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Enzyme histochemical demonstration of hairy cell leukaemia in paraffin-embedded tissue sections

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Summary. Hairy cell leukaemia is a form of leukaemia difficult to diagnose since pancytopenia is often present. Hairy cells contain tartrate-resistant acid phosphatase, and this factor is utilised in the diagnosis of the condition. This study confirms that it is also possible to demonstrate tartrate-resistant acid phosphatase in leukaemic infiltrates in formalin fixed paraffin-embedded tissue sections.

Key words: Hairy cell leukaemia – Enzyme histochemistry – Paraffin sections

Introduction

Hairy cell leukaemia (HCL), or leukaemic reticulo-endotheliosis, is characterised by invasion and proliferation of mononuclear "hairy" cells in blood, bone marrow, spleen, liver, and lymph glands (Bouroncle et al. 1958). The diagnosis is made on detection of the pathognomic hairy cells in the blood and bone marrow. These cells are histochemically characterised by the cytoplasmic content of tartrate-resistant acid phosphatase (TRAP) (Yam et al. 1971).

Diagnosis may be difficult, as patients often have pancytopenia and few of the cells in blood and bone marrow smears are in a good state of preservation (Burke et al. 1974). Liver biopsy is generally done as part of the initial diagnostic assessment, or later during laparotomy for splenectomy.

Some authors have shown that TRAP activity can be demonstrated in routine formalin fixed paraffin-embedded tissue sections (Grouls and Stiens 1984; Grouls 1980), whereas others have been unable to confirm this (Yam et al. 1983).

The aim of the present study was to examine whether TRAP activity can be demonstrated in leukaemic infiltrates in formalin fixed paraffin-embedded tissue sections from patients with HCL.

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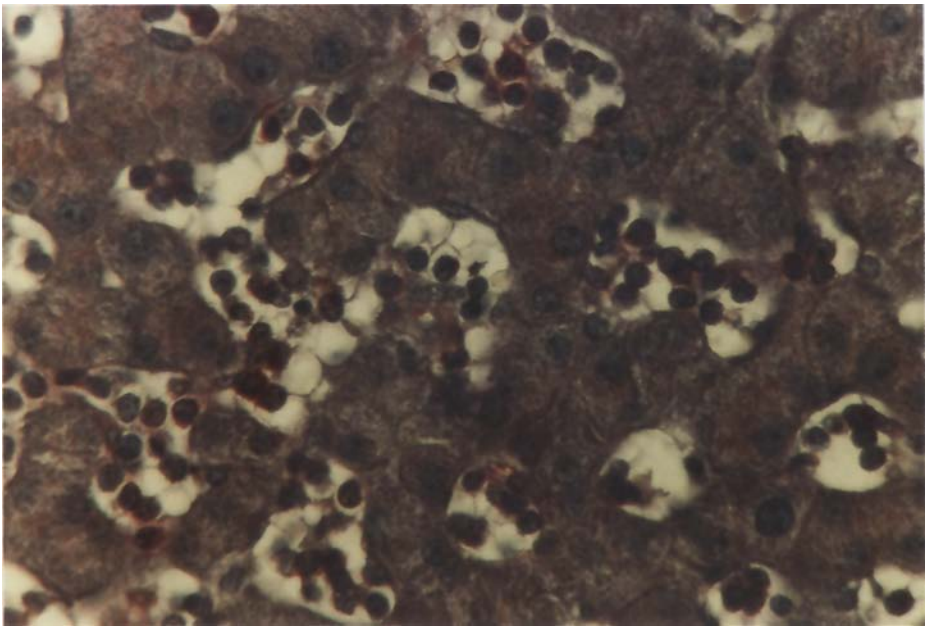

Fig. 1. Hairy cell leukaemia, liver tissue, paraffin section: Positive tartrate-resistant acid phosphatase activity in hairy cells × 400. Hæmatoxylin counterstained

Material and methods

The presence of TRAP was investigated in specimens of liver (3), spleen (7) and lymph node (1) tissues of 7 patients with HCL. Furthermore 6 decalcified needle biopsy specimens of bone marrow were investigated to evaluate the effect of decalcification. The control material was hepatic and splenic tissue from patients with non-Hodgkin lymphoma (2 patients), chronic lymphatic leukaemia (2 patients), small cell anaplastic lung carcinoma (1 patient), and normal splenic tissue (1 patient). The tissue sections measured up to 1,5 cm in length and 0,3 cm in thickness. The specimens were fixed in 10% buffered neutral formalin for up to 24 h at room temperature. Hereafter the material was processed in a Histokinette with ethanol dehydration, xylene and paraffin-embedding (60° C). Finally the sections were dried at 60° C for 30 min. TRAP was demonstrated using Naphtol-AS-BI-phosphate (Sigma No N2250) as the substrate and hexazotised pararosailine as coupler (Leder and Stutte 1983). The specimens were incubated at room temperature for 4 and 6 h. Half of the sections were treated with pronase 0,1% (Type 7, Sigma P 5253) at room temperature for 5 min to "unmask" the enzyme activity. Haematoxylin was used for nuclear counterstaining. The sections were mounted with Aquamount.

The results were assessed for the purpose of establishing the location and the intensity of the reaction product and the effect of pronase.

Results

TRAP activity was shown as a diffuse red staining of the cytoplasm of the hairy cells.

All the specimens of liver, spleen and lymph node tissues from patients with HCL showed weak to strong TRAP activity with the exact location in the cytoplasm of the hairy cells (Fig. 1 and Table 1). The decalcified

Table 1. The activity of tartrate-resistant acid phosphatase in paraffin-embedded tissue sections of liver, spleen and lymph node tissue from patients with hairy cell leukaemia

No. biopsies	Organ	TRAP without pronase	TRAP with pronase
3	Liver	+++/÷	+++/÷
7	Spleen	++/+	+++
1	Lymph node	+++/++	+++

TRAP: tartrate-resistant acid phosphatase, ÷: Negative, +: Weak activity, ++: Moderate activity, +++: Strong activity, /: Variation in the enzyme activity from one biopsy to another

needle biopsy specimens of bone marrow showed no activity. A few of the Kupffer cells in the liver tissue exhibited weak activity. In 1 liver biopsy and 1 splenic section no TRAP activity was demonstrated until pronase was added. Treatment with pronase generally intensified the reaction. The 6 control sections were all negative, excepting a few histiocytes in the splenic tissue. These were located outside the tumour infiltrates.

Discussion

The present study shows that TRAP activity can be demonstrated in formalin fixed paraffin-embedded tissue sections, as others have shown (Grouls and Stiens 1984; Chilosi et al. 1981; Grouls 1980). TRAP activity could not be demonstrated in decalcified needle biopsy specimens of bone marrow, while others have been able to show this (Grouls 1980). The individual positive Kupffer cells have been described earlier (Yam et al. 1983) and morphologically are unlikely to be confused with hairy cells. Yam et al. (1983) were unable to demonstrate TRAP activity in 19 liver biopsies. The explanation may perhaps be found in one or more of the following factors: 1) Some hairy cells contain very little or no enzyme activity. In such cases the TRAP reaction would be negative (Schaefer et al. 1975). 2) The size of the tissue sections is important. Large specimens may be incompletely fixed in formalin, which would lead to inactivation of the enzyme. The activity is best preserved in small tissue specimens fixed immediately after removal (Grouls and Stiens 1984). 3) The duration of fixation is important. Formalin fixation longer than 5 h leads to greatly reduced enzyme activity (Fujimori et al. 1981). 4) Compared to smears and cryostat sections the incubation time have to be prolonged in order to achieve a stronger enzyme reactivity (Chilosi et al. 1981).

The enzyme activity must be considered to be specific because of the precise location in hairy cell cytoplasm and because of the negative controls. The study showed that pretreatment of the sections with pronase increased the enzyme activity probably due to unmasking of the enzyme through cleavage of the cross bindings between the tissue an fixative (Curran and Gregory 1978; Bourne 1983). The demonstration of TRAP in pronase-treated formalin fixed paraffin-embedded tissue sections can be used in the diagnosis of hairy cell leukaemia.

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