Lysosomal localisation of parallel tubular arrays in chronic lymphocytic leukaemia of T cell origin: an ultrastructural cytochemical study

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SUMMARY An ultrastructural cytochemical study of lysosomal acid phosphatase was performed on leukaemic cells in a case of chronic lymphocytic leukaemia of T cell origin (T-CLL). The cells showed inclusion bodies known as parallel tubular arrays, which often lay within acid phosphatase-positive, membrane-bound spaces. This suggests their lysosomal location.

Chronic lymphocytic leukaemia of T cell origin (T-CLL) is a rare but well known disease.^{1,2,3} The leukaemic cells exhibit characteristic cytoplasmic inclusions known as parallel tubular arrays (PTA) or Hovig bodies. Following Hovig's first description in a case of chronic rheumatoid arthritis,⁴ these bodies have been observed in circulating lymphocytes in several other diseases⁵⁻⁹ and in normal subjects.¹⁰ In 1978, McKenna *et al*¹¹ reported that PTA corresponded to prominent cytoplasmic azurophilic granules on light microscopy.

The nature of the PTA and their association with known subcellular organelles is not fully understood. Because they are often in vacuoles bounded by a single membrane, we investigated their possible relation to the lysosomal system. Catovsky *et al*¹² reported acid phosphatase positivity in these bodies and we have performed an ultrastructural cytochemical study of acid phosphatase in lymphocytes from a patient with T-CLL in order to confirm this.

Case report

The patient was a 69-year-old woman, with a 15 year history of a lymphocytosis. An abnormal white cell count (leucocytes $22.9 \times 10^9/l$, lymphocytes $21 \times 10^9/l$) was first noticed at a medical evaluation for cutaneous lesions. The subsequent course has been chronic with persistent dermatitis and a gradual increase of the lymphocyte count.

Eleven years later the patient was admitted to hospital because of haemolytic anaemia and a

splenomegaly. The skin lesions were diffuse pruriginous angiomatous plaques. Microscopical examination of both a needle-biopsy and an aspirated specimen of bone marrow revealed a diffuse infiltration by small and large lymphocytes with convoluted nuclei. A year later, splenectomy and liver biopsy were performed. Microscopical examination disclosed splenic changes consistent with haemolytic anaemia and an infiltration of both spleen and liver by small and large lymphocytes with convoluted nuclei. At that time no precise diagnosis was established nor any treatment given.

Three years later a second bone marrow biopsy showed little change. Irregularities of the nuclear membrane resembled those observed in T cell leukaemia¹³ and a membrane receptor study demonstrated T cells.

Material and methods

CELL PREPARATION

Leukaemic cells were separated from heparinised peripheral blood samples on a Ficoll gradient and washed in Hank's medium.

MEMBRANE RECEPTORS

E rosettes were performed according to the method described by Jondal *et al*.¹⁴ Rosettes were detected in a cell suspension using a phase microscope at a high magnification ($\times 1200$). Smears with E rosettes were also examined and thermostable E rosettes were looked for after a 30 min incubation at 37°C.

Membrane immunoglobulins were investigated with commercial fluorescent polyvalent and mono-

specific antisera (Wellcome). Our normal values for peripheral lymphocytes are:

E rosettes formed at 4°C: $60 \pm 8\%$.

E rosettes formed at 37°C: $<2\%$.

sIg : $22 \pm 5\%$.

The trypan blue dye exclusion test with a rabbit antihuman T lymphocyte antiserum (Merieux Institute, Lyon) was performed in microchambers (Möller Coates, Norway). Tumour cells at $5 \cdot 10^6/\text{ml}$ were incubated for 20 min at 37°C with the antiserum, and rabbit complement was added for 30 min. The cytotoxicity index (% of dead cells) in a panel of chronic lymphocytic leukaemia of B cell type was always below 20%. Normal values for peripheral blood lymphocytes were $75 \pm 7\%$. Controls for toxicity of complement were always below 5%.

ULTRASTRUCTURAL CYTOCHEMISTRY AND MORPHOLOGY

The cells were fixed for 15 min with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4), at 4°C. A 90 min washing in cold cacodylate preceded a 15 min rinse in 0.2 M citrate buffer (pH 4.8) and a quick rinse in 0.05 M acetate buffer (pH 5). The cells were incubated for 30 min for acid phosphatase

activity in Barka and Anderson's modification of the Gomori¹⁵ medium. Control samples were incubated in a substrate-free solution. The cell suspension were then rinsed in cacodylate buffer, post-fixed in 2% osmium tetroxide and dehydrated in 70% alcohol. The cells were centrifuged in a heat-liquefied 3% agar medium, the clot obtained was cooled, then processed as a tissue specimen through graded alcohols, propylene oxide and Epon 812. Ultrathin sections were observed in a Siemens Elmiskop 101 with and without uranyl acetate and lead citrate staining.

Results

IMMUNOLOGY

Leukaemic cells did not exhibit surface immunoglobulins but receptors for sheep red blood cells (SRBC) were demonstrated in 65% of the cells. The cytotoxicity test using antihuman T lymphocyte antiserum was highly positive (cytotoxicity index: 90%). All these findings indicated the T nature of the leukaemic cells. Thermostable receptor for SRBC, as seen in acute T cell lymphoid leukaemia, was not present.

Fig. 1 A leukaemic cell, incubated for acid phosphatase, with an irregular nuclear outline, numerous inclusion bodies in the cytoplasm (arrows), and mitochondria. Uranyl acetate and lead citrate $\times 15\,000$. (a) PTA in an acid phosphatase-positive vacuole with, above, a vacuole containing PTA but no acid phosphatase reaction product $\times 38\,500$. (b) free cytoplasmic PTA $\times 38\,500$.

MORPHOLOGY

The cell preparations processed for electron microscopy contained mostly lymphocytes which showed uniform features. Cell size ranged between 9 and 15 μm . The central nucleus usually presented an irregular outline, with nuclear pore in a hollow space (Fig. 1). The chromatin was dispersed with slight accumulation next to the nuclear membrane. One small nucleolus was usually seen. The cytoplasm was limited by a membrane with no projection, or associated coated or smooth vesicles. Mitochondria were few and scattered. Rough endoplasmic reticulum was seen, as well as a Golgi apparatus. In many cells (80%) the typical PTA inclusion bodies were found. They appeared scattered in the cytoplasm and occasionally close to a centriole or a Golgi apparatus. They consisted of tubules arranged in various ways: parallel arrays of tubules formed typical stacks (free or set within a membrane-limited space); curved or coiled tubules packed together gave a more complex picture, and transverse sections yielded images resembling a honeycomb mesh.

ULTRASTRUCTURAL CYTOCHEMISTRY

Acid phosphatase reaction product was easily

recognised as a well defined, dark, granular precipitate. Some, but not all, membrane-bound structures containing PTA were strikingly positive. The granular precipitate was either linearly spread at the margin of the vacuole or scattered as bulky dark dots between the microtubules. The very small, as well as the large, inclusion bodies yielded a positive reaction. Positive bodies were seen next to totally negative ones in the same cell (Figs. 1-3).

The demonstration of acid phosphatase reaction product within bodies containing PTA identified them as lysosomes (Fig. 2b). The free cytoplasmic tubular arrangements seemed mostly negative for the enzyme. Nevertheless, a few tubules seemed to contain a patchy, zebra-like precipitate where no limiting membrane could be resolved. Positive reaction was also visualised in well defined Golgi apparatus and associated vesicles (Fig. 2a) and, in a few cells, small lysosomes were seen containing no tubular structures. No particular relation between the Golgi apparatus and the bodies was noticed. No reaction product was seen in control sections and the inclusion bodies did not bind any lead in control preparations incubated in medium free of substrate. A few cells containing PTA had a positive reaction in the perinuclear cisternae (Fig. 4).

Fig. 2 (a) Leukaemic cell incubated for acid phosphatase with a positive reaction in a membrane-bound space containing PTA (arrow) and free cytoplasmic tubular structures with acid phosphatase positivity (double arrow). Golgi apparatus (G) also shown. Uranyl acetate and lead citrate $\times 34\,000$. (b) PTA in a lysosome.

Fig. 3 Cell incubated for acid phosphatase with positive PTA. Uranyl acetate and lead citrate $\times 12\,000$.

Discussion

The particular inclusion bodies that we have been studying are called PTA or Hovig inclusion bodies. They have been described in lymphoid cells and can also be seen in non-malignant diseases especially connective tissue disorders. However, a small fraction of the lymphocytes originating from healthy persons also contains PTA.

These bundles of tubules, arranged in a parallel or anarchic manner, either lie free in the cytoplasm or are limited by a unit membrane. Inside the vacuoles the background is usually clear with darker areas described as a dense matrix. Our cytochemical investigation, demonstrating acid phosphatase activity, suggests the lysosomal nature of some of the organelles containing the tubular structures. These PTA could possibly be the result of lysosomal digestion of some unknown material, either produced or phagocytosed by the cell, since Payne *et al* showed that PTA-containing lymphocytes could phagocytose complement-coated bacteria.¹⁶ Parallel tubular arrays have been described in circulating lymphocytes in non-malignant diseases and in normal donors so that T-CLL could be generated by the proliferation of this subset of T lymphocytes.

Fig. 4 Leukaemic cell incubated for acid phosphatase, PTA (arrow), positive reaction in the nuclear cisternae.

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