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Comparison of S-100 and OKT6 Antisera in Human Skin

ROBERT L. MODLIN, M.D., GEOFFREY ROWDEN, PH.D., CLIVE R. TAYLOR, M.D., D. PHIL., AND THOMAS H. REA, M.D.

Departments of Dermatology (RLM, THR) and Pathology (CRT), Los Angeles County/University of Southern California Medical Center, Los Angeles, California, U.S.A., and Department of Pathology (GR), Dalhousie University Medical School, Halifax, Nova Scotia, Canada

The monoclonal antibody OKT6 and antisera against S-100 protein have both been advocated as immunologic markers of Langerhans cells in the skin. S-100 antiserum has an advantage in its ability to stain Langerhans cells in paraffin tissues. In order to evaluate whether these antibodies stain equivalent numbers of Langerhans cells in skin, we compared the staining patterns of S-100 antiserum and OKT6 antibody on biopsy specimens from 40 patients with leprosy using immunoperoxidase techniques. Utilizing OKT6 antibody, greater numbers of positive Langerhans cells were found in the epidermis in tuberculoid leprosy, reversal reaction, and erythema nodosum leprosum than in lepromatous leprosy. However, these differences were not observed with the S-100 antiserum and, overall, fewer cells were found as compared with the OKT6 antibody. In the dermis both antibodies stained "dendritic cells" that were found encircling granulomas in tuberculoid leprosy and reversal reaction. Staining in lepromatous leprosy granulomas, in contrast to the epidermal staining pattern, revealed rare OKT6-positive cells, while S-100 cells were numerous and were more diffusely distributed throughout the granuloma. Our results indicate that antiserum to S-100 protein and OKT6 antibody stain morphologically similar cells (dendritic cells), but do not provide comparable results concerning distribution and frequency of these cells.

The availability of monoclonal antibody, OKT6, that reacts with Langerhans cells [1–3] has provided a new tool for the investigation of Langerhans cells in the epidermis of a variety of dermatoses [4–7]. This antibody recognizes a 49,000 dalton cell surface glycoprotein [8]. The use of this antibody is limited to frozen sections so that tissues routinely processed in paraffin cannot be used. An antibody that reacts with Langerhans cells in paraffin sections would facilitate investigation of the frequency and distribution of these cells in a variety of skin diseases.

S-100 protein is an acid calcium binding protein present in the cytoplasm of a variety of cells including Langerhans cells [9,10]. Unlike OKT6 antibody, S-100 antiserum can be used for routine immunostaining of paraffin sections. However, it is not known whether the two antisera produce equivalent results on human skin. Therefore, we undertook to compare the immunostaining patterns of OKT6 antibody and S-100 antiserum on human skin biopsy specimens. Because we have found distinct differences in the number of epidermal Langerhans cells throughout the spectrum of leprosy [4,5], and because we have "banked" 40 frozen blocks of leprosy skin lesions with corresponding paraffin blocks from the same biopsy specimen, we elected to perform our study on these tissues.

Both OKT6 and S-100 antisera may react also with Langerhans cells in the human dermis [10,11]. Therefore, we also undertook to compare these antisera in their ability to stain cells in the dermis of leprosy lesions. Our results show that the numbers of cells identified by these antisera in human epidermis and dermis are not equivalent.

MATERIALS AND METHODS

Skin biopsy specimens obtained from 40 patients with leprosy were bisected. Half the specimens were fixed in formaldehyde for routine paraffin embedding; half were coated in OCT, snap-frozen in liquid nitrogen, and then stored at −70°C. Classification (Table I) was according to the criteria of Ridley [12], with the reactional states identified as previously described [13].

OKT6 antibody was utilized in an indirect immunoperoxidase technique [5]. Cryostat sections were cut to 6 μm and fixed for 10 min in acetone. After 5 min in phosphate-buffered saline (PBS), mouse monoclonal OKT6 (Ortho) antibody protein level/ml at 1:50 was applied for 15 min, followed by peroxidase-conjugated goat antimouse IgG (Tago) at 1:10 for 15 min, with a 5-min PBS wash between incubations. A colored reaction product was obtained using aminoethyl carbazole and hydrogen peroxide as substrate. Slides were counterstained with hematoxylin and mounted in glycerol jelly. Controls consisted of the use of an antibody of irrelevant specificity at the same concentration and of omission of the primary antibody in the indirect peroxidase method.

Antiserum was prepared as follows. For comparison to the commercial Dako antiserum, an S-100 protein fraction was isolated from fresh calf brain homogenate by ammonium sulfate precipitation and column chromatography with diethylaminoethyl cellulose-sephacel and Bio-Gel P-60 [14]. The resulting S-100 fraction was assayed for amino acid composition, molecular weight, electrophoretic mobility, and reacted with a defined anti-S-100 antiserum (courtesy of Dr. S. E. Pfeiffer, University of Connecticut) by double diffusion and immunoelectrophoretic methods. All findings were consistent with published values for the aforementioned features [15]. A heteroantiserum was produced in rabbits by hyperimmunization with Freund's complete and incomplete adjuvants.

For identification of the S-100 antigen, checkerboard titrations were employed to determine the optimal antibody concentrations in the peroxidase-antiperoxidase technique [10]. The serum was stored at −20°C until utilized at dilutions of 1:10 to 1:200. Initial antibody protein levels in the undiluted serum were 8 ng protein/ml. Paraffin sections were deparaffinized in xylene and rehydrated in graded ethanol concentrations, then treated for 20 min in 0.5% hydrogen peroxidase in methanol to block endogenous peroxidase activity. After washing in PBS the following reagents were sequentially applied, with 5-min washes with PBS between incubations: normal swine serum (Dako) 1:20 for 10 min; anti-S-100 protein antibody (Dako) at 1:50 for 30 min; swine antirabbit immunoglobulin serum (Dako) at 1:20 for 30 min; rabbit peroxidase-antiperoxidase complex (Dako) at 1:30 for 30 min. Visualization of peroxidase activity was obtained with aminoethyl carbazole and hydrogen peroxide, and slides were counterstained in hematoxylin. Trypsinization of paraffin sections was not routinely performed, because staining of sections with or without trypsinization [16] yielded identical results. Identification of S-100 in representative acetone-fixed frozen sections produced equivalent results to paraffin sections, although frozen sections showed a "weaker" staining reaction. Because the goal of this study was to evaluate S-100 staining on paraffin sections, staining on frozen sections was not routinely performed.

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Reprint requests to: Thomas H. Rea, M.D., Department of Dermatology, Room 8441, LAC/USC Medical Center, 1200 N. State Street, Los Angeles, California 90033.

Abbreviations:
PBS: phosphate-buffered saline

TABLE I. *Numbers of specimens graded by density of Langerhans cells in epidermis according to percentage of cells exhibiting cell–cell contact*

Patient classification	OKT6		S-100	
	<10%	>10%	<10%	>10%
Tuberculoid	1	7	8	0
Borderline	2	6	8	0
Lepromatous	7	0	7	0
Erythema nodosum leprosum	4	4	8	0
Reversal reaction	3	4	7	0
Lucios reaction	1	1	2	0

Numbers were graded according to density as to whether less than 10% of the positive cells showed visible cell–cell contact of dendritic extensions with other positive cells, or whether greater than 10% of cells exhibited positive cell–cell contact (i.e., a dense interconnecting meshwork).

Controls

The specificity of the staining for S-100 was assessed as follows:

Tissue controls: Normal brain, gut (for myenteric plexi) and normal skin (for Langerhans cells, melanocytes, and peripheral nerve Schwann cells) embedded in paraffin and treated as for test sections served as positive controls. Peripheral Schwann cells in the experimental section also served as reliable indications for the successful staining of S-100 protein.

Reagent controls: (a) Specific immune blocking: Control consisted of exposure of test and control sections to unlabeled anti-S-100 antiserum followed by exposure to directly labeled anti-S-100 horseradish peroxidase. A significant total diminution of staining was necessary and was noted not only in Langerhans cells but also in Schwann cells.

(b) Antibody: Control consisted of antigen preabsorption. Anti-S-100 antiserum was incubated overnight at 4°C with 1 mg/ml S-100 protein. Staining was performed with the resultant filtered centrifuged supernatant. Failure of the staining of Schwann cells and Langerhans cells was necessary and was noted.

(c) Sections were incubated in preimmune rabbit serum at equivalent dilution instead of the anti-S-100 antibody.

(d) Sections were incubated for endogenous peroxidase activity (minus blocking).

(e) Sections were exposed to free horseradish peroxidase followed by demonstration of peroxidase activity as above.

Langerhans cells were identified in the epidermis of immunostained preparations by their oval cell bodies and dendritic extensions. OKT6-positive and S-100-positive cells in the dermis, oval with dendritic extensions, were evaluated for numbers and microanatomic location.

RESULTS

Epidermis

OKT6-positive cells (presumptive Langerhans cells) were identified in the epidermis of tuberculoid and borderline specimens in greater numbers than in lepromatous specimens. Also, OKT-6 positive cells were identified in great numbers in the epidermis of the reactional states of reversal reaction and erythema nodosum leprosum.

By contrast, anti-S-100 antibody demonstrated few positive cells in all specimens; S-100-positive Langerhans cells were well spaced with rare interconnections of dendrites (Table I, Fig 1). Dilutions of both antisera did not significantly reduce the numbers of positive cells in a selective fashion; weakening of the staining reaction was a constant feature of all Langerhans cells at levels of dilution in excess of 1:200 for S-100 antiserum, and 1:50 for OKT6 activity. At further dilution of S-100 antiserum, significant staining of Schwann cells was still visible, while Langerhans cell staining faded, reflecting the quantitative differences in amounts of S-100 protein in the two cell types.

Dermis

Considered as a whole, the tuberculoid and borderline specimens, including those cases with reversal reaction, had large numbers of OKT6-positive cells in the dermis; the positive cells morphologically exhibited dendritic extensions and often encircled the granulomas. Similar positive cells were rare in the dermis from lepromatous patients with or without erythema nodosum leprosum.

S-100-positive cells in the dermis were also of dendritic morphology and were usually more numerous than OKT6-positive cells, in contrast with epidermis. S-100-positive cells were frequent in tuberculoid and borderline specimens with or without reversal reaction and in lepromatous specimens. They were, however, absent (or very rare) in specimens from patients with erythema nodosum leprosum (Table II). In tuberculoid specimens S-100-positive cells often encircled granulomas (Fig

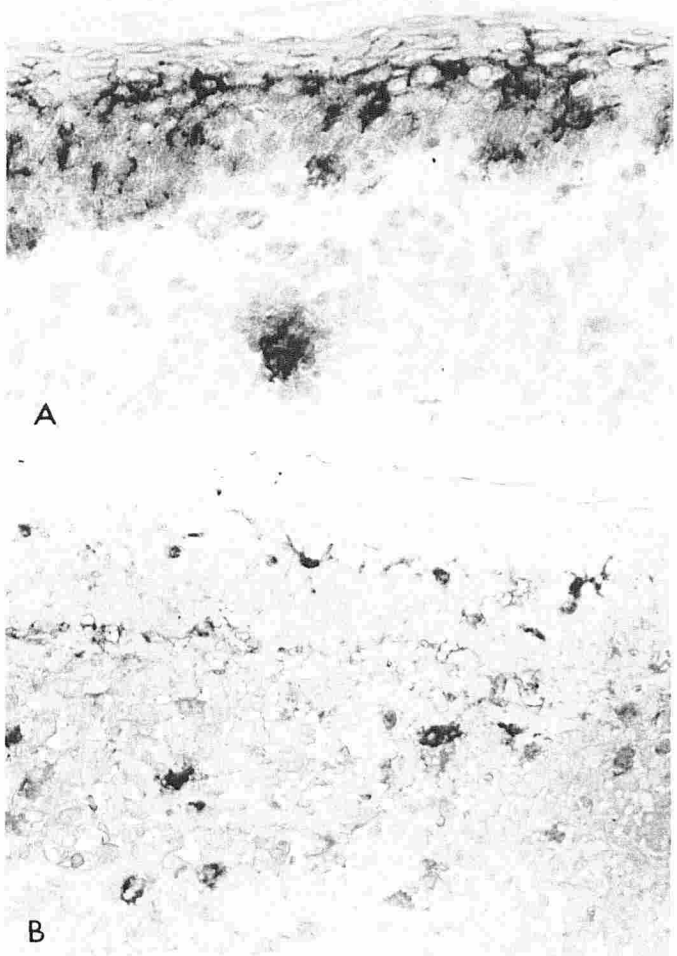

FIG 1. Epidermis of tuberculoid leprosy. *A*, OKT6 immunostaining reveals numerous ovoid cell bodies with dendrites exhibiting many interconnections resulting in a meshlike network. *B*, S-100 immunostaining on specimen from same patient as (*A*) reveals few cell bodies and less than 10% of dendritic extensions exhibiting interconnections. (Immunoperoxidase, hematoxylin, × 180.)

TABLE II. *Number of specimens with positive cells in the dermis of lepromatous tissues*

	OKT6		S-100	
	<5 cells/ tissue	>5 cells/ tissue	<5 cells/ tissue	>5 cells/ tissue
Lepromatous without erythema nodosum leprosum	7	0	1	6
Lepromatous with erythema nodosum leprosum	8	0	7	1

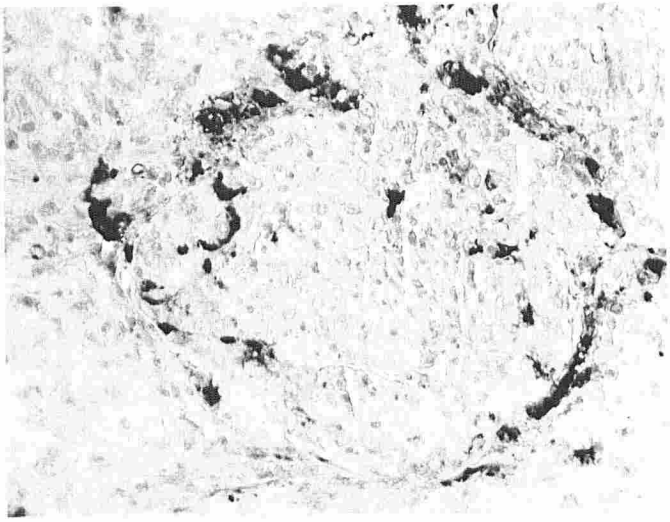

FIG 2. Dermis of tuberculoid leprosy. S-100 immunostaining demonstrates positive dendritic cells surrounding a granuloma. (Immunoperoxidase, hematoxylin, × 180.)

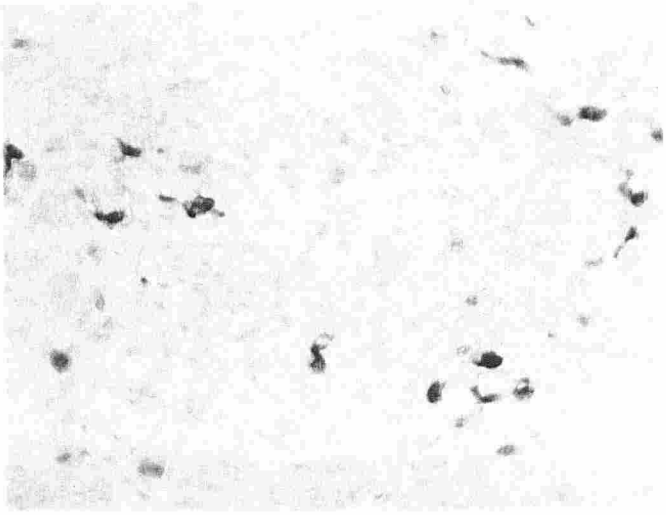

FIG 3. Dermis of lepromatous leprosy. S-100 immunostaining reveals scattered positive dendritic cells throughout granuloma.The corresponding OKT6 stain was negative in the dermis. (Immunoperoxidase, hematoxylin, × 180.)

2), while in lepromatous specimens they were distributed throughout the granuloma (Fig 3).

DISCUSSION

Our results indicate that OKT6 antibody and S-100 antiserum do not have identical staining patterns in cutaneous specimens from leprosy patients. The OKT6 antibody is superior to the S-100 antibody for identification of Langerhans cells in the epidermis in terms of number of positive cells identified. Further, OKT6 antibody gives different staining patterns of epidermal cells in different disease states; specimens from patients with immunologically active tuberculoid leprosy, reversal reaction, and erythema nodosum leprosum contained greater numbers of OKT6-positive Langerhans cells in the epidermis, as compared to lepromatous leprosy. By contrast, anti-S-100 failed to reveal any differences in the number of positive cells in the epidermis.

Of unexpected interest was the dermal staining pattern of S-100. S-100 positive cells were generally more numerous in the dermis as compared with OKT6-positive cells. In tuberculoid and borderline specimens with or without reversal reaction these cells surrounded granulomas in a manner similar to OKT6-positive cells. They were present throughout lepromatous granulomas but absent from those with erythema nodosum leprosum.

It has been claimed that S-100 antibody stains Langerhans cells in the dermis [9]; while the dendritic appearance and immunoelectron microscopic examination of OKT6-positive cells in the dermis suggest that they may also be Langerhans cells [11], or at least close relatives. The functional role of these cells is speculative, although they appear to correlate in numbers and locations with aspects of the host immune response.

That S-100 stained fewer epidermal cells but greater numbers of dermal cells as compared with OKT6 suggests that these antibodies do not stain identical populations of cells. This finding, combined with the observation that increased concentration of S-100 antisera does not increase the numbers of positive epidermal cells, implies that the heterologous S-100 antisera is not "weaker" than the monoclonal OKT6 antibody. Instead, the different populations of cells identified by these antisera may be related to functional characteristics perhaps of Langerhans cells.

We conclude that although both antiserum to S-100 and OKT6 "identify" cells that are morphologically consistent with Langerhans cells in the epidermis, they do not recognize such cells in equivalent numbers. Similarly, these two antibodies give discrepant results in relation to "dendritic cells" in the dermis. Use of S-100 antisera on paraffin sections cannot substitute for, but may provide additional information to, OKT6 staining on frozen sections. Further investigation is required to determine whether these patterns represent different phenotypic subsets of a single cell population and whether such differences have any biologic significance or diagnostic and prognostic value.

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