T-cell nature of leukaemic cells in a case of Sézary’s syndrome with ‘null-cell’ features

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SUMMARY α-naphthyl acetate esterase (ANAE) activity has been investigated in leukaemic cells from peripheral blood in a typical small-cell Sézary syndrome (SS) case in which cerebriform mononuclear cells failed to form E rosettes. The ‘dot-like’ ANAE positivity found in the majority of these neoplastic cells strongly supports a T-cell origin. In addition, a non-monocytic, non-B-cell nature of Sézary cells is indicated by the lack of Ia-like antigens. Finally, there is evidence of a distinct portion of Sézary cells simultaneously expressing ANAE activity and Fc IgM receptors.

Sézary’s syndrome (SS) is a human lymphoproliferative disease in which malignant cells with T-cell characteristics are found in skin infiltrates as well as in peripheral blood (Edelson et al., 1974a; Preud’-Homme and Seligmann, 1974; Zucker-Franklin et al., 1974). The T-cell nature of Sézary cells is supported by the capacity to form rosettes with sheep red blood cells (E rosettes). However, some typical SS cases have been reported in which the neoplastic cells failed to form E rosettes (Wybran and Fudenberg, 1973; Edelson et al., 1974b; Braylan et al., 1975; Goldstone et al., 1976). In these cases the T-cell nature of the neoplastic cell proliferation remains putative. Therefore the search for other distinguishing T-cell markers could lead one to ascertain the origin of these undefined cells.

In addition to the capacity to form rosettes with sheep red blood cells, a distinctive, dense, localised, ‘dot-like’, non-specific acid α-naphthyl acetate esterase (ANAE) positivity has been demonstrated in the vast majority of human T-cells (Ranki et al., 1976; Horwitz et al., 1977; Kulenkampff et al., 1977; Tötterman et al., 1977; Knowles et al., 1978; Ranki, 1978) as a distinguishing feature of thymus-derived lymphocytes. Human T lymphocytes have recently been shown to express receptors that bind the Fc portion of IgG (Tg) or IgM (TM) (Dickler et al., 1974; Ferrarini et al., 1975; Moretta et al., 1975); up to 95% of the TM cells are esterase-positive and 90% of the TG cells are completely esterase-negative (Grossi et al., 1978).

It was shown that, in acute lymphoblastic leukaemias with T-cell surface characteristics, ANAE positivity may be a further specific marker for thymus-derived lymphocytes (Kulenkampff et al., 1977; Knowles et al., 1978).

In the present study we investigated the ANAE activity in leukaemic cells from the peripheral blood of a case of SS in which typical cerebriform mononuclear cells failed to form E rosettes but showed in very high proportion an ANAE ‘dot-like’ positive reaction. Moreover, in order to obtain more detailed information into the origin of these Sézary cells, we tried to find if Ia-like molecules were expressed by the leukaemic population. Ia-like molecules were recognised initially as alloantigens, primarily represented on B lymphocytes. Later, in addition to their presence on B cells and monocytes, they have been detected on leukaemic blasts in cases of acute lymphocytic leukaemia, acute myelogenous leukaemia, and chronic myelogenous leukaemia in blastic crisis (Fu et al., 1975; Schlossman et al., 1976; Winchester et al., 1975, 1976). On normal human T-cells, the presence of Ia-antigens has not been well documented.

Case report

A 55-year-old man was referred to this hospital because of a persistent peripheral blood lymphocytosis associated with exfoliative erythroderma and generalised adenopathy. The haemoglobin was 13·5 g/dl and the white cell count 22 × 10⁹/l with 33% neutrophils, 65% lymphocytes, and 2% monocytes.
After separation of the mononuclear cells on Ficoll-Hypaque gradient, more than 89% of the cells under consideration showed the typical cerebriform nucleus when examined under the electron microscope. Bone marrow and lymph node biopsy showed infiltrating cells with the morphological features of atypical peripheral blood lymphocytes.

**Material and methods**

**CELL SEPARATION**
Peripheral blood mononuclear cells were isolated on Ficoll-Hypaque density gradient (Böyum, 1968), and adherent cells were removed by incubation at 37°C for 60 minutes in plastic Petri dishes. Enumeration and separation of cells rosetting with neuraminidase-treated sheep erythrocytes (EN-RFC) were performed as previously reported (Piantelli *et al.*, 1979).

**SURFACE MARKER STUDIES**
Surface immunoglobulin was investigated by the direct immunofluorescence test, as previously reported by us (Salsano *et al.*, 1979). Detection of lymphocytes with receptors for the Fc portion of IgG or IgM was performed as described in a previous paper (Lauriola *et al.*, 1979).

The presence of Ia-like antigens on mononuclear cells was investigated by Dr Rosa Sorrentino, Laboratory of Cell Biology, CNR, Rome. An inhibition test was performed, using human purified radioiodinated Ia-molecules prepared from the RPMI 8057 cell line as antigen and a rabbit anti B-cell membrane heteroantiserum produced with membrane material of a B-cell type line 5329-1117 (Koyama *et al.*, 1977; Tosi *et al.*, 1978). Briefly, Renex-30 solubilised material from Sézary cells or chronic lymphatic leukaemia of B origin (10 × 10⁶

![Fig. 1 Low-power survey electron photomicrographs of typical Sézary cells showing characteristic convoluted nuclei and large nucleoli. (× 6800).](http://jcp.bmj.com/ on December 19, 2017 - Published by group.bmj.com)
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Table Comparative percentages of immunological and cytochemical markers in peripheral mononuclear cells* from Sézary syndrome patient and two normal subjects

<table>
<thead>
<tr>
<th>Peripheral mononuclear cells</th>
<th>RFCs</th>
<th>Surface Ig</th>
<th>ANAE activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EN</td>
<td>EA IgM</td>
<td>EA IgG</td>
</tr>
<tr>
<td>Sézary syndrome patient</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfractionated</td>
<td>7</td>
<td>35</td>
<td>12</td>
</tr>
<tr>
<td>EN-enriched</td>
<td>94</td>
<td>61</td>
<td>16</td>
</tr>
<tr>
<td>EN-depleted</td>
<td>&lt;1</td>
<td>29</td>
<td>2</td>
</tr>
<tr>
<td>Normal subjects (A/B)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfractionated</td>
<td>73/78</td>
<td>39/34</td>
<td>22/16</td>
</tr>
<tr>
<td>EN-enriched</td>
<td>97/99</td>
<td>59/64</td>
<td>16/10</td>
</tr>
</tbody>
</table>

*Adherent cells depleted preparations.

cells/ml in both cases) was incubated with the anti B-cell membrane heteroantiserum, followed by the addition of radiiodinated antigen; goat anti-rabbit immunoglobulin was then added and immunoprecipitate radioactivity was determined. The results were expressed as per cent inhibition of binding of radiiodinated Ia-antigens by rabbit antiserum.

Cytotoxic Demonstration of ANAE activity

The demonstration of esterase activity was performed using α-naphthyl acetate as substrate under conditions described by Mueller et al. (1975).

Ultrastructural Studies

Mononuclear cell preparations were fixed in 2.5% glutaraldehyde in 0.1 m phosphate buffer pH 7.4 and processed for electron microscopy.

Response of Mononuclear Cells to Phytomitogens

Blast cell transformation after stimulation with Phytohaemagglutinin, Concanavalin A, and Pokeweed mitogen was performed as previously described (Lauriola et al., 1979).

Results

The morphology (Fig. 1) of the leukaemic cells from peripheral blood appeared similar to that described in the small cell variant of Sézary's syndrome by

Fig. 2 Cytocentrifuge smear of Sézary cell enriched population. The majority of the cells display a typical 'dot-like' cytoplasmic reaction product adjacent to the cell membrane. (× 1000).
several authors (Lutzner et al., 1973; Edelson et al., 1974b; Goldstone et al., 1976). The abnormal mononuclear cells showed an infolded nucleus with less prominent irregularity than the classic Sézary cell. Frequently, a well-developed nucleolus was observed. The nuclear/cytoplasmatic ratio was, in general, high.

**Immunological Markers**

The percentage of several surface markers in the peripheral blood mononuclear population, E\(_N\) rosetting population (E\(_N\)-RFC), as well as E\(_N\) rosette forming cells depleted preparation (E\(_N\)-RFC depleted), from the leukaemic patient are shown in the Table. In the same table the values obtained in the peripheral blood mononuclear cells of two healthy donors are given.

The E\(_N\)-RFC depleted preparation from a Sézary syndrome patient on ultrastructural examination showed the vast majority of cells with typical Sézary small cell features (94%); on the other hand, the E\(_N\) rosetting fraction revealed a typical lymphocytic population with rare, contaminating Sézary cells.

A 50% inhibition of binding between specific antiserum and radioiodinated Ia antigens was attained with 16 \(\mu\)l of chronic lymphatic leukaemia preparation but failed to be reached even with a 30-fold amount of E\(_N\)-RFC depleted cell preparation from the Sézary syndrome patient.

**Cytochaemic Investigations**

The results of cytochemical investigations on the SS leukaemic population in toto and on the E\(_N\) rosetting population, as well as in the E\(_N\)-RFC depleted preparation, are reported in the Table and compared with data from two normal subjects. The typical pattern of ANAE activity exhibited by the Sézary cell enriched population is shown (Fig. 2).

**Response to Phytotherapeutics**

The peripheral blood SS mononuclear preparation depleted of E\(_N\)-RFC failed to respond when cultured with phytotherapeutics (data not shown).

**Discussion**

The present study provides a more detailed insight into the characteristics of the neoplastic population in SS and supplies further criteria for the assessment of the true cellular origin when Sézary cells lack the E rosetting capacity.

In our SS case, the neoplastic population, which shared the morphological appearances of the small cell variant, failed to form E rosettes. Only in one of several SS null-cell variant cases reported has the T nature of leukaemic cells been corroborated by the presence of human thymic lymphocyte antigen-positive cells (Edelson et al., 1974b).

‘Dot-like’ ANAE positivity of a distinctive lymphocyte population in normal human peripheral blood and lymphoid tissues correlates with an E rosette forming cell population. Moreover, an association between E marker positivity and ANAE ‘dot-like’ activity has been shown in leukaemic cells (Kulenkampff et al., 1977; Knowles et al., 1978). Thus, the demonstration of ANAE activity appears to be a useful T-cell marker and a reasonable alternative to E rosetting capacity. The ‘dot-like’ ANAE positivity that we have found in the vast majority of the peripheral blood Sézary cell enriched population (E\(_N\)-RFC and monocytic cells depleted) strongly supports a T-type origin.

The absence of E rosetting capacity in our SS case, as well as in a single childhood acute lymphoblastic leukaemia case reported by Kulenkampff et al. (1977), may be interpreted as an alteration in the maturative process leading to a dissociation in the normal expression of the two markers.

A non-monocytic, non-B cell origin of Sézary cells is further supported by the lack of Ia-like antigens. In fact, Ia-like antigens have generally been thought not to be expressed by normal or leukaemic T cells in man, although very recently Ia molecules have been demonstrated on a small population of normal peripheral T cells, on T cells grown in long-term cultures (Fu et al., 1978), and on T lymphocytes sensitised in a mixed leucocyte culture (Evans et al., 1978).

The finding that a portion of Sézary cell enriched population bear receptors for the Fc portion of IgM is consistent with the recent findings of Worman et al. (1978) and Gupta et al. (1978), although Sézary cells in these reported cases exhibited a very high percentage of E RFC. In the present study, as shown in the Table, the sum of ANAE ‘dot-like’ positive and Fc-IgM receptors bearing cell values exceeded 100%, suggesting the presence of cells simultaneously expressing both markers.

Peripheral blood E rosetting cells from our SS patient were morphologically normal T lymphocytes; in addition, they expressed Fc-receptors for IgM and IgG in the same ratio as peripheral T cells from normal subjects.

In conclusion, our observation in a typical SS case shows that non E rosetting leukaemic cells may express other T cell features and emphasises the usefulness of several techniques in order better to classify lymphoproliferative disorders.

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References


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