

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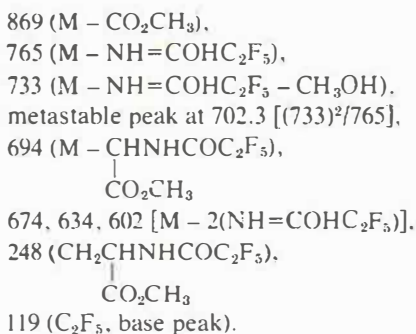
O-methylated dopa can be studied by means of combined gas chromatography – mass spectrometry (GC–MS) after formation of derivatives with methanol–HCl and pentafluoropropionic anhydride (for details, see 1). With the aid of these methods, three different isomers of cysteinyl-dopa and four O-methylated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

appeared in three distinct peaks in the gas chromatogram. 2-S-cysteinyl-dopa (II) had the shortest retention time, 6-S-cysteinyl-dopa (III) an intermediate retention time, and 5-S-cysteinyl-dopa (I) appeared last.

Mass spectrometry was performed, using ionizing voltage 70 eV. The 5-S-cysteinyl-dopa derivative has a molecular ion at 928. Two isomers (2-S- and 6-S-cysteinyl-dopa) were found, having the same molecular ion but with other fragmentation patterns. Some of the most important peaks (*m/e*) for the various derivatives are given below.

I. 5-S-cysteinyl-dopa. Molecular ion peak of the derivative at 928. Fragment ions at



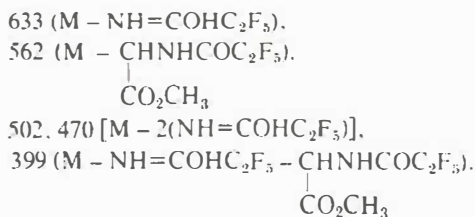
II. 2-S-cysteinyl-dopa. Molecular ion peak of the derivative at 928. Fragment ions at 869, 765 (relative intensity of the peak much smaller than that of I), 733 (small), metastable ion at 702.3 not present, 694 and 674 not present, 634 (less than in I), 620 (larger than in I), 602 (less than in I), 543 (larger than in I), 471 (larger than in I), 248 (base peak).

III. 6-S-cysteinyl-dopa. Molecular ion peak of the derivative at 928. The fragmentation pattern is similar to that of II. However, 733 is present in II but not in III. 484 is larger in III than in II, 248 is slightly larger in II than in III, 235 is slightly larger in II than in III.

It also proved possible to study the O-methylated cysteinyl-dopa isomers occurring in melanoma urines, by GC-MS after purification on ion exchange column Dowex 50 W-X4, (15×1.8 cm), using the same derivation technique. The two isomers of O-methyl 5-S-cysteinyl-dopa appeared after elution with 550 ml 2 M HCl. Two O-methyl isomers of 2-S-cysteinyl-dopa were observed and showed peaks at an elution volume of 125 ml 2 M HCl. The two methyl derivatives of 5-S-cysteinyl-dopa were named Ia and Ib, respectively, and the

O-methyl derivatives of 2-S-cysteinyl-dopa II a and II b, respectively.

Ia. Molecular ion peak of the derivative at 796. Fragment ions at



Ib. Molecular ion peak at 796. Fragment ions at 633 (larger than in Ia), 601 ($M - \text{NH}=\text{COHC}_2\text{F}_5 - \text{CH}_3\text{OH}$, not present in Ia), 562 (much smaller than in Ia), 502 (much larger than in Ia), 470 and 399 (much smaller than in Ia).

IIa. Molecular ion peak at 796. Fragment ions at 633, 562, 502, 399.

IIb. Molecular ion peak at 796. Fragment ions at 633, 562 (slightly smaller than in IIa), 502 and 399 (both much larger than in IIa).

NMR and enzymic techniques were also applied for the structural elucidation. The present study illustrates the usefulness of GC-MS for investigations of cysteinyl-dopa isomers, one of them, 6-S-cysteinyl-dopa, previously unknown. Four O-methyl derivatives of 5-S- and 2-S-cysteinyl-dopa are also described.

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