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Staining of Langerhans cells with monoclonal antibodies to macrophages and lymphoid cells

(interdigitating dendritic cell/Mac antigen series/Fc receptor antigen/follicular dendritic cell/Kupffer cell)

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ABSTRACT **Langerhans cells are Ia-bearing antigen-presenting cells in the epidermis that share many functions with macrophages. We have used monoclonal antibodies to the macrophage antigens, Mac-2 and -3, Ia antigen, Fc fragment receptor, and the common leukocyte antigen CLA to compare the cell surface antigens of these cells with those of interdigitating and follicular dendritic cells and of macrophages in lymphoid tissues. Immunoperoxidase staining was carried out with epidermal sheets from BALB/c mice and epidermal cell suspensions enriched for Langerhans cells by Fc rosetting. Langerhans cells stained for all of these antigens. Comparison with the staining properties of other dendritic cells and macrophages, in combination with previous observations, indicates a close relationship of Langerhans cells to the interdigitating cells of lymphoid tissues.**

The Langerhans cells (LC) of the epidermis were originally identified by their dendritic morphology and property of staining with gold chloride (1) and later by the presence of Birbeck granules in their cytoplasm (2). Only recently was it found that LC are the antigen-presenting cells of the skin. They are Ia antigen$^+$ (3) and appear to mediate the induction of contact sensitivity *in vivo* (4, 5) and the induction of antigen-specific T-lymphocyte responses *in vitro* (6). These findings raised the question of the relationship of LC to antigen-presenting cells in other tissues. LC (7), like macrophages (8), are derived from the bone marrow, but the relationship of their precursors is unknown.

In the present study, we have used monoclonal antibodies for the recently defined macrophage antigens Mac-1, -2, and -3 (9–11) to compare the cell surface antigen phenotypes of LC, interdigitating and follicular dendritic cells (DC) of lymphoid tissues, and macrophages. We have previously shown that interdigitating cells are Mac-1$^-$, -2$^+$, -3$^+$ whereas follicular DC are Mac-1$^-$, -2$^-$, -3$^-$ (12, 13) and macrophages are Mac-1$^+$, -2$^+$, -3$^+$ (9–11). LC show electron micrographic similarities (14, 15) to interdigitating DC, including the presence of Birbeck granules in both cell types (2, 16). This suggests that LC and interdigitating cells may be more closely related to one another than to follicular DC or to macrophages. In addition, we have found (12) that LC bear the CLA (17) antigen, which is identical to the Ly-5 antigen (18), common to all leukocytes and found on hematopoietic stem cells but not in nonhematopoietic tissues (19). By studying epidermal cell suspensions, highly enriched for LC, we have now been able to examine LC for the presence of Mac-2 and Mac-3. This was not possible on skin sheets or sections because keratinocytes also contain these antigens (12).

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MATERIALS AND METHODS

Mice. Male BALB/c mice were purchased from Charles River Breeding Laboratories and were used when 8–16 wk old.

Cell Preparations. LC were studied by immunoperoxidase staining using epidermal sheets and epidermal cell suspensions enriched for LC by Fc fragment rosetting (6).

Nine to 11 mice were killed, shaved, depilated, rinsed with 95% ethanol, and skinned. The pelts were cleaned of underlying tissue, cut into 1-cm^2 pieces, floated, dermal side down, on 0.5% trypsin (Millipore, Freehold, NJ)/20 mM EDTA in phosphate-buffered saline (pH 7.4), and incubated for 2 hr at 37°C, after which the dermis was peeled away. The epidermis was teased into a cell suspension in medium 199 (GIBCO)/10% fetal bovine serum (GIBCO)/1% antibiotic/antimycotic mixture (GIBCO). Dead cells were removed by Ficoll-Hypaque gradient centrifugation. The interface cells were collected, washed twice, mixed with sheep erythrocytes sensitized with the IgG fraction of rabbit anti-sheep hemolysin (EA), and incubated overnight at 4°C. The rosettes were then separated from other cells by layering them over 70% isotonic Percoll (Pharmacia) and centrifuging for 30 min at 800 × *g*. The resulting LC-enriched pellet was gently resuspended, an aliquot was removed to count the rosettes, and the sheep erythrocytes were subjected to hypotonic lysis.

Monoclonal Antibodies. The sources of the monoclonal antibodies used in these experiments have been described: anti-Mac-1 = M1/70 (9), anti-Mac-2 = M3/38 (11), anti-Mac-3 = M3/84 (20), anti-Ia = M5/114 (21), anti-CLA = M1/89.18.2 (17), anti-LFA-1 = M17.5.2 (22). The anti-Fc receptor antibody 2.4G2 (23) was provided by Jay Unkeless (Rockefeller University).

Staining Technique. The LC-enriched epidermal cell suspensions were mixed at 10^7 cells per ml with agarose (0.1 to 0.2 ml; final concentration, 2%) in Hanks' balanced salt solution at 45°C, allowed to gel at 4°C, and fixed in cold alcohol for 1 hr. They were then embedded in polyester wax (BDH, Poole, England). The sections were dewaxed, washed in Tris-buffered saline (pH 7.6), and overlaid with the appropriate monoclonal antibody for 1 hr at room temperature. The sections were washed three times in Tris-buffered saline overlaid with a 1:20 dilution of rabbit anti-γ2b antibody (Pel-Freez) and incubated for 30 min. They were again washed and then overlaid with a 1:200 dilution of a peroxidase-rat antiperoxidase complex (Sternberger Meyer Immunocytochemicals, Jarretsville, MD). Subsequent staining for peroxidase activity with diaminobenzidine revealed the localization of the antigens. Epidermal sheets were

Abbreviations: LC, Langerhans cells; DC, dendritic cells; EA, sensitized erythrocytes.

stained in the same manner except that they were floated on 50 μl of the appropriate dilution of antibody.

RESULTS AND DISCUSSION

The results of three experiments are presented in Table 1. The percentage of LC is expressed as the percentage of cells forming Fc rosettes with EA and corresponds well with the values obtained by staining for Ia and for CLA. The results indicate that Mac-2 and Mac-3 are present in LC as well as in keratinocytes (12), since more than 95% of the cells stained for both of these antigens. The presence of these antigens on keratinocytes was not unexpected because in previous studies they were found in a number of epithelial cells (12). It is to be emphasized that these antigens are found in the cytoplasm of the LC and that it is therefore unlikely that LC derive Mac-2 and Mac-3 from the surrounding keratinocytes. In lymphoid tissues, these antigens are restricted to macrophages and DC. Lymphocytes are Mac-1$^-$, -2$^-$, -3$^-$.

In the second series of experiments, epidermal sheets were stained for antigens absent from keratinocytes, including Ia antigen, Mac-1, Fc receptor, CLA, and LFA-1 (Fig. 1). Regardless of whether the epidermal sheets were prepared with 0.5% trypsin in phosphate-buffered saline, 1 M sodium bromide, or 0.5 M ammonium thiocyanate in 0.1 M phosphate buffer (pH 6.8) (24), staining for Mac-1 in LC was not obtained. In contrast, LC staining for Ia antigen succeeded with all three methods of separation. LC were also positive with the antibodies to Fc receptors and to CLA, as previously shown (12, 13). Staining was negative with antibody to the lymphocyte antigen LFA-1 (12, 13). LC, therefore, express neither the macrophage-specific Mac-1 nor the lymphocyte-specific LFA-1 antigens of this interesting group of proteins recently identified by Kurzinger *et al.* (25).

The present findings for murine LC are compared with those previously described for other DC in Table 2. The nomenclature for DC used here has recently been discussed by Tew *et al.* (46). LC are Ia$^+$ (3), Fc receptor$^+$ (47), and ATPase$^+$ (48). Interdigitating cells, in addition to their previously mentioned electron microscopic similarities are also Ia$^+$ (26) and ATPase$^+$ (44) but have not been studied for Fc receptors. In contrast, follicular DC (49, 50) studied in tissue sections and recently also in cell suspensions (31), are Fc receptor$^+$ (31) but Ia$^-$ (31) and ATPase$^-$ (32) and differ in electron microscopic appearance (33). In sections of lymphoid tissue, Mac-3 is invariably associated with granule-like structures inside positive cells while Mac-1 and -2 are homogeneously distributed in the cytoplasm (12). Antibody to Mac-1 stains free-lying macrophages but none of the DC, not even Kupffer cells (12, 13). Anti-Mac-2 and -3, however, stain interdigitating cells and Kupffer cells but not follicular DC (12, 13). The cytoplasm of free-lying macrophages is stained by all three antibodies.

It is difficult to relate findings on lymphoid tissue sections to results obtained on dispersed cells from lymphoid organs.

Table 1. Properties of epidermal cells enriched for LC

Cell population	Property	% cells		
		Exp. 1	Exp. 2	Exp. 3
Preenrichment	Fc rosetting	2	3	3
Postenrichment	Fc rosetting	35	30	21
Postenrichment	Ia$^+$	35	37	27
Postenrichment	Mac-2$^+$	97	99	96
Postenrichment	Mac-3$^+$	96	96	96
Postenrichment	CLA$^+$	ND	33	30

ND, not done.

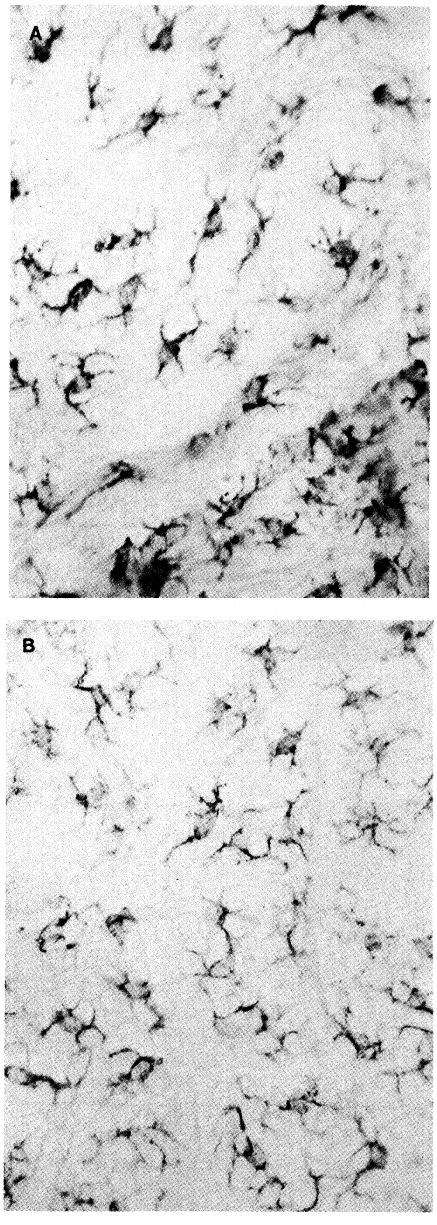

FIG. 1. LC in epidermal sheet of BALB/c mouse stained for Ia antigen (rat monoclonal antibody M5/114) (*A*) or Fc receptor antigen (rat monoclonal antibody 2.4G2) (*B*).

This complicates the comparison between lymphoid DC, described by Steinman and Cohn (34, 35), and other DC. As far as known, the properties of murine lymphoid DC differ significantly from those described for LC and for other DC (Table 2). This is particularly true for Fc receptors, which are absent from lymphoid DC, both by EA rosetting (27, 35) and by staining with the anti-Fc antibody 2.4G2 (36), and for ATPase activity (33).

Recent studies on rat dendritic cells suggest that the low-density cells from skin cell suspensions that can function as accessory cells lack Fc receptors (51). However (unpublished data), we have found that separation of LC on density gradients prepared with commercial bovine serum albumin results in per-

Table 2. Properties of various DC and macrophages

Species	Surface receptor or cytoplasmic staining	LC*	DC			Kupffer cell¶	Macrophage‖
			Interdigitating†	Follicular‡	Lymphoid§		
Mouse	Mac-1	−	−	−	−	−	+
	Mac-2	+	+	−	ND	+	+
	Mac-3	+	+	−	ND	+	+
	Fc receptor	+	ND	+	−	+	+
	C3b receptor	+**	ND	+	−	+	+
	ATPase	+	ND††	+	−	ND	+
	Ia	+	+	−	+	+	+/−
Rat	MRCOX2	ND	−	+	ND	ND	−

ND, not done.
*See refs. 13 and 26–29.
†See refs. 12, 26, and 30.
‡See refs. 12 and 30–33.
§See refs. 12 and 34–36.
¶See refs. 12, 37, and 38.
‖See refs. 20, 30, 36 and 39–41.
**The C3 receptor on LC is C3cb. It was recently shown that the Mac-1 antigen identifies the C3bi receptor (42), which, like C3d, is absent on LC (43).
††Not done in mouse, positive in human (44, 45).

manent inability of murine LC to form Fc rosettes because of contamination of the albumin preparations by Ig. Preliminary results suggest that LC are indeed much less able to regenerate Fc receptors than are common macrophages. Thus, absence of Fc receptors, as judged by the ability to form rosettes, may not correlate with the presence of Fc receptors by staining with 2.4G2, particularly after bovine albumin gradient centrifugation.

Although the presence of C3b receptors on lymphoid DC from human peripheral blood has been described recently (52), this receptor is lacking on lymphoid DC from mouse spleen (35). It is possible that the DC that are most readily obtained in cell suspensions are the less mature forms of DC, which may not be as consolidated with their dendrites into the reticular structure of the lymphoid tissue, as are the majority of the cells seen in tissue sections stained with anti-Mac-2. The large number of DC seen in lymph node sections (12, 13) stained with anti-Mac-2 is quite surprising considering the low percentage of lymphoid of DC obtained in cell suspensions (34). This suggests that most of the interdigitating cells are not represented in cell suspensions obtained by teasing of lymph node tissues. It is known from the work of Humphrey and Grennan (31) that it is also very difficult to obtain follicular DC in cell suspensions.

Thus, the present results are in agreement with earlier observations and further illustrate the striking similarity between LC and interdigitating cells, as well as the differences between these two types DC and follicular DC. These results with mouse DC also parallel findings in other species. A recent study by Barclay (30) has shown staining of rat follicular DC by a monoclonal antibody that does not stain any other DC examined. Moreover, similar to Mac-2 in the mouse, S100 antigen, detected in human lymphoid tissue by a rabbit antibody to S100 of neural origin, is present in human interdigitating DC (53) and LC (54) but is absent from follicular DC and macrophages (55). Ia antigens are present on all DC listed except follicular DC (30). Taken together, these findings suggest that LC and interdigitating cells are close relatives (if not the same cell population) but that follicular DC might be regarded as a different cell type. The differences between free-lying macrophages and LC include staining properties for Mac-1 and S100 as well as the functional difference in phagocytic activity, which is low to absent in all DC except Kupffer cells.

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