

SHORT REPORTS

Thymus-derived Origin of Sezary Cells Demonstrated by Peroxidase-conjugated Anti-HTLA Serum

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Abstract. A horse anti-serum rendered specific for human T lymphocytes was conjugated with peroxidase and used for identification of Sezary cells extracted from skin infiltrates. Po-anti-HTLA serum stained all the cells showing morphological characteristics of Sezary cells, which confirmed their thymus-derived origin.

Key words: Sezary cells; Ultrastructure; Peroxidase; Conjugated anti-HTLA serum; T lymphocytes markers

The Sezary syndrome is characterized by a generalized erythrodermia and large, abnormal mononuclear cells in skin and blood. Recent studies have shown Sezary cells to possess T cell properties (for review see 2). They are able to bind sheep erythrocytes and are killed by anti T lymphocyte serum, whereas they bear neither surface immunoglobulins (slg) nor complement receptors. We have prepared a horse anti-serum specific for human T lymphocytes which, after conjugation with peroxidase, allows ultrastructural identification of T lymphocytes (Schmitt, D., Brochier, J., Revillard, J., and Thivolet, J., submitted for publication). We report its use for identification of Sezary cells extracted from cutaneous infiltrates.

MATERIAL AND METHODS

Patient

A 76-year-old male showing generalized erythrodermia and typical Sezary cells recognized on routine blood smears (6).

Extraction of lymphocytes

Circulating cells were obtained from heparinized blood by centrifugation on Ficoll-Metrizoate. Lymphocytes from cutaneous infiltrates were obtained according to a previously described technique (7). Briefly, biopsies were minced with scissors, homogenized in Potter and washed in Hanks' medium.

Identification of T cells by anti-HTLA serum

Anti-HTLA serum was prepared from anti-human thymocyte immunoglobulins from a horse (Institut Mérieux, Lyon, France), sequentially absorbed on AB erythrocytes, polymerized Ig, fixed placental tissue and lymphoblastoid cell lines (Brochier, J., Abou-Hamed, Y. A., Gueho, J. P. and Revillard, J. P., submitted for publication). Peroxidase-conjugated anti-HTLA Ig (Po-Anti-HTLA) were prepared according to the Avrameas technique and lymphocytes were labelled according to a previously described technique.

Other techniques for T and B cell identification

T cells were also characterized by the E rosette test and B lymphocytes by the detection of membrane Ig with a fluoresceinated anti-human Ig (Behring) serum or by Po-Fab anti-human Ig conjugate (Institut Pasteur, Paris) using an immunocytochemical technique (5). The EAC rosette technique was performed according to Bianco et al. (1).

Table 1. *Percentage of T and B lymphocytes obtained from skin infiltrates and blood from a patient with Sezary syndrome*

n. d. = not done

	Skin	Blood	
		Sezary syndrome	Healthy donors ^d
EAC rosettes	6	10	11
slg ^a	4	9	13
slg ^b	3	n. d.	10
E rosettes	62	55	61
HTLA ^c	79	n. d.	n. d.

^a Fluoresceinated anti-Ig conjugate.^b Po-Fab anti-Ig conjugate.^c Po-anti-HTLA conjugate.^d Mean from 5 donors.

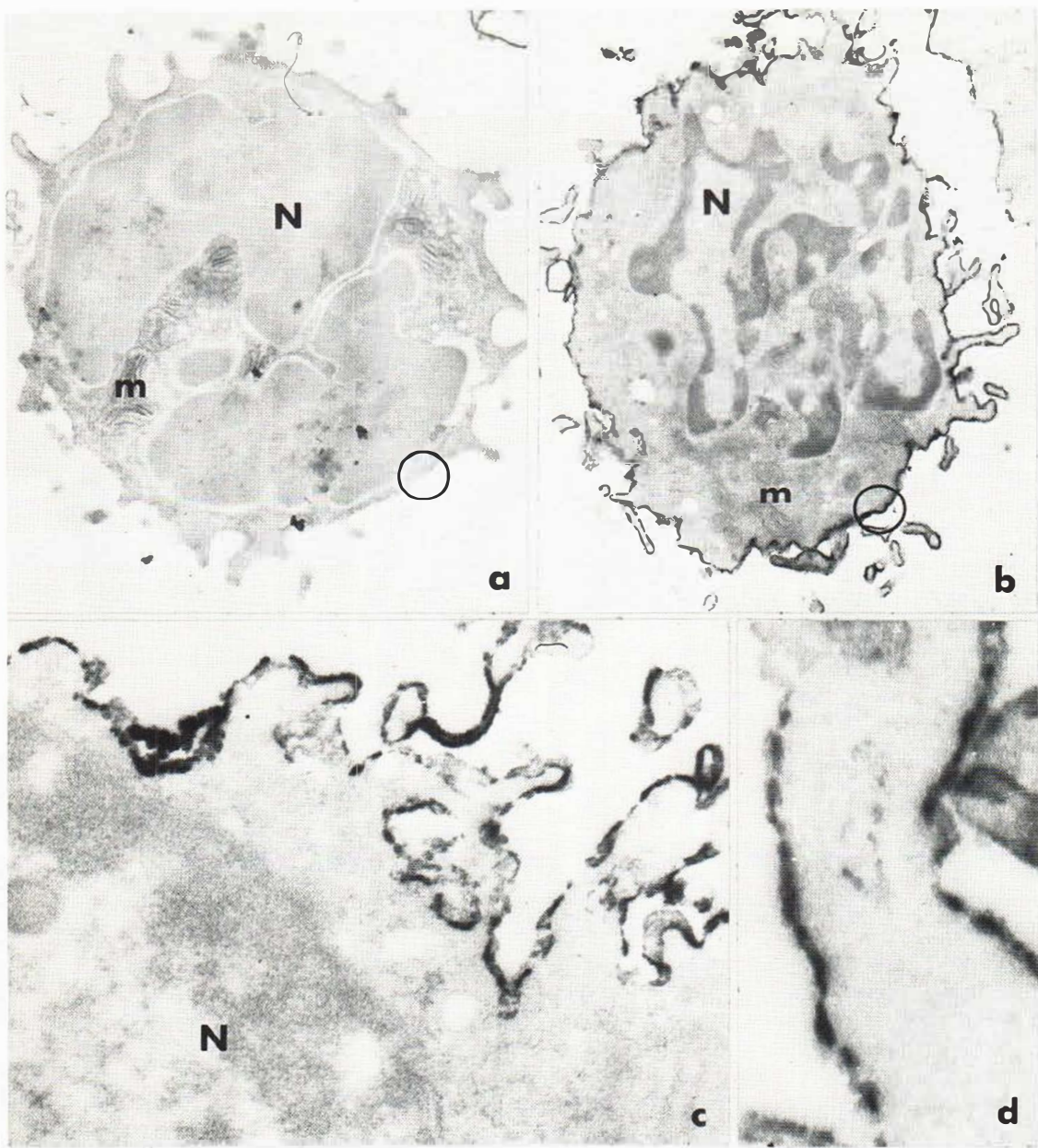


Fig. 1. Immunocytochemical aspects of Sezary cells extracted from cutaneous infiltrates as seen by electron microscopy. (a) Stained with Po-Fab anti-Ig conjugate ($\times 9\,160$). (b) Stained with Po-anti-HTLA conjugate

($\times 9\,160$). (c) Detail of membrane staining ($\times 36\,600$). (d) Detail of membrane staining ($\times 82\,500$). N, nucleus; m, mitochondria.

RESULTS

Table I shows the percentage of B and T lymphocytes in cell suspensions obtained from blood and skin infiltrates. Their proportion in the patient's blood did not appear to differ from healthy donors.

Cells extracted from cutaneous infiltrates appeared to contain a large proportion of T lymphocytes (E rosette-forming cells and HTLA-positive cells).

Ultrastructural studies of cells from skin infiltrates showed nearly all the cells to be stained by

Po-anti-HTLA conjugate, whereas very few were labelled by Po-Fab anti-Ig. All the cells stained by Po-anti-HTLA conjugate showed ultrastructural characteristics of Sezary cells (Fig. 1*b*). Conversely, these cells were not stained when Po-Fab anti-Ig conjugate was used (Fig. 1*a*). The staining was found on the cytoplasmic membrane only as irregular spots (Fig. 1*c, d*).

DISCUSSION

A number of immunological studies have already suggested the thymus-derived nature of Sezary cell. The present data present direct evidence of the T cell nature of these cells, by immunocytochemical and ultrastructural techniques, and using a Po-anti-HTLA serum. The direct labelling technique showed that all the cells with morphological characteristics of Sezary cells (cerebriform nucleus, microvilli) were stained by Po-anti-HTLA conjugate, whereas they were not stained by Po-Fab anti-Ig. Previous results have clearly shown that Po-anti-HTLA Ig did stain tonsillar T lymphocytes, whereas they left B cells and monocytes unstained (Schmitt, D., Brochier, J., Revillard, J. P. and Thivolet, J., submitted for publication). Other studies were performed using Sezary cells from blood; we used cells from skin infiltrates and, therefore, confirm their cutaneous homing (4). A few B lymphocytes were isolated from skin infiltrates: their number was found to be lower than in blood, although they were generally found in the same proportion in other skin disorders or even in increased numbers in melanoma (3). The present report illustrates a new approach to the identification and characterization of T cell proliferations by using a specific anti-serum conjugated with peroxidase, in light and electron microscopy.

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REFERENCES

1. Bianco, C., Patrick, R. & Nussenzweig, V.: A population of lymphocytes bearing a membrane receptor for antigen-antibody complement complexes. I. Separation and characterization. *J Exp Med* 132: 702, 1970.
2. Claudy, A.: The immunological identification of the Sezary cell. *Br J Dermatol* 91: 597, 1974.
3. Claudy, A. L., Schmitt, D., Viac, J., Alario, A., Staquet, M. J. & Thivolet, J.: Morphological, immunological and immunocytochemical identification of lymphocytes extracted from cutaneous infiltrates. *Clin Exp Immunol* 23: 61, 1976.
4. Edelson, R. L., Kirkpatrick, C. H., Shevach, E. M., Schein, P. S., Smith, R. W., Green, I. & Lutzner, M.: Preferential cutaneous infiltration by neoplastic thymus-derived lymphocytes. *Ann Int Med* 80: 685, 1974.
5. Schmitt, D., Viac, J., Alario, A. & Thivolet, J.: Technique immunohistochimique rapide d'identification des cellules lymphoïdes de la lignée B. *Experientia* 31: 700, 1975.
6. Thivolet, J., Perrot, H., Bouchet, B., Hermier, C. & Michel, F.: Syndrome de Sezary. *Bull Soc Fr Derm Syph* 81: 503, 1974.
7. Viac, J.: Contribution à l'étude des sous-populations lymphocytaires au niveau du sang et des tissus chez l'homme et chez l'animal. Thèse de Doctorat de Sciences. Lyon n° 39, 1975.

Analysis of Cysteinyl-dopa Isomers and Some of Their O-methyl Derivatives in the Urine of Melanoma Patients by Means of Gas Chromatography – Mass Spectrometry

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O-methylated dopa can be studied by means of combined gas chromatography – mass spectrometry (GC–MS) after formation of derivatives with methanol–HCl and pentafluoropropionic anhydride (for details, see 1). With the aid of these methods, three different isomers of cysteinyl-dopa and four O-methylated cysteinyl-dopa isomers have been detected in the urine of melanoma patients.

Urine was obtained from patients with widespread melanoma metastases. Cysteinyl-dopa isomers were purified by alumina adsorption (2). The derivatives of the isomers of cysteinyl-dopa