A case of small-cell Sézary's syndrome with null-cell features

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SYNOPSIS A case of the small-cell variant of Sézary's syndrome (SS) is reported in which the SS cells lacked the surface-marker characteristics of both T- and B-cells. In particular, the SS cells failed to form E-rosettes even with a sensitive technique using 2-amino-ethylisothiuronium bromide (AET)-treated sheep red blood cells. The significance of these findings is briefly considered in relation to the existing literature.

Although the majority of human lymphoreticular tumours appear to be B-cell proliferations, Sézary's syndrome (SS) has come to be regarded as one of the few examples of human T-cell neoplasia (Preud'homme and Seligmann, 1974). This view has been based on the ability of Sézary cells to form rosettes with washed unsensitized sheep red cells (E-rosettes). However, in one report (Wybran and Fudenberg, 1973) the Sézary cells failed to form E-rosettes, and it was suggested that this was due to the relative insensitivity of the technique used. Very recently, Braylan *et al* (1975) have examined the surface marker properties of three cases of SS and have also failed to demonstrate the formation of E-rosettes using the standard E-rosetting technique.

In this paper we report a further case of SS in which the cerebriform cells failed to form E-rosettes even when the more sensitive method of Kaplan and Clark (1974) was employed.

Material and methods

CLINICAL FEATURES

This 73-year-old man presented with an erythematous rash on the abdomen. This developed into a psoriasiform erythroderma with involvement of the palms and soles and was associated with grossly dystrophic nails. He had no lymphadenopathy or hepatosplenomegaly and his general condition was good. A blood count showed a Hb of 14.0 g/dl, platelet count of $250 \times 10^9/l$, and a WBC count of

$30 \times 10^9/l$ with 32% neutrophils, 60% mature-looking small lymphocytes (which at $\times 100$ displayed some clefting of the nucleus), and occasional monocytes and eosinophils. The ESR was < 40 mm/h. Skin biopsy showed a dense infiltration of the upper dermis by abnormal mononuclear cells, some of which showed cerebriform nuclei. Atypical chronic lymphocytic leukaemia was excluded on bone marrow examination where a cellular normal marrow without lymphocytic infiltration was found. The lymphoid cells were further investigated by surface marker studies and electron microscopy (see below).

Initially, treatment was with topical steroids and a regimen of low-dose oral prednisone. When this failed to control the eruption, β ray therapy was started but was poorly tolerated. On a combination of prednisone (20 mg/day), triamcinolone (24 mg/day), and chlorambucil (4 mg/day) he improved. The improvement appeared markedly steroid-dependent since, when steroids were tapered steeply because of steroid-induced diabetes, he again relapsed. The WBC fell to $14 \times 10^9/l$ with 50% neutrophils and 46% lymphocytes. Since the haemoglobin also fell from 14.0 g/dl to 10.9 g/dl without evidence of blood loss, the chlorambucil was later stopped. After reinstating full steroid dosage and tapering more slowly, control of the eruption was regained and he was discharged in a much improved state on triamcinolone, 8 mg three times a day and prednisone, 5 mg twice daily. Subsequently he developed hyponatraemia, the aetiology of which was not elucidated. He died at home soon afterwards of bronchopneumonia.

Figure A typical Sézary cell; uranyl acetate and lead citrate staining ($\times 19\,000$). The cell contains the folded nucleus typical of the mononuclear cells of SS. As in the majority of our patient's SS cells, the nucleus contains moderately heavy peripheral chromatin condensation and a well-developed nucleolus. The relatively scanty cytoplasm is of non-specific appearance; in particular, accumulations of glycogen and the Golgi apparatus were not prominent in our patient's cells.

ULTRASTRUCTURE

Buffy coat preparations were fixed in 1.5% glutaraldehyde and processed for electron microscopy according to routine methods (Cawley and Hayhoe, 1973).

SURFACE-MARKER STUDIES

Heparinized blood was sedimented with 0.6% dextran, and the leucocyte-rich plasma was centrifuged on a mixture of Ficoll and sodium metizoate and washed with HEPES buffer Hank's BSS

containing 0.2% BSA, as described previously (Collins *et al*, 1974). E-rosette formation was measured using 0.143M 2-aminoethylisothiuronium bromide (AET)-treated sheep RBCs as described by Kaplan and Clark (1974). Fc-receptored cells were detected by rosette formation with IgG rabbit anti-ox RBC-treated ox RBCs (Haegert *et al*, 1974). C3 receptors were measured by rosette formation with IgM rabbit anti-ox RBC-treated ox RBCs which had been treated with AKR mouse serum as a source of complement (Ross *et al*, 1973). Surface Ig was detected by the indirect immunofluorescence technique, treating the cells with a polyvalent rabbit antiserum to human Ig, raised in this laboratory, and known to react with γ , μ , α , κ , and λ chains followed by fluorescein-labelled goat anti-rabbit IgG (Mehz Laboratories, Springfield, Va). The cells were suspended in PBS:glycerol (1:1 u/v) and examined by epifluorescence.

Results

CYTOLOGY AND ULTRASTRUCTURE

The Romanowsky cytology and ultrastructure (figure) of our patient's circulating mononuclear cells closely resembled that described in small-cell SS by a number of authors (Lutzner *et al*, 1973; Rosas-Urbe *et al*, 1974).

The abnormal mononuclear cells showed a considerable morphological heterogeneity but, in general, contained a complexly infolded nucleus (figure). The nuclei showed moderately heavy peripheral chromatin condensation and frequently contained a well-developed spherical nucleolus. The nuclear/cytoplasmic ratio was somewhat variable but was in general high. The relatively scanty cytoplasm contained scattered ribosomes, mitochondria, microtubules, and microfibrils, but RER, the Golgi apparatus, and glycogen particles were rarely seen.

SURFACE-MARKER STUDIES

The results of our surface-marker studies are summarized in the table. The mononuclear cells showed only a small percentage of cells with B-cell markers (SIg, Fc, and C3 receptors). Furthermore, only 3.5% of the patient's mononuclear cells form E-rosettes with AET-treated SRBC. The small percentages of marked cells probably represent the normal lymphoid populations present in our mononuclear preparation.

Discussion

Both the clinical and haematological features of our patient are those of the small cell SS. He had

typical skin changes, clinically and histologically without significant lymphadenopathy or hepatosplenomegaly. The ESR was normal and the peripheral blood contained numerous typical cerebriform cells, while no such cells were present in a marrow aspirate.

Patient	Marker			
	SIg	Fc Receptor	C3 Receptor	AET-SRBC
JW	5%	3%	6%	2.5%

Table Percentages of mononuclear cells showing surface markers in a case of Sézary's syndrome

The results reported here show that our patient's Sézary cells lack the characteristic surface markers of both T- and B-cells. These results confirm the findings of Braylan *et al* (1975) but extend them by demonstrating a failure of SS cells to form E-rosettes even with the more sensitive technique of Kaplan and Clark (1974). In our hands the AET pretreatment in general gives a higher percentage of rosetting cells and, in particular, in a cultured cell line derived from a T-cell acute leukaemia yielded a significantly higher percentage of rosette-forming cells (Karpas *et al*, 1976).

Although the single negative feature of failure to form E-rosettes cannot be considered to prove that our patient's Sézary cells are not T-cell in type, it nevertheless suggests that this may be so, and represents a significant point of difference from most previous studies, in which a high percentage of the Sézary cells have formed E-rosettes (Preud'homme and Seligmann, 1974). Use of other T-cell markers such as acid phosphatase reactivity, PHA transformation, and antithymocyte antisera, would further clarify this point but were precluded in our patient by his early death. However, since the diagnosis of SS in our patient seems certain, our findings at least raise the possibility that SS may be composed of two subgroups, one with T-cell features and the other lacking either T- or B-cell characteristics.

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