DEMONSTRATION AND CHARACTERIZATION OF
Ia-POSITIVE DENDRITIC CELLS IN THE INTERSTITIAL
CONNECTIVE TISSUES OF RAT HEART AND OTHER
TISSUES, BUT NOT BRAIN*

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Nonlymphoid accessory cells play an important role in antigen presentation (1, 2). This accessory cell function has been attributed to macrophages, but more recent data suggest that a specialized dendritic cell, probably in the macrophage lineage but quite distinct from conventional macrophages, is probably the cell that is primarily involved in antigen presentation (3). The best-characterized cell of this type is the dendritic cell, described by Steinman et al. (4), in the spleen and lymph nodes of the mouse. Another cell type of similar dendritic morphology, but with some distinctive features, is the Langerhans's cell (5), which has been definitely implicated in antigen presentation (6). The precise relationship of the Steinman dendritic cell and the Langerhans's cell to one another and to other Ia-positive cells of similar morphology in thymus (7) and spleen and lymph nodes (8) is unknown. However, that these dendritic cells are likely to play a vital role in the physiology of the immune system is demonstrated not only by the capacity of at least some dendritic cells to function as accessory cells, but also by the recent demonstration that the Steinman cell is an extraordinarily potent stimulator of T lymphocyte division, both in allogeneic (9) and syngeneic culture (10).

To date, the Steinman cell has been reported only in the spleen and lymph node, with the liver being reported as lacking these cells (11), whereas the Langerhans's cell has been described in skin (5) and the oral and esophageal (12) mucosa. These are sites of potentially high antigen exposure, so the presence of antigen-presenting cells in these areas might be expected. However, in this paper we demonstrate that dendritic cells similar in every respect to the Steinman cell are present in large numbers in the interstitial connective tissues of all rat tissues we have examined (heart, liver, pancreas, thyroid, skin, skeletal muscle, kidney, ureter, bladder) except brain. The presence of such cells in secluded organs such as the heart in numbers similar to that seen in areas such as skin was unexpected. It is further evidence that these cells are likely to have a vital function in immunity, and suggests that their role might be broader than the presentation of environmental antigens.

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Materials and Methods

Rats. Inbred male DA (RT1\(^{a}\)) rats were used in most experiments, but to test for possible strain variations in the findings, other rat strains including the nude rat were studied. AGUS (RT1\(^{a}\)) and F344 (RT1\(^{b}\)) rats were supplied by Bantin and Kingman Ltd. (Hull, England). PVG/c (RT1\(^{c}\)), WAG (RT1\(^{i}\)), and the homozygous nude rat (Ola: RNU) were obtained from Olac 1976 Ltd (Bicester, Oxon, England). AS (RT1\(^{a}\)) and AUG (RT1\(^{-}\)) rats were a gift from the McIndoe Research Unit (Sussex, England) and the BN (RT1\(^{b}\)) rats were a gift from Dr. I. V. Hutchinson (St. Mary's Hospital, London).

Monoclonal Antibodies. The following mouse monoclonal antibodies were used: (a) F17-23-2, which is directed against rat RT1.B (la-like, or class II) antigens. This antibody has been fully characterized (13, 14) and reacts with inbred rat strains of the a, l, and n haplotypes but not with c or u haplotypes. (b) F16-4-4, which is directed against rat RT1.A (SD-like, or class I) antigens. This antibody has been described elsewhere (14, 15) and reacts with all haplotypes examined so far, including a, l, n, c, and u. (c) MRC OX1, which is directed at the rat leukocyte common (LC)\(^{1}\) antigen, a leukocyte-specific molecule widely distributed among leukocytes but absent from other tissues (16, 17). (d) W3/13, which is directed at an antigen present on rat T lymphocytes, granulocytes, and macrophages (18). The control monoclonal antibody used in these studies was F3-20-7, which is directed at the canine homologue of Thy-1 (19) and does not react with rat tissues.

Conventional Antisera. Immunoadsorbent purified rabbit F(ab')\(_2\) anti-mouse F(ab')\(_2\) (RAM), rabbit F(ab')\(_2\) anti-rat F(ab')\(_2\) (RAR) and goat F(ab')\(_2\) anti-rabbit F(ab')\(_2\) (GAR) were prepared as described previously for GAR (20). In addition, RAM depleted of antibodies cross-reacting with rat immunoglobulins was prepared by passing the rabbit anti-mouse F(ab')\(_2\) serum through a rat F(ab')\(_2\) column before passage through and elution from the mouse F(ab')\(_2\) column. Mouse immunoglobulins were pepsin degraded at a pH of 4.1.

The immunoadsorbent-purified antibodies were labeled with fluorescein isothiocyanate by incubating in bicarbonate buffer, pH 9.5, at room temperature for 3 h, using standard techniques (21). Uncoupled fluorescein was removed by use of small desalting columns containing Sephadex G-25 (PD-10; Pharmacia Fine Chemicals, Inc., Uppsala, Sweden).

Immunofluorescence. Tissues were removed from exsanguinated rats and immediately frozen in liquid nitrogen. Cryostat sections of -6 \(\mu\)m were cut, transferred to gelatinized slides, and air dried for 15 min at 4°C. The smaller specimens such as ureter were mounted on blocks of liver to facilitate the cutting of sections. The sections were washed three times in phosphate-buffered saline (PBS) (Dulbecco's A and B media, Oxoid Ltd., London) and then incubated 30 min with monoclonal antibody at a dilution known to saturate all the antigen sites on the section. The antibodies raised in this laboratory (F17-23-2, F16-4-4, and F3-20-7) were partially purified from immune ascites by ion-exchange chromatography (22). Saturating concentrations of antibody were calculated from preliminary titrations of the antibody on lymph node lymphocyte targets, using saturating binding assays (23). The sections were washed three times and then incubated with 50 \(\mu\)g/ml of RAM depleted of antibodies cross-reactive with rat immunoglobulin as described in the preceding section. In addition, the RAM was incubated with 125 \(\mu\)g/ml of rat F(ab')\(_2\) for 1 h before use. After a further three washes, the slides were incubated with fluorescein-labeled GAR at 50 \(\mu\)g/ml. The GAR had cross-reactive antibodies blocked by incubating it with 125 \(\mu\)g/ml of rat F(ab')\(_2\) for 1 h before use. After a further three washes, the sections were mounted in 90% glycerol (vol:vol) in PBS and examined with a Leitz Ortholux II fluorescence microscope (E. Leitz, Inc., Rockleigh, N. J.).

Double labeling with anti-LC and anti-Ia was performed by first labeling the section with one monoclonal antibody and fluorescein-labeled RAM, photographing the field with the section mounted in PBS, then labeling with the second monoclonal antibody and fluorescein-labeled RAM, and rephotographing the same field.

Abbreviations used in this paper: LC, leukocyte common; GAR, goat F(ab')\(_2\) anti-rabbit F(ab')\(_2\); NSE, nonspecific esterase; PBS, phosphate-buffered saline; RAM, rabbit F(ab')\(_2\) anti-mouse F(ab')\(_2\); RAR, rabbit F(ab')\(_2\) anti-rat F(ab')\(_2\).
Immunoperoxidase Labeling. This was performed with the same techniques used for immunofluorescence except that the sections were fixed in cold acetone for 4 min at 4°C to preserve the tissue architecture in subsequent steps. The other modification was the use of a horse radish peroxidase-conjugated immunoglobulin fraction of a swine anti-rabbit immunoglobulin serum (Dako Immunoglobulins Ltd., Copenhagen, Denmark) in place of GAR. This was used at a 1:20 dilution and preblocked with rat IgG at 250 μg/ml. The sections were developed with diamino tetrahydrochloride and 0.01% H2O2. The sections were counterstained with hematoxylin and mounted in DPX (Raymond Lamb, London).

Histochemistry

Histochemical stains were applied to unfixed fresh-frozen sections.

Non-Specific Esterase. Nonspecific esterase activity was determined by the method of Ornstein et al. (24) using a commercial kit (Sigma Chemical Co., London) and α-napthyl butyrate as a substrate at pH 6.0. The effects on the staining pattern of substituting α-napthyl acetate as a substrate and adding the enzyme inhibitor sodium fluoride in a concentration of 1.5 mg/ml were each tested on a single occasion. The sections were either counterstained with cresyl blue or hematoxylin to demonstrate nuclei, or fixed with cold acetone at 4°C for 4 min to allow the stain to be retained during additional fluorescence labeling.

Acid Phosphatase. Acid phosphatase activity was demonstrated by the use of sodium naphthol AS-BI phosphate (Sigma Chemical Co., London, England) as substrate at pH 5.0 (25). The sections were either counterstained or fixed for additional fluorescence studies as mentioned in the preceding section.

β-Glucuronidase. β-Glucuronidase staining was performed using sodium naphthol AS-BI β-d-glucuronic acid (Sigma Chemical Co.) as substrate at pH 5.0 (26).

ATPase Staining. ATPase staining was kindly performed by courtesy of Dr. M. Esiri, Neuropathology, Radcliffe Infirmary, Oxford (27).

Phagocytic Function Studies. Colloidal carbon (India ink, Rowney Kandohar, England) was administered intravenously (300 μl in 1 ml of saline) at various times before killing the rats to obtain tissue samples. Sections were processed for immunofluorescence, and the use of a conventional light source and a yellow filter allowed clear visualization of carbon particles in contrast to the unstained tissue. The same field could thus be examined for phagocytic cells and in addition the Ia density of each cell of interest detailed.

Cyclophosphamide Pretreatment. Cyclophosphamide (Endoxana, WB Pharmaceuticals, Bracknell, Berkshire, England) was injected intraperitoneally at a dose of 300 mg/kg. This resulted in a 50% mortality in the first 24 h, but a sufficient number of animals survived in satisfactory health to allow tissue samples to be obtained daily for up to 7 d.

Irradiation/Reconstitution Experiments. DA rats, anesthetized with 30 mg chloral hydrate and 0.04 mg atropine sulphate given intraperitoneally, were irradiated by exposure to 1,000 rad (given over 2 min) from a Cs source (Gammacell 1000; Atomic Energy of Canada, Ltd., Ottawa). The animals were either not reconstituted or were reconstituted with 5 × 10^7-7 × 10^8 syngeneic nucleated bone marrow cells (prepared from tibias, femurs, and humeri) injected intravenously in 2 ml of PBS a few hours after irradiation.

Results

Tissue Distribution of Ia Antigen-positive Dendritic Cells. Immunofluorescence studies with the F17-23-2 monoclonal antibody were performed in the first instance on frozen sections of heart, and the results are given in Fig. 1. There was a striking and unexpected scatter of very bright, isolated structures, which appeared to have an orientation parallel with the sweep of the fibers (Fig. 1 a). High-power views (Fig. 1 b) showed that these structures had a remarkable dendritic appearance, similar to that described for the Steinman (4) and Langerhans's cells (5). The irrelevant antibody control (Fig. 1 c) showed no staining at all. Similarly, the genetic control using sections of heart from the PVG/c strain, with which the F17-23-2 antibody does not react (13), gave no staining. With immunoperoxidase staining, we could demonstrate that
Fig. 1. In-positive dendritic cells in rat heart. Frozen sections of DA heart were incubated with a 1:50 dilution of F17-23-2 immune ascites (a, b, d) or with an irrelevant monoclonal antibody (c) and then with fluorescein-labeled RAM (a–c) or RAM followed by peroxidase-labeled swine anti-rabbit immunoglobulin (d) as described in Materials and Methods. a × 100; b × 400; c (control) × 100; d × 400. Arrows in (d) indicate the peroxidase-positive cells. The genetic control using frozen sections of heart from the PVC/c strain (with which the F17-23-2 antibody does not react) in fluorescence studies gave no staining at all as in (c).
Fig. 2. In positive dendritic cells, frozen sections of (a) thyroid, (b) brain, (c) liver, and (d) pancreas were incubated with a 1:50 dilution of P1/23-2 immune sera and then with fluorescein-labeled RAM, as described in Materials and Methods. a and b x 100; c and d x 250. Arrows indicate position of dendritic cells; (b) 1, choroid plexus; 2, floor of ventricle. (c) Region of portal tract. (d) 1, 1.
the structures being stained were cells with a central nucleus and long processes (Fig. 1 d). The myocardial fibers themselves gave no obvious staining with the F17-23-2 antibody.

Frozen sections of thyroid, cerebrum, liver, pancreas (Fig. 2 a–d, respectively) and skin with panicultus carnosus (data not shown) were also studied. All of these tissues except brain had many la-positive dendritic cells, similar in every respect to those seen in heart, scattered in the interstitial connective tissues. Under high power, the characteristic dendritic morphology (as in Fig. 1 b) was quite clear. In liver, the dendritic cells were concentrated mainly around the portal triads (Fig. 2 c), with only occasional cells seen within the liver lobule. The hepatocytes, and in particular the Kupffer's cells, were not stained at all. With skin, the dendritic cells were seen in the skeletal muscle of the panicultus carnosus as well as in the subcutaneous connective tissue, although they appeared less frequently in the muscle layer. In contrast to the large numbers of dendritic cells in the dermis and subcutaneous connective tissues, we could only very occasionally see such cells in the epidermis, where Langerhans's type cells would be expected to be located. However, Rowden et al. (28) have previously noted the difficulty in demonstrating dendritic cells in the epidermis of the mouse, and in this respect, the rat appears to be similar to the mouse.

In addition to the tissue studied above, we have previously noted dendritic cells in the kidney and in the mucosal connective tissues of ureter and bladder (14). The complete absence of dendritic cells in brain (Fig. 2 b) is, therefore, most unusual, because in every other tissue we have examined dendritic cells have been readily demonstrable. Dendritic cells could be seen in the meninges and chorioid plexus, but never in the brain substance.

Using our F17-23-2 monoclonal antibody, we could demonstrate dendritic cells in the density seen in Fig. 1 a and b (where DA heart was used) on frozen sections of heart from the AGUS, F344, AS, and BN strains. To demonstrate dendritic cells in the PVG/c, AUG, and WAG strains, with which F-17-23-2 does not react, we used the MRC OX4 antibody (29) (Sera Laboratories Ltd., Crawley Down, Sussex, England). With this antibody, we could show that the hearts and kidneys of the PVG/c, AUG and WAG strains had dendritic cells in the amounts seen with the DA strain. Also of interest was the examination of frozen sections of heart and kidney of a nude rat, with the MRC OX4 antibody, because the nude genotype was on a PVG/c background. This animal had normal, and possibly increased, numbers of dendritic cells in heart and kidney.

It is worth noting that in heart, liver, thyroid, brain, pancreas, and skin, the only la-positive structures seen were dendritic cells, except for the epidermal cells of skin.

Further Phenotyping of Dendritic Cells in the Heart. That the only la-positive structure seen on frozen sections of many tissues were the dendritic cells meant that surface phenotyping these cells by immunofluorescence on frozen sections would be a particularly useful approach. All phenotyping was done on frozen sections of DA heart. Studies with fluorescein-labeled RAR to detect surface immunoglobulin and with the W3/13 antibody (which interacts with T lymphocytes and granulocytes) failed to give any staining in rat heart, so that the dendritic cell is W3/13 and surface immunoglobulin negative. The F16-4-4 antibody directed at SD antigens and the MRC OX1 antibody directed at the LC antigen both gave staining of cells, which was almost certainly of dendritic cells. The pattern of staining and the number of
cells stained was similar to that seen with the F17-23-2 antibody (directed at Ia antigens), although the brightness was much less and the dendritic appearance not nearly as marked.

To be certain that it was the Ia-positive dendritic cell that was staining for the LC antigen, we performed experiments where the same section was stained sequentially for the two antigens. In these experiments, a section of DA heart was stained with the MRC OX1 antibody and fluorescein-labeled RAM, and several fields were photographed. The section was then stained with the F17-23-2 antibody and fluorescein-labeled RAM, and the same fields were rephotographed. Given that the number of Ia-positive and LC-positive cells seen when these antibodies were used separately was approximately equal, one would expect a doubling of the number of fluorescent cells if the two labels were on separate cell populations, and smaller increases if there was partial overlap. However, only very occasionally could we see extra cells staining when the anti-Ia antibody was used after the anti-LC, and similarly in the converse experiments. Any increase in the number of labeled cells on addition of the second monoclonal antibody was <5%, and the results of a typical experiment where the anti-LC antibody was followed by the anti-Ia antibody are given in Fig. 3a and b. Not only was there no increase in the number of fluorescent cells, but the cells staining for the LC antigen appeared brighter after the anti-Ia antibody. These experiments therefore demonstrate that the Ia-positive dendritic cell is also LC positive, and is almost certainly positive for the SD (class I) antigens of the major histocompatibility complex.

**Histochemical Characteristics of Dendritic cells in Rat Heart.** Frozen sections of DA heart were examined for nonspecific esterase, acid phosphatase, β-glucuronidase and ATPase activity. In the case of each of the last three histochemical stains, only very occasional and isolated positive cells were seen, clearly indicating that at least the vast majority of the dendritic cells was negative for these enzymes. In all experiments involving histochemistry, frozen sections of spleen were included, as the red pulp served as a good positive control.

With the nonspecific esterase (NSE) stain, large numbers of isolated, positive structures were seen. When the sections were counterstained, the NSE activity was frequently seen to overlay nuclei in the interstitial connective tissue, strongly suggesting that we were staining interstitial cells rather than particular areas of the myocardial fibers. The frequency and distribution of the NSE-positive cells was approximately that of the Ia-positive dendritic cells, suggesting that the dendritic cell might be NSE positive, but double-labeling experiments showed that this almost certainly was not the case. Frozen sections of DA heart were first fixed and stained for NSE, and then were stained with the F17-23-2 antibody and fluorescein-labeled RAM. The results given in Fig. 3c and d show that cells staining for NSE activity were not positive for Ia antigen by fluorescence, and vice versa. It is clear, therefore, that at least some of the Ia-positive dendritic cells are NSE negative, but because the NSE stain might interfere with fluorescence, we cannot say with certainty that the NSE-positive cells are all Ia negative. That this is likely to be so, however, comes from studies with irradiated animals. As will be shown in a subsequent section, 12 d after irradiation with 1,000 rad, no Ia-positive dendritic cells are seen in rat heart. At that time, however, there are large numbers of NSE-positive cells present in rat heart (Fig. 5e). Fig. 5f shows staining of the same irradiated heart for the LC antigen, showing one
positive cell in the field; similar results were obtained with the anti-Ia antibody. Very clearly, none of the NSE-positive cells in the irradiated heart are Ia positive.

Double-labeling experiments using the acid phosphatase stain followed by staining for Ia by fluorescence demonstrated that the few acid phosphatase-positive cells could not be shown to be Ia positive (Fig. 3 e and f).
We would interpret these results as establishing that the dendritic cell of rat heart is acid phosphatase, ATPase and β-glucuronidase negative, and that at least some of the dendritic cells are NSE negative. It is very likely, however, that virtually all the dendritic cells are NSE negative, and that the NSE-positive cells represent a separate, radioresistant population of Ia- and LC-negative resident macrophages.

**Phagocytic Capacity of Dendritic Cells.** Rats injected intravenously with colloidal carbon were killed 90 min later and the livers, kidneys, and hearts removed and frozen in liquid nitrogen for subsequent processing. Frozen sections were stained for Ia antigens to demonstrate the dendritic cells, whereas the colloidal carbon in the same field could be visualized with a yellow filter and the conventional light source. As illustrated in Fig. 4, carbon could readily be seen in the hepatic lobules, presumably in the Kupffer cells, but none was associated with the dendritic cells in the portal triad. Very few accumulations of carbon could be seen in sections of rat heart, even up to 18 h after the injection of carbon. In the kidney glomerulus, mesangial cells containing colloidal carbon could be clearly distinguished from dendritic cells that had failed to ingest any carbon. These data suggest that the dendritic cell has little or no phagocytic ability, although one should note that the location of the dendritic cells might not have permitted optimal exposure to the carbon particles, in contrast to the situation with the Kupffer’s cells lining the sinusoids.

**Bone Marrow Origin and Radiosensitivity of the Dendritic Cell or its Precursor.** Initial studies showed that 7 d after a lethal dose of cyclophosphamide (300 mg/kg) or 1,000 rad of γ-irradiation, the heart and most other tissues examined were virtually completely depleted of dendritic cells. The most likely explanations for this result are either that the dendritic cell is radiosensitive, or that its precursor is radiosensitive and that the dendritic cell itself is short-lived or normally circulates fairly rapidly through the tissues. That this latter explanation is probably correct is suggested by the finding that the dendritic cells in the mucosal connective tissues of the bladder appeared to be unaffected by irradiation or cyclophosphamide, perhaps because of the presence of cystitis which might cause the dendritic cells to be retained.

The next series of experiments involved irradiating rats with 1,000 rad, and dividing...
Fig. 5. Irradiation and reconstitution experiments. DA rats were given 1,000 rad of γ-irradiation, and hearts were removed from nonreconstituted animals at 1, 3, and 12 d after irradiation and from a bone-marrow-reconstituted animal at 12 d after irradiation. Results of staining with anti-Ia (F17-23-2) immune ascites at a 1:50 dilution and fluorescein-labeled RAM are in (a–d), (a–c) are from the 1-, 3-, and 12-d nonreconstituted animals, and (d) is from the 12-d reconstituted animal. (e and f), frozen sections of heart from nonreconstituted rat irradiated 12 d previously and stained for NSE (e) and with anti-LC antibody and fluorescein-labeled RAM (f). The section in (e) was not counterstained for nuclei, so all of the darker areas represent the numerous NSE-positive cells present. The arrow in (f) indicates the solitary LC-positive cell. This heart was also negative for Ia antigens. a–e X 100; f X 250.

them into a nonreconstituted group from which the rats were killed daily, and a group reconstituted with $5 \times 10^6 - 7 \times 10^6$ syngeneic bone marrow cells from which animals were killed at day 9 and at day 12. The results, illustrated in Fig. 5, showed that 24 h after treatment with 1,000 rad, the number of dendritic cells in rat heart were normal, although the cells appeared unusual in having unusually prominent dendritic
processes. The numbers then declined rapidly, although some dendritic cells were readily seen at 3 d after irradiation. By the 5th d after irradiation, few or no dendritic cells could be seen. The hearts of the reconstituted animals killed at day 9 had readily demonstrable, but probably smaller than usual, numbers of dendritic cells. By day 12, the number of dendritic cells was normal, and possibly even somewhat higher than normal. Nonreconstituted animals killed at day 9 and 12 had no dendritic cells present in their hearts.

As mentioned in a preceding section, the hearts from nonreconstituted rats examined at days 9-12 after irradiation, had normal numbers of NSE-positive cells (Fig. 5 e), although there were few if any Ia- or LC-positive cells present at that time (Fig. 5 f). In addition, the irradiated heart had an increased number of acid phosphatase-positive cells, although whether this represented an influx of new cells or activation of the NSE-positive cells already present was not possible to determine.

Discussion

Our studies demonstrate that an intensely Ia-positive dendritic cell is widely distributed in the interstitial connective tissues of the rat, being found in all the tissues we have examined (heart, liver, thyroid, pancreas, skin, skeletal muscle, kidney, ureter, and bladder) except brain. The precise relationship of this interstitial dendritic cell to other dendritic cells is uncertain, but it appears to have all the characteristics ascribed to the Steinman dendritic cell, which has previously been found only in spleen and lymph node (4). Its Ia-positive nature and dendritic morphology are striking features, and like the Steinman cell (4, 11, 30, 31) it lacks the histochemical markers typical of macrophages (NSE, acid phosphatase, β-glucuronidase); it lacks surface immunoglobulin; it is of bone marrow origin; either the cell itself or its precursor is radiosensitive; the kinetics of disappearance after irradiation are similar; and it is probably poorly or not phagocytic. Like the Steinman cell but in contrast to the Langerhans's cell, the interstitial dendritic cell lacks ATPase activity (32). In addition, we demonstrate that the interstitial dendritic cell is present in the nude rat and that it is positive for the LC antigen and negative for the W3/13 antigen found on brain, granulocytes, and T lymphocytes (18).

The relationship of the Ia-positive dendritic cell to other interstitial cells in connective tissues is an important question to consider. It is clearly distinguishable from fibroblasts, because these have been reported as Ia-negative in both mouse (33) and man (34), and fibroblasts are unlikely to be positive for the LC antigen. Certainly, the human homologue of the LC antigen (22) is absent from cultured human fibroblasts (J. L. McKenzie and J. W. Fabre, unpublished observations). The relatively sparse distribution and radiation sensitivity are also features that distinguish the dendritic cell from fibroblasts. These last two features and the dendritic morphology would distinguish the dendritic cell from endothelial cells and other cells associated with capillaries and the vasculature. In addition, endothelial cells of the rat (14) are negative for Ia antigens. The absence of NSE and other hydrolases distinguish the DC from conventional macrophages in the connective tissues. As far as we can tell, the Ia-positive dendritic cell we report in this study has not hitherto been described as a discrete entity in the connective tissues of the body.

The precise function, physiology, and interrelationships of the various Ia-positive dendritic cells are intriguing problems. Such a widespread distribution of these cells
was unexpected, and this fact adds a wider dimension to our understanding of these cells, as does their striking and total absence from brain. A role in antigen presentation is clearly possible for the interstitial dendritic cell, because the Langerhans's cell (35) and dendritic cells in spleen and lymph node (3) have been shown to function in this way. However, in contrast to the Langerhans's cell (found in skin [5] and mucosal surfaces [12]) and the other dendritic cells (found in lymph node and spleen [4]), the interstitial dendritic cell is located in areas, such as heart and thyroid, that one would expect to be relatively secluded from environmental antigen. It is possible that their presence in these tissues is simply a safeguard against the widespread dissemination of environmental antigens, but it is equally possible that the interstitial dendritic cell might have a wider role, such as in the immune surveillance for the development of neoantigens on malignant cells, or perhaps even in self tolerance.

The striking similarity of the interstitial dendritic cell to the Steinman cell suggests that these might be identical cells, and that the interstitial cell circulates from the tissues to the organized lymphatic tissues, perhaps via the lymph. The precursor cell, of bone marrow origin, is likely to enter the tissues by the blood, and if this is so, the blood-brain barrier might explain their absence from brain. This absence might explain, in part, why the brain is an immunologically privileged site.

The widespread distribution of dendritic cells is of interest in relation to the reported capacity of liver (36), heart (37), and kidney (38) cells to stimulate allogeneic lymphocytes and in mixed culture. The allogeneic mixed lymphocyte culture reaction is believed to be dependent on Ia antigen differences, but we could not demonstrate Ia antigens with the F17-23-2 antibody on hepatocytes or myocardial cells in this study, although renal tubular cells were demonstrated to have Ia antigens in previous studies (13, 14). On the other hand, the Steinman dendritic cell has recently been shown to be an extremely potent stimulatory of both allogeneic (9) and syngeneic (10) lymphocytes, and it has been suggested that it is the main stimulating cell in mixed lymphocyte cultures, even though it is present in only very small numbers. It would seem likely that the lymphocyte stimulation observed when hepatocytes, kidney cells, and myocardial cells are mixed with allogeneic lymphocytes is in fact a result of small levels of contamination of these cells by the interstitial dendritic cell.

Another area of interest is in relation to transplantation. We have presented evidence to suggest that the dendritic cell of kidney is the so-called passenger leukocyte of transplantation (39). Much effort has been directed at depleting grafts of passenger leukocytes by a variety of techniques, especially treatment of the donor by irradiation or cytotoxic drugs (40, 41). However, grafts have been harvested from such donors usually only a few hours after treatment. Our irradiation experiments (Fig. 5) demonstrate that even 24 h after a lethal dose of irradiation, the number of interstitial dendritic cells is undiminished, that they are still present though in much diminished numbers 3 d after irradiation, and that for complete depletion, one must wait for ~5 d. Clearly, if the interstitial dendritic cell is indeed the passenger leukocyte, grafts must be harvested at much longer intervals after donor treatment than is currently the practice. An alternative is to use drugs directly toxic to the cells. In any case, the capacity to visualize these cells using anti-Ia antibodies on frozen sections means that precise experiments in this area are now possible, because previously the term “passenger leukocyte” was used to describe an ill-defined cell that could not be visualized, and the presence of which could only be inferred from functional studies.
In this study, we have used a mouse monoclonal antibody to rat Ia (RT1-B or class II) antigens to demonstrate, by immunofluorescence on frozen sections, intensely Ia-positive dendritic cells in the interstitial connective tissues of every tissue we have examined (heart, liver, thyroid, pancreas, skin, kidney, ureter, and bladder) with the striking exception of brain. The characteristics of the interstitial dendritic cell found in heart were studied in detail, and this cell was shown to be negative for acid phosphatase, β-glucuronidase, and ATPase activity, and certainly some and probably all of the cells were negative for nonspecific esterase activity. Experiments with colloidal carbon suggested that the cell was either poorly or not at all phagocytic. The cells were negative for surface immunoglobulin and the W3/13 antigen, but positive for the leukocyte common antigen and the SD (Class I) antigens of the major histocompatibility complex. The cell was shown to be of bone marrow origin, and either the cell itself, or more probably its precursor, was shown to be sensitive to irradiation and to cyclophosphamide. All strains tested—including the nude rat—had large numbers of interstitial dendritic cells. The widespread distribution, except in brain, of this cell, which resembles in every respect the dendritic cell described by Steinman et al. (4) in the spleen and lymph nodes of the mouse, is of interest, and the implications in this finding are discussed.

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