spleens were taken from groups of three or four animals, and the total number of viable nucleated cells (x—x), as well as dendritic cells (O—O) were determined. The values plotted are the means from four experiments. Dendritic cells appear to be more radiosensitive than the total nucleated cell population of spleen. Their numbers diminish exponentially with the dose of Co^{60} administered. The D_0 is 100 rads, compared to 195 rads for total spleen cells.

marrow as a source, and 2 wk using spleen, but the rate of reconstitution once begun appeared to be similar with both sources. The doubling time during reconstitution was 2–3 days. When donor spleen cells were depleted of dendritic cells by glass adherence, the recovery of dendritic cells in the recipient was identical to that observed with whole spleen. When either spleen or marrow from F_1 donors were transferred, the dendritic cells appearing in the recipient were of donor origin (Fig. 3 B), i.e., they exhibited in mixed hemadsorption assays the $H\text{-}2^b$ surface antigen unique to donor cells.

We conclude from these data that dendritic cells must originate from precursors found in both bone marrow and nonadherent spleen suspensions. Since neither population contains dendritic cells per se, there must be a morphologically distinct precursor to splenic dendritic cells. The precursors in spleen

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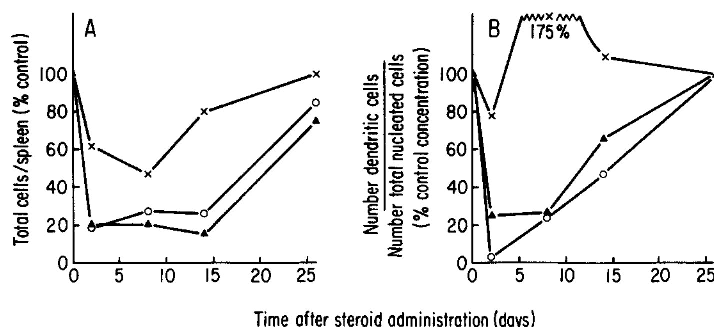

FIG. 2. Steroid sensitivity of dendritic cells. 1.0 (x—x), 2.5 (▲—▲), and 5.0 (O—O) mg of hydrocortisone acetate were administered subcutaneously to groups of 3-mo old NCS mice. The total numbers of viable spleen cells (Fig. 2 A), and the concentration (no. dendritic cells/no. total nucleated cells) of dendritic cells (Fig. 2 B) were determined at varying time points after drug administration. All doses of steroid produced an initial decrease in the number and concentration of splenic dendritic cells 2 days later. With time the percentage of dendritic cells recovers to adult levels, though this takes 3–4 wk after the 2.5 and 5.0 mg doses. The data are means of two experiments.

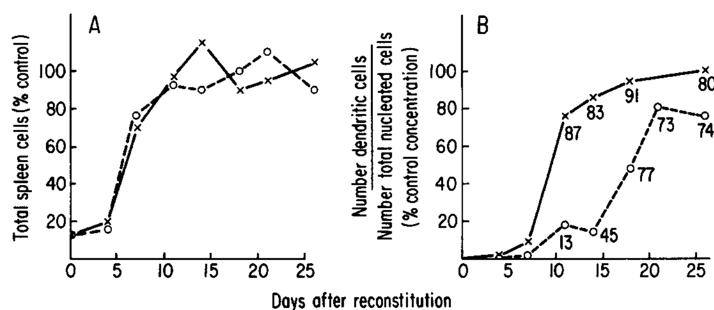

FIG. 3. Origin of dendritic cells. DBA/2 (H-2^d) mice were exposed to 900 rads of Co^{60} irradiation, a dose sufficient to eliminate more than 95% of splenic dendritic cells. Immediately after irradiation, the mice were reconstituted intravenously with 10^7 bone marrow (x---x) or 4×10^7 spleen (O---O) cells from DBA/2 × C57BL (H-$2^d \times H$-2^b) F_1 hybrids. At the doses of bone marrow and spleen employed, the recovery of total spleen cell numbers (Fig. 3 A) is similar and reaches control levels by 11 days. Dendritic cells also repopulate the recipients' spleens, although the onset of recovery is more delayed following adoptive transfer of spleen vs. bone marrow cells (Fig. 3 B). In both instances however, the majority of new dendritic cells are of donor (F_1) origin (percentages next to designated points) since they exhibit H_2b surface antigens in mixed hemadsorption tests.

presumably derive from the marrowlike regions of red pulp. The data suggest that marrow is a richer source of precursors than spleen, since dendritic cells repopulate recipients of marrow faster, even with lower doses of transferred cells.

Kinetics and Turnover.—Splenic glass adherent dendritic cells are not rapidly proliferating. Their labeling index is only 1.5–2.5%, 1 h after an intravenous or

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intraperitoneal pulse of [^{3}H]thymidine. Similar values were obtained when splenic dendritic cells were exposed to [^{3}H]thymidine in vitro (14). These data confirm that dendritic cells are not large lymphocytes, which label with a much higher frequency in vitro (6) and in vivo (5, 11).

In spite of their low rate of proliferation, splenic dendritic cells appear to undergo substantial turnover, i.e., following pulse or continuous labeling with [^{3}H]thymidine, labeled dendritic cells rapidly replace nonlabeled ones. Steady state conditions pertain in these experiments as the total numbers of dendritic cells in spleen remains constant. The rate of turnover, defined as the rate at which labeled dendritic cells replace unlabeled ones, can then be determined by measuring the labeling index at varying time points after the start of the experiment.

Following a pulse of [^{3}H]thymidine (a single intraperitoneal injection or four intramuscular injections over 12 h), labeled dendritic cells appear at a constant rate of 4–6% per day for 5 days, and then begin to disappear (Fig. 4). The y-intercept of these plots is about 2% and represents the initial level of cell division in spleen. Splenic macrophages turnover more slowly, less than 1% per day (Fig. 4 B). The influx of labeled dendritic cells during the first 5 days after a thymidine pulse is accompanied by an increase (about twofold) in the total number of grains per 1,000 dendritic cells (Fig. 4), even though the mean grain count of the unlabeled cells is decreasing (Fig. 5). It is unlikely that re-

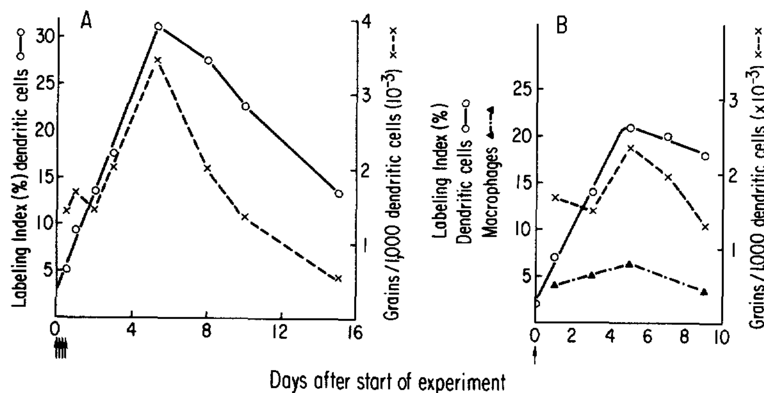

FIG. 4. Turnover of splenic dendritic cells following pulse labeling. Groups of NCS mice were pulse labeled with [^{3}H]thymidine, 6.0 Ci/mmol, using four, equally spaced, 1.0 μc/gm intramuscular injections (arrows) over 12 h (Fig. 4, A) or a single intraperitoneal injections of 4.0 c/gm (Fig. 4 B). With both regimens, the labeling index or percentage of labeled dendritic cells (O—O) increases linearly at a rate of 4–6% per day for 5 days, and then falls off more slowly. The influx of labeled splenic macrophages (▲-·-▲) following the intraperitoneal pulse proceeds at a slow rate of 0.5% per day. Concomitant with the influx of labeled dendritic cells in both experiments, there is an increase in the total number of grains per 1,000 total labeled and unlabeled dendritic cells (x---x), indicating that the turnover involved influx of labeled cells into the spleen.

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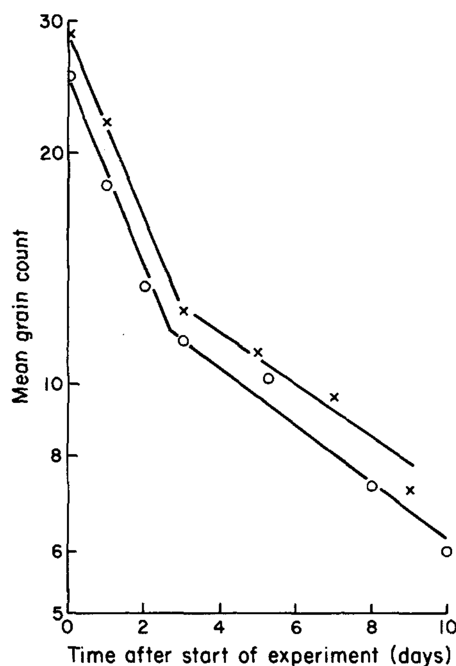

FIG. 5. Mean grain counts of dendritic cells following pulse labeling. Following both intramuscular (O—O) and intraperitoneal (x—x) pulses of [^{3}H]thymidine depicted in Fig. 4, the mean grain count of labeled dendritic cells decreases exponentially. The average generation time of dividing cells in this system, as indicated by the initial half-life of mean grain count decay, is 60 h.

utilization of [^{3}H]thymidine could account for these results. The increase in labeled dendritic cells and in total label occurring over the first 5 days must therefore represent an influx of labeled cells into the spleen from a precursor compartment also labeled during the pulse. We assume that this compartment is located in bone marrow, and/or in the marrowlike regions of splenic red pulp, since both sources could reconstitute dendritic cells depleted by Co^{60} irradiation (Fig. 3 B).

The average generation time (T_{GEN}) of cells proliferating in these pulse experiments can be estimated from an analysis of the mean grain counts of labeled cells. If one assumes that T_{GEN} is similar for both dendritic cells labeled initially in spleen, and cells which migrate into the spleen from the precursor compartment, then the mean grain count of labeled dendritic cells should fall exponentially with time, the half-life representing the T_{GEN}. Instead, a rapid and a slow exponential component was observed during the grain count decay (Fig. 5). Several explanations are possible, but it is likely that the slow component is an artefactual one. Since one only considers labeled cells in obtaining mean grain counts, it is likely that one overlooks progeny that contain insufficient [^{3}H]thymidine to produce the threshold number (three or more) of autoradiographic

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grains (9). Omission of such "labeled" but undetectable cells artificially raises the mean grain count of the detectable ones. In any case, the initial slope of the mean grain count decay curve suggests that the T_{GEN} in this system is 50–60 h. This value is similar in magnitude to the rate at which the number of dendritic cells double in irradiated spleens repopulated with spleen or bone marrow (Fig. 3 B).

The turnover rate (4–6% per day) of dendritic cells in pulse labeling experiments represents a minimum value, as one overlooks the possible influx of cells not incorporating thymidine at the time of the pulse. A closer approximation of the actual turnover rate can be obtained by "continuous" labeling, i.e., intraperitoneal injections of precursor every 12 h throughout the experiment. Under these conditions (Fig. 6), labeled splenic dendritic cells appear at a rate of 9–12% per day, and most if not all cells participate in this turnover. This enables one to state that the equivalent of a total pool of splenic dendritic cells must turnover at least every 8–11 days (100%/9–12%), a value which might be even more rapid if a truly continuous labeling regime had been employed. Splenic macrophages turn over four times more lowly than dendritic cells in these same animals (Fig. 6).

The turnover of splenic dendritic cells can be further characterized by a cohort analysis of the grain counts of labeled cells after pulse labeling. Shortly after administration of [^{3}H]thymidine, most of the grain counts fell in the 21–40 range (Figs. 7, 8). Cohorts were therefore selected containing 21–40,

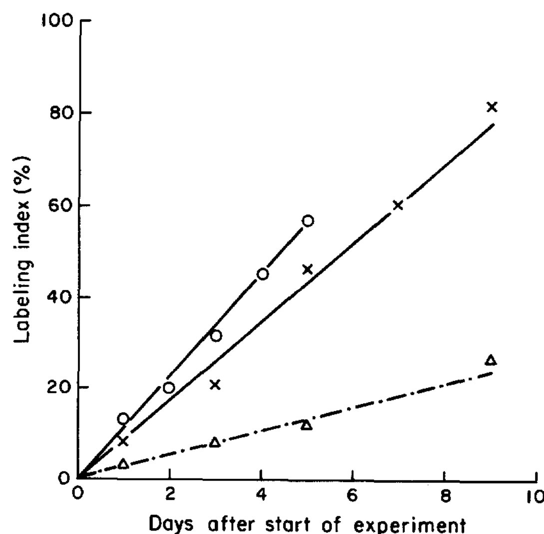

FIG. 6. Turnover of splenic dendritic cells following intraperitoneal [^{3}H]thymidine, 0.25 μc/gm, every 12 h. With more frequent injections of labeled precursor, it can be seen that labeled dendritic cells replace unlabeled ones at more rapid rates than observed with pulse labeling (Fig. 4). In two experiments the turnover rate was 9% (x—x) and 12% (○—○) of the total splenic pool per day. The turnover of splenic macrophages (△—△) is only 2.5% per day.

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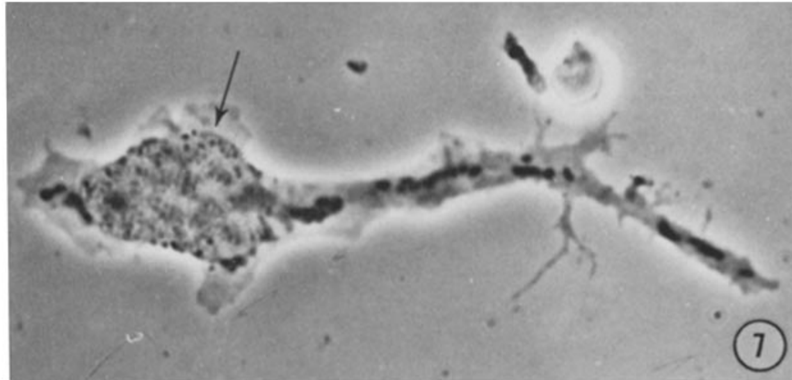

FIG. 7. A phase contrast micrograph of a labeled dendritic cell shortly after administration of an [$_3$H]thymidine pulse in situ. The supranuclear silver grains (arrow) are smaller than the larger phase dense mitochondria. × 5,000.

11–20, 6–10, and 3–5 grains. The assumption in these studies is that the grain count of a cohort reflects the amount of DNA that was synthesized during the pulse label; halving of the cohort's grain count indicates the completion of one additional cell cycle relative to the next more heavily labeled cohort.

During the first five days following administration of labeled DNA precursor, the number of labeled cells with 21–40 grains changes very little while cells with lower grain counts increase in number (Fig. 8). The rate of increase is greater in the more lightly labeled cohorts, implying that these cells are progeny of an expanding population. Although assignment of cells to a given cohort is arbitrary, the dilution in grain counts from 21–40 to 3–5 grains indicates that some 3–4 cycles of division are taking place.

After reaching a peak at 5 days, the labeling index of many of the cohorts of splenic dendritic cells decreases in exponential fashion. This might suggest that the turnover of dendritic cells (here defined as the rate at which unlabeled cells replace labeled ones) is a random process, since a constant fraction of cells disappears per unit time. Unfortunately, the system is too complex to permit this conclusion since cell division may be occurring in "mature" glass adherent splenic dendritic cells, as well as in the precursor compartment. In addition, division may occur preferentially in spleen cells recently derived from the precursor pool. The exponential decrease in the number of labeled cells may thus simply reflect a loss of cells from a cohort due to a cell division, rather than some other random turnover process.

Response to Antigens.—We examined three situations to see if the number of dendritic cells in peripheral lymphoid organs is altered during a primary immune response. In all instances, the phase contrast morphology of dendritic cells was not altered.

5 days after intravenous immunization with varying doses of sheep red blood cells, the total number of dendritic cells in spleen was not changed (Table

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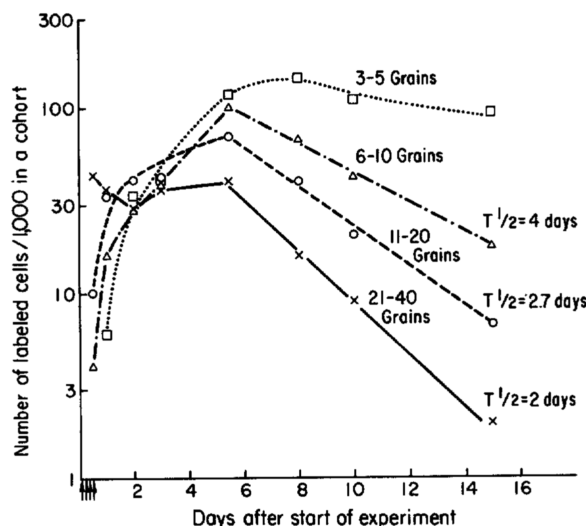

FIG. 8. Cohort analysis of pulse labeled dendritic cells. Cells were assigned to different cohorts according to their grain counts after four intramuscular injections of thymidine at the start of the experiment (Arrows). The cohorts were 3–5 (□···□), 6–10 (△–·–△), 11–20 (○---○), and 21–40 (x—x) grains. The number of cells in the most heavily labeled cohort changes little for 5 days and then diminishes exponentially with a half-life of 2 days. The labeling indices of other cohorts first increases and then also diminishes exponentially, although the half-lives are longer. Similar cohort data were obtained following a single intraperitoneal pulse of [^{3}H]thymidine.

TABLE I

Response of Spleen 5 Days after a Primary Injection of Sheep Red Blood Cells i.v.

Dose of sheep red blood cells i.v.	PFC/10^6 spleen cells		Total cells/spleen ($\times 10^{-7}$)	Dendritic cells/spleen ($\times 10^{-5}$)
	Direct	Indirect		
0	2	0	10.8	9.9
5×10^5	20	12	10.5	9.8
5×10^6	50	100	11.8	10.5
5×10^7	338	363	14.0	9.8
5×10^8	187	975	16.2	11.0

With increasing numbers of sheep red cells administered, the number of PFC as well as the number of total nucleated cells increase, but the number of dendritic cells remains constant.

I), whereas the total number of nucleated cells did increase with increasing doses of antigen. When a single dose of 5×10^8 sheep cells was given intraperitoneally, the number of nucleated cells and dendritic cells in spleen were not altered on days 4 (Table II) and 8 (data not shown). However the mediastinal lymph nodes, the nodes to which intraperitoneally administered particles drain (7), exhibited a detectable increase in both dendritic and total nucleated

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cells. These increases are small relative to the dramatic rise in specific antibody-secreting, or plaque-forming, cells.

The failure to observe an increase in splenic dendritic cell numbers following immunization may reflect a high level of ongoing antigenic stimulation, e.g., from antigens entering via the gastrointestinal tract. We observed that both the total number and concentrations of dendritic cells was increased (13- and 2-fold respectively) in germ-free mouse spleens, 2 wk after a return to a non-axenic environment (Table III). Similar observations were made on the mesenteric lymph nodes in these mice (Table III).

Ontogeny.—The total number of nucleated cells in mesenteric lymph nodes and spleens of NCS mice increases progressively during the first two months of life, and reaches adult levels by 8 wk. The total number of dendritic cells also increases, but the number of dendritic cells/nucleated cell does not reach adult levels until 3 wk in lymph node and 3–4 wk in spleen (Fig. 9). The number of splenic macrophages was not quantitated in these preparations, but even neonates had considerable numbers of typical macrophages, in contrast with a total absence of dendritic cells. These data are consistent with morphologic observations on the ontogeny of splenic white pulp nodules in situ (R. M. Steinman,

TABLE II

Response of Spleen and Mediastinal Lymph Nodes 4 Days after Sheep Red Blood Cells (5×10^8) i.p.

Organ	Day assayed	PFC/10^6 cells		Total cells/organ ($\times 10^{-7}$)	Dendritic cells/organ ($\times 10^{-5}$)
		Direct	Indirect		
Spleen	0	6	3	11.6	6.2
Spleen	4	960	500	11.2	6.2
Node	0	0	0	0.7	0.08
Node	4	220	84	1.5	0.5

Both spleen and nodes show substantial increases in the numbers of PFC. Dendritic cells do not increase in spleen during this immune response, and increase moderately in node.

TABLE III

Response of Spleen and Mesenteric Lymph Nodes in Germ-Free Mice 2 wk after Returning to a Nonaxenic Environment

Organ	Total cells/organ ($\times 10^{-7}$)	Dendritic cells/organ ($\times 10^{-5}$)
Axenic spleen	2.3	0.6
Nonaxenic spleen	14.5	8.0
Axenic node	0.2	0.03
Nonaxenic node	2.1	0.9

Both total nucleated cells and dendritic cells increase substantially. The concentration of dendritic cells also increase two- to three-fold.

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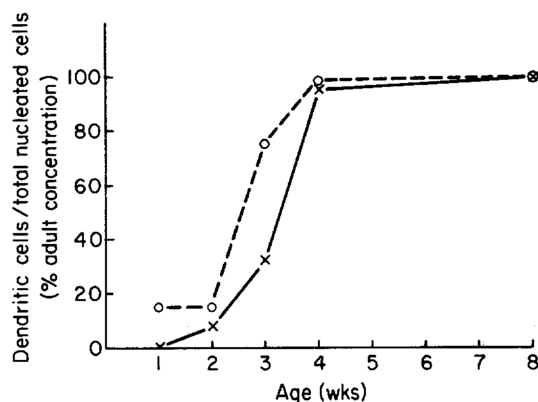

FIG. 9. Ontogeny of dendritic cells. Spleens and mesenteric lymph nodes were obtained from groups of four or more NCS mice. For each cell suspension, 10^6 nucleated cells were plated on 100-mm cover slips and the number of dendritic cells per 200 oil immersion fields were counted. The concentration of dendritic cells, i.e., the number of cells/total nucleated cells reaches adult levels at 3 wk in mesenteric node (O—O) and at 4 wk in spleen (x—x).

unpublished observations). Cells with cytological features similar to dendritic cells in vitro are found only in white pulp of spleen (15), while macrophages are found both in red pulp and white pulp. Neonatal spleen contains little white pulp, and typical adult amounts do not appear until 3–4 weeks of age.

DISCUSSION

This study documents several properties of lymphoid dendritic cells in situ. The central feature concerns their origin and kinetics, for which a tentative model has been proposed (Fig. 10). The details of this model include: (*a*) Splenic dendritic cells are not rapidly proliferating since their pulse labeling index is only 1.5–2.5%. (*b*) Although the total number of splenic dendritic cells remains remarkably constant in the mice we have studied, they are constantly being replaced or turned over at a rate of 10% or more per day. (*c*) New dendritic cells are derived from a precursor compartment in which several (three or more) cycles of cell division appear to take place and have a generation time of 2–2½ days. (*d*) Adoptive transfer experiments demonstrate that dendritic cell precursors exist in bone marrow and in the nonglass-adherent population of spleen, the latter presumably including cells from the marrowlike regions of the red pulp. (*e*) Since dendritic cells cannot be identified as such in marrow and nonglass-adherent spleen cells, the precursor cell must have different morphological features from the "mature" elements which are being characterized in spleen.

There are many questions related to this model of dendritic cell kinetics and compartmentalization which are unclear at this time. These include: Is the turnover of dedritic cells a random or linear process? Do all dendritic cell precursors undergo a specified number of cell divisions? What is the transit time and route taken by precursors to arrive in spleen? Unfortunately, thymidine-

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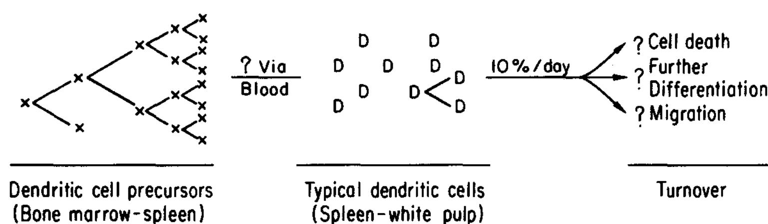

FIG. 10. A model to explain several of the observations on the kinetics of dendritic cells in spleen. The typical dendritic cells (*D*) which can be isolated from spleen probably are derived from white pulp nodules. Relatively few divide in spleen, but all appear to turnover at a substantial rate, 10+% per day. The mechanism of turnover and the anatomical compartment responsible are not known. The turnover of splenic dendritic cells is balanced by an influx of new cells from a precursor compartment. This precursor pool can be labeled with [^{3}H]thymidine, and appears to be capable of three or more cycles of cell division. The precursor cells (*x*) are located in bone marrow and spleen, probably the marrowlike regions of splenic red pulp. They cannot be identified directly and lack the morphological features of dendritic cells that are identified in the glass adherent population of spleen.

labeling data are too complex to answer these questions. The chief difficulty is that proliferation is possible in both precursor and mature dendritic cell compartments, so that one cannot assess the relative contributions of each to the overall measurements. In vitro experiments are also not yet practical since dendritic cells cannot be studied at length in tissue culture (14), and the presumptive precursor cells cannot be directly identified.

The mechanism responsible for dendritic cell turnover is unknown. Several possibilities exist (Fig. 10): dendritic cells may have a short life span; they may emigrate from the spleen to other sites; they could be converted to another cell type; or they may assume properties which make it impossible to recover them by current methods, i.e., by preparing glass adherent populations from single cell suspensions. The latter possibility seems relevant since we have recently observed that dendritic cells in suspension can form aggregates with a subpopulation of splenic lymphocytes. Following aggregrate formation, there is also a substantial decrease in the ability of the component dendritic cells to adhere to glass. Conceivably a similar process is occurring in situ; i.e., dendritic cells that turnover as a result of aggregate formation are replaced by an influx from the precursor pool.

Several of the properties described for dendritic cells distinguish them from members of the mononuclear phagocyte lineage, a distinction previously established by morphological criteria (13), and functional tests (14) in vitro. Dendritic cells can be identified in adequate numbers in mouse spleen and lymph nodes at only 3–4 wk of age whereas even neonates contain typical macrophages. Dendritic cells cannot be recovered in adherent cell populations following administration of low doses of steroids (2.5 mg hydrocortisone acetate s.c.) or ionizing radiation (D_o for Co^{60} of 100 rads), whereas macrophages in spleen (this study) and peritoneal cavity (16) are readily identified under these conditions. The kinetics and turnover of splenic dendritic cells are also quite

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different from mononuclear phagocytes. Tissue macrophages of mouse spleen (this study) and peritoneal cavity (18) turnover more slowly (2–3% per day) while blood monocytes, the precursors of tissue macrophages, are replaced at a more rapid rate, 40% of the total pool per day (18).

Although dendritic cells lack the morphological and functional properties of lymphocytes (13, 14) many of their responses in vivo are superficially similar to lymphoid cells. Both dendritic cells and B lymphocytes do not reach adult concentrations for some time after birth (12), are sensitive to steroids (2) and ionizing radiation (1, 3), and originate from bone marrow. In contrast to B cells we have demonstrated in this study that dendritic cells do not undergo a profound increase in numbers, during an immune response. However, the properties of both dendritic cells and B lymphocytes should be considered in interpreting variations in the functions of whole lymphoid organs. For example, lymphoid germinal centers and the development of immunologic memory are both disrupted following administration of steroids (4) or ionizing radiation (1, 3, 17). The effects may in part reflect the sensitivity of dendritic cells to these agents, and suggest a cooperative role for dendritic cells in the immune response. These notions are speculative at this time and must await the results of current studies on the functions of dendritic cells.

SUMMARY

Several properties of lymphoid dendritic cells in situ have been determined, and contrasted to information previously established for lymphocytes and mononuclear phagocytes. Dendritic cells are not found in newborn mice, and their concentration in both spleen and mesenteric lymph node does not reach adult levels until 3–4 wk of age. Dendritic cells largely disappear from adherent populations following administration of steroids (2.5 mg hydrocortisone acetate s.c.) and ionizing radiation (D_o of 100 rads for Co^{60}). Splenic dendritic cells can originate from precursors located in both bone marrow and spleen itself, probably the red pulp. The mature splenic population does not actively divide (pulse labeling index with [^{3}H]thymidine of 1.5–2.5%), but does turnover at substantial rate, 10+ % of the total pool per day. The influx of new cells appears to be derived from a proliferating precursor compartment, but the mechanism for efflux or turnover is not known. Dendritic cells in spleen and node undergo little or moderate increase in numbers during development of a primary immune response. These in vivo characteristics, taken together, further distinguish dendritic cells as a novel cell type, distinct from mononuclear phagocytes and lymphocytes.

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