Mononuclear Phagocyte Biology

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3

THE RELATION OF LANGERHANS CELLS TO OTHER DENDRITIC CELLS AND MACROPHAGES

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Within the lymphoid system, there is a group of dendritic cells that is thought to play a major role in the immune response, even though they represent a small percentage of the cell population. In particular, evidence has been presented that some of these cells are important for the retention and presentation of antigen and as stimulators of mixed leukocyte reactions. This group of cells includes the Langerhans cells, interdigitating dendritic cells of the T-dependent areas in lymphoid tissue, follicular dendritic cells, lymphoid dendritic cells, Kupffer cells, and thymic dendritic cells.

Langerhans cells (LC) were first described by Paul Langerhans in 1868 (27), when he noted dendritic cells in the epidermis stained by a gold chloride method. He thought that these cells were neural in origin. It was not until Silberberg and co-workers noted the apposition of lymphocytes and LC in contact sensitivity reac-

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tions (28) that their possible role in the immune response was appreciated. Since that time, experiments have shown that LC can present antigen to T cells (29) and that they are important in the contact sensitivity reaction (30,31). Like macrophages, LC bear Fc (1,13) and C3 receptors (7,13) and are able to migrate from skin to draining lymph nodes (30). Because of these functional and morphological similarities to macrophages, discussed in more detail previously (32), and their even greater similarities to some of the other dendritic cells, interest in the LC has generated more attention for dendritic cells in general.

Lymphoid dendritic cells, first described by Steinman and Cohn (4,12) have not been located in tissue sections, but can be isolated and purified from spleen, Peyer's patch, blood, and lymph node cell suspensions. These cells appear to be potent stimulators of the mixed leukocyte reaction (33) and can provide accessory functions in vitro (34).

Interdigitating cells have been described by Veldman (35) and Veerman (36). With the electron microscope, these cells have been identified in the thymus and in T-dependent areas of the spleen and lymph nodes. No functional information is available because they have not been isolated. It is interesting that, by electron microscopy, they have a strikingly similar appearance to LC and that they are invariably located in proximity to T cells. LC granule-containing cells which by electron microscopic criteria could be LC or interdigitating cells, are seen in the paracortex of lymph nodes draining sites of antigen injection (37), and in the thymic medulla of the rat (38). Dendritic cells of both mesenchymal and epithelial origin have been described in the thymus and both of these bear Ia antigens (39). Although a definitive function for these cells has not been shown, they are thought to be of importance for thymocyte proliferation and functional development.

Kupffer cells are dendritic cells lining the sinusoids of the liver. In contrast to other cells of dendritic morphology, these cells are highly phagocytic. Recent studies with cell populations

enriched for Kupffer cells have shown that these cells are able to present antigen to T cells (40) and are potent stimulators of mixed lymphocyte reactions (41).

Follicular dendritic cells have been shown by electron microscopy to have irregular nuclei with extensive membrane invaginations and protrusions, which often enfold electron-dense material and viruses (42). These cells bear antigen or antigen-antibody complexes on their surfaces which can be detected long after their injection (43,44). Recently, Humphrey and Grennen have succeeded in isolating follicular dendritic cells and have reported them to be Ia negative, and to have Fc and C3 receptors (3).

In the present study we have examined the tissue distribution of the macrophage antigens, Mac-1, Mac-2, and Mac-3, described by Springer et al (45-48), they are present on cell surface membranes of thioglycollate induced peritoneal macrophages and not on lymphocytes. Since only little attention has been given so far to their intracellular localization (49), we decided to stain sections of a variety of mouse tissues with the monoclonal rat antibodies M1/70 = anti-Mac-1 (45,46), M3/38 = anti-Mac-2 (47), M3/84 = anti-Mac-3 (48), and, for comparison, M5/114 = anti-Ia (50) and 2.4G2 = anti-mouse Fc receptor (51), donated by Dr. J. Unkeless. We used a sandwich staining technique in which the antibody is linked via rabbit anti-rat Ig with a peroxidase-rat anti-peroxidase complex (purchased from Sternberger-Meyer Immunocytochemicals Inc., Jarretsville, Maryland). Subsequent staining of peroxidase activity (52) revealed the localization of the antigens. Preliminary experiments showed that only Mac-2 was stable to conditions used in Carnoy's fixation, followed by paraffin embedding. Mac-1 was destroyed by alcohol and therefore had to be studied on frozen sections. For Mac-3 and Ia antigen, fixation in cold alcohol followed by embedding in polyester wax (BDH Chemicals, Ltd., Poole, England) gave satisfactory results. Cell suspensions were mixed at 10^7 cells/ml with 2% agarose (final concentration) in Hanks' balanced salt solution at 45°C, allowed to gel at

TABLE 1

Staining Patterns with Monoclonal Antibodies in the Thymus

Antigen	Thymocytes	Free Round Macrophages	Dendritic-like Cells	
			Cortex	Medulla
Mac-1	-	+	-	-
Mac-2	-	+	+	++
Mac-3	-	+	++	++

4°C, and fixed in cold alcohol for 1 hour (for a 0.2 ml volume). They were then embedded in polyester wax.

Tables 1-3 show the results obtained for localization of Mac-1, -2, and -3 in thymus, spleen, and lymph node. Mac-1 and -2 always showed a homogeneous localization throughout the cytoplasm of the positive cells, whereas Mac-3 was invariably localized in discrete granules. Lymphocytes or lymphoblasts never stained for any of the three macrophage antigens.

In all these organs Mac-1 was found in free-lying, round macrophages in thymic cortico-medullary junctions, splenic red pulp, and lymph node sinuses. Mac-2 was present in dendritic cells as well as in free macrophages. The dendritic cells which stained for this antigen had the typical localization previously described for interdigitating cells (35-39), including a high frequency in thymic medulla with much less staining in the cortex, numerous cells in the splenic periarteriolar sheaths and the paracortex of the lymph nodes (T-cell zones). Striking staining patterns of

TABLE 2

Staining Patterns with Monoclonal Antibodies in the Spleen

Antigen	Red Pulp		Dendritic-like Cells in Periarteriolar Sheaths	Germinal Centers	T and B Lymphocytes
	Round Macrophages	Dendritic Cells			
Mac-1	+	-	-	-	-
Mac-2	+	+	+	+[a]	-
Mac-3	+	+	+	+[b]	-

[a] A few positive cells in each germinal center, but none in corona area as would have been expected for follicular dendritic cells.

[b] Dendritic-like cells with the appearance of endothelial cells.

TABLE 3

Staining Patterns with Monoclonal Antibodies in the Lymph Nodes

Antigen	Free Cells in Sinuses and Medullary Cords	Dendritic-like Cells in Paracortex	Germinal Centers	Endothelial Cells of Postcapillary Venules	T and B Cells
Mac-1	+	-	-	-	-
Mac-2	+	+	+[a]	-	-
Mac-3	+	+	+[a]	+	-

[a] Staining not in the corona as expected for follicular dendritic cells.

typical tingible body macrophages in germinal centers were also observed with anti-Mac-2. However, staining for Mac-2 of dendritic cells in the corona of follicles, where follicular dendritic cells are located (42-44), was never observed. The localization of Mac-3 was somewhat more difficult to evaluate in view of its presence in endothelial cells. A dendritic staining pattern was seen in the thymus, which was equally dense in cortex and medulla. The frequency of Mac-3 positive cells in the thymus, however, was still lower than that seen for Ia^+ cells. In the spleen a dendritic staining pattern, primarily in the red and less dense in the white pulp, was present in addition to heavily staining hematopoietic cells, including megakaryocytes. In lymph nodes, the dendritic pattern formed a background against heavy staining in sinus lining cells, and of free macrophages in sinuses and medullary cords. Particularly noteworthy was the staining for Mac-3 in postcapillary venule endothelium. Cells staining for Mac-3 in coronas of follicles were less numerous than in most other areas of the lymphoid tissue. The intensity of the dendritic pattern in the paracortex of lymph nodes suggested that interdigitating cells were positive, but the degree to which staining of reticular cells of the lymphoid tissue contributed to this pattern was hard to evaluate.

Free-lying macrophages in lymphoid organs, as well as in lung, and resident peritoneal cells were positive for all three antigens (Table 4). Cell surface staining for Mac-2 was previously found on

TABLE 4

Staining of Macrophages in Various Locations by Monoclonal Antibodies

Antigen	Peritoneal Cell	Alveolar Macrophage	Kupffer Cell	Endothelial Cell	Granulocyte	Lymphocyte
Mac-1	+	+	-	-	+	-
Mac-2	+	+	+	-	-	-
Mac-3	+	+	+	+	?[a]	-

[a] 50% of bone marrow cells stain, but differentiation in sections is difficult.

a low percentage of resident peritoneal cells and on a high percentage of thioglycollate induced exudate cells. It is of interest to compare these findings with the present ones for intracellular staining. Resident peritoneal cells showed strong staining of 5% and much weaker but definite staining of the remaining cells for Mac-2, whereas thioglycollate induced exudate cells stained strongly for Mac-2. Therefore, it was likely that only those cells with strong internal staining for Mac-2 also had readily detectable Mac-2 on their surface.

Alveolar macrophages stained strongly with all three antibodies. Alveolar lining cells stained only with anti-Mac-3. In contrast, Kupffer cells were negative for Mac-1, but stained with both anti-Mac-2 and -3.

Epithelial cells never stained for Mac-1. However, Mac-2 and -3 were frequently present in epithelial cells of a variety of organs. In lung, bronchial columnar epithelial cells were positive for both. In the liver, the bile canuliculi were Mac-3^+. In the ileum a definite staining pattern of the epithelium was noted for both Mac-2 and Mac-3. Mac-2 was more concentrated in the tips of the villi, whereas Mac-3^+ granules were present in the apical portions of all the columnar epithelial cells. The staining pattern of kidney sections were even more complex. Glomeruli were Mac-3^+, Mac-2^-. The epithelium of some tubuli stained for Mac-2 and some for Mac-3, but the patterns for the two antigens were quite distinct. Stratified squamous epithelial cells were no exception: they were negative for Mac-1, but stained strongly for both Mac-2 and -3.

In the intact epidermis, the question whether Langerhans cells stained could, therefore, only be studied for Mac-1, Ia antigens, and Fc receptors, but not for Mac-2 and Mac-3. Langerhans cells were more readily studied in stained epidermal sheets than in skin sections. For this purpose epidermal sheets were stained while floating on 50 µl of the appropriate antibody dilution. Regardless of whether the epidermal sheets were prepared with 0.5% trypsin in PBS, with 1M sodium bromide, or with 0.5 M ammonium thiocyanate in 0.1% phosphate buffer, pH 6.8 (53), staining for Mac-1 in Langerhans cells was not obtained. In contrast, staining for Ia antigen succeeded with all three methods of separation, and LC were also positive with the antibody to Fc receptors (Fig. 1).

The presence of Mac-2 and -3 antigens in Langerhans cells was then studied in sections of epidermal cell suspensions, enriched for LC. Groups of 9-11 BALB/c mice (Charles River Laboratories,

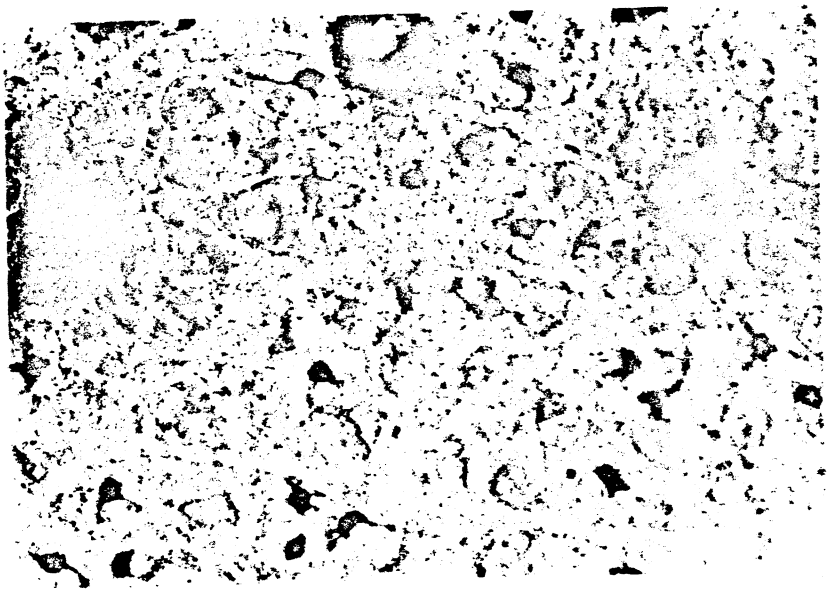

FIG. 1. Photomicrograph of a whole mount of BALB/c epidermis stained for Fc receptors by the monoclonal antibody 2.4G2 using the immunoperoxidase method showing positive Langerhans cells (X 300).

Wilmington, MA) were sacrificed, shaved, depilated with Neet cream hair remover (Whitehall Laboratories, NY, NY), washed, rinsed with 95% ethanol, and skinned. Subcutaneous fat, blood vessels and any lymph nodes adhering to the pelts were removed by dissection, the isolated pelts were cut into 1 x 1 cm pieces, and floated, dermal side down, in a sterile solution of 0.5% w/v trypsin (Millipore Corp., Freehold, NJ) and 20 mM EDTA in PBS and incubated at 37°c for two hours. The pieces were blotted dry, the dermis peeled away and the cells were teased into suspension in Medium 199 (GIBCO, Grand Island, NY), supplemented with 10% v/v fetal bovine serum (GIBCO) and 1% antibiotic-antimycotic mixture (GIBCO). The cells were filtered through gauze, washed once, layered over Ficoll-Hypaque (specific gravity 1.094) and centrifuged at 400 g for 30 minutes. The resulting interface was collected and washed twice. The cells were then rosetted with sheep erythrocytes coated with the IgG fraction of rabbit anti-sheep hemolysin at a cell to EA ratio of 1:25. The rosette mixture was partially pelletted by centrifugation at 100 x g for 5 min. and left to incubate at 4°C overnight. The rosettes were gently resuspended, layered over 70% isotonic Percoll® (Pharmacia Fine Chemicals, Upsala, Sweden) and centrifuged at 800 g for 30 min. The pellet was gently resuspended, an aliquot removed for counting and erythrocytes were subjected to hypotonic lysis. The resulting LC enriched epidermal cell suspension was washed three times in supplemented Medium 199 and mixed with the agarose as described above. The results of a typical experiment are presented in Table 5. The percentage of Langerhans cells is given by the percent of cells giving Fc rosettes with EA and corresponds very well with the percent of cells staining for Ia. The results suggest strongly that Mac-2 and Mac-3 are also present on Langerhans cells, since 97 and 96% of the cells, respectively, stained for these antigens.

A comparison of properties of Langerhans cells with other dendritic cells is presented in Table 6. Lymphoid dendritic cells are characterized only in cell suspensions and their surface properties differ from those described for Langerhans cells and for other

TABLE 5
Staining of Epidermal Cells Enriched for Langerhans Cells

Property	% Positive
Fc rosetting	35
Ia +	37
Mac-2 +	97
Mac-3 +	96

dendritic cells as far as they are known. This is particularly true for Fc receptors and ATPase activity. Recent studies on human lymphoid dendritic cells obtained from peripheral blood have shown the presence of C3 receptors, although these cells were still negative for Fc receptors (14). It is possible that these cells are precursors of some of the other dendritic cells and are still lacking in receptors which they will develop upon maturation. It is remarkable that, while one observes very many Mac-2 and -3 positive dendritic cells in sections of spleen and lymph node, only few dendritic cells are detectable in suspensions made from these organs and that all these are ATPase negative, Fc- and C3 receptor (4, 12 ,34). It seems likely that the dendritic cells stained in sections are not readily dispersed into cell suspensions upon teasing of the lymphoid organs and that this may be related to

TABLE 6
Comparison of Surface Properties of Various Dendritic Cells

Species	Property	Langerhans Cell	Interdigitating Dendritic Cell	Follicular Dendritic Cell	Lymphoid Dendritic Cell	Kupffer Cell
Rodent	FcR	+[a]	ND	+(2,3)	-[b]	+(6)
	C3R	+(7)	ND	+(3,8)	-(4)	+(6)
	ATPase	+(9)	ND	+(11)	-(12)	ND
Human	FcR	+(13)	ND	ND	-(14)	ND
	C3R	+(13)	ND	ND	+(14)	ND
	ATPase	+(15)	+(16)	-(10)	ND	ND

[a] Demonstrated both by binding of immune complexes (1) and by staining with the 2.4G2 monoclonal anti-Fc-receptor (this manuscript).
[b] Absent both by binding of immune complexes (4) and by staining with monoclonal anti-Fc-receptor (5).

their degree of differentiation. The difficulties involved in obtaining dendritic follicular cells into suspensions are illustrated in the studies of Humphrey and Grennan (3).

A summary of staining properties of dendritic cells, shown in our laboratory as well as in those of other investigators, is given in Table 7. These findings emphasize the similarity between Langerhans cells and interdigitating cells on the one hand, and the differences between both of these and follicular dendritic cells on the other. A recent study of Barclay (26) has shown staining of rat follicular dendritic cells with a monoclonal antibody to thymocytes, which does not stain any other dendritic cells examined. Moreover, in addition to Mac-2, the S100 antigen, detected in lymphoid tissue by antibody to S100 of neural origin, is present in interdigitating cells (24) and Langerhans cells, but absent in follicular dendritic cells and in macrophages (unpublished observations). Ia antigens are present on all dendritic cells listed, except on follicular dendritic cells (3).

In summary, use of the three monoclonal antibodies to macrophage antigens, Mac-1, -2, and -3, may distinguish two groups of cells within the macrophage-dendritic cell system. Staining for

TABLE 7
Similarities Between Langerhans Cells and Dendritic Cells

Species	Antigen	Macrophage	Langerhans Cell	Interdigitating Dendritic Cell	Follicular Dendritic Cell	Lymphoid Dendritic Cell
Mouse	Ia	+/-(17)	+(1)	+(18)	-(3)	+(4)
	Mac-1	+	-	-	-	-(5)
	Mac-2	+	+	+	?[a]	ND
	Mac-3	+	+	+[b]	-	ND
	Ly-5	+(19)	+	ND	ND	+(5)
Human	Ia	+/-(20)	+(21)	+(22)	ND	+(14)
	OKT6	-(22)	+(23)	+/-(22)	-(22)	-(14)
	S100	-(24)	+(25)	+(24)	-(24)	ND
Rat	MRCOX2	-(26)	ND	-(26)	+(26)	ND

[a] Some cells in follicles stain positively, but their location does not correlate with that expected for follicular dendritic cells.
[b] The staining pattern observed is most consistent with the interpretation that these cells are positive.

both Mac-1 and -2 (and also Mac-3) characterizes free-lying, round macrophages. Staining with Mac-1 alone is not a sufficient criterion to distinguish these cells from all other cells, since granulocytes are also known to be Mac-1^+, but are Mac-2^-. The second group is Mac-1^-, Mac-2^+ (and Mac-3^+). These include cells of dendritic morphology of which large numbers are present in lymph node and thymus, and Kupffer cells. This group probably does not include the follicular dendritic cells.

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RECENT DEVELOPMENTS

Recently we have found that murine low density non-adherent Ig^- cells, obtained after density gradient centrifugation of collagenase-treated minced lymph node, are heterogeneous with respect to the presence of Fc receptors (FcR) on their cell surface (Bienenstock et. al., submitted). Even after removal of Ig^+ cells, cells stimulating in syngeneic mixed lymphocyte reactions (SMLR) are found in these low density cells,

presumably corresponding to the lymphoid dendritic cells. When such FcR^+ fractions of low density nonadherent lymph node cells are prepared in parallel with epidermal Langerhans cells (LC), they are found to stimulate the SMLR equally well. The FcR^- fraction of the lymph node cells also stimulates, but not the FcR^- cells from epidermis. However, at least when working with murine cells, one has to be careful to avoid the presence of Ig in the BSA used for the density gradients, since Fc rosetting does not succeed after exposure of the cells to Ig. In addition, the Fc rosettes with these cells form very slowly at 4°C and not well at all at 37°C (K.A. Haines et al., unpublished observations). Thus epidermis LC and lymph node dendritic cells may not differ as much with respect to the absence or presence of FcR as was expected originally, and the FcR of both cells differs from the macrophage FcR.

If LC represent the epidermal outposts of dendritic cells (for nomenclature and comparison of various dendritic cells see also Flotte et al., Am. J. Pathol. 111:112, 1983, and Tew et al., J. Res. 31:371, 1982) with important immunomodulating and accessory cell function, enumeration of LC may be helpful in the evaluation of accessory cell function in man. Recent studies on patients with acquired immunodeficiency syndrome (AIDS) show, indeed, that LC numbers in the epidermis of these immunosuppressed as compared to a variety of control individuals are significantly reduced, both by staining for Ia and for ATPase activity. Staining for OKT6 also reveals significant differences, but some patients (approximately 20%) have normal LC counts with this stain, suggesting that the LC are present but fail to express certain surface markers (Belsito et al., N. Engl. J. Med., in press, 1984). In view of the importance of Ia in antigen-presentation, this means that AIDS patients have impaired LC function in addition to the previously shown $OKT4^+$ T cell deficiency.