IgM lambda cytoplasmic crystals in three cases of immunocytoma: a clinical, cytochemical, and ultrastructural study

W. W. FEREMANS, P. NEVE, AND M. CAUDRON

From the Laboratories of Electron Microscopy, Pathology, and Experimental Medicine, Departments of Haematology and Medicine, Hôpital Saint-Pierre, Université Libre de Bruxelles, Brussels, Belgium

SUMMARY Endoplasmic reticulum-associated crystals were seen in 1-10% of the bone-marrow lymphocytes and in the lymphocytes of the peripheral blood in three cases of immunocytoma. Their crystalline nature and their location in the cisternae of the rough endoplasmic reticulum was proved by ultrastructural study. IgM lambda in the crystals was demonstrated by fluorescent and peroxidase-labelled antibody methods. The crystals did not stain with the PAS reaction, suggesting that the immunoglobulin was not bound to a carbohydrate group. A defect in glucosyltransferase activity with failure to modify the immunoglobulins could explain the absence of the PAS reaction and the accumulation of immunoglobulin in crystalline form before reaching the Golgi region.

Three patients originally diagnosed as suffering from chronic lymphocytic leukaemia showed intracytoplasmic crystals in their lymphocytes. Inclusions of this type were first reported by Bessis (1951) in a case of chronic lymphocytic leukaemia. Bernard *et al.* (1959) observed these crystalline bodies with the electron microscope. The presence of gamma-globulins and absence of PAS staining were noted by Goldberg (1960) and De Man and Meiners (1962). Flandrin *et al.* (1971) and Hurez *et al.* (1972) found IgM lambda in similar crystals, using a fluorescent-labelled antibody method. Crystals containing IgA lambda were described by Cawley *et al.* (1973).

In none of the 15 cases reported so far were the lymphocytes normal (Bessis, 1951; Bernard *et al.*, 1959; Goldberg, 1960; De Man and Meiners, 1962; Flandrin *et al.*, 1971; Hurez *et al.*, 1972; Cawley *et al.*, 1973, 1976; Clark *et al.*, 1973; Stefani *et al.*, 1977). Inclusions of a different structure have been studied in leukaemic lymphocytes by Zucker-Franklin (1963), Nardo and Norton (1972), Anday *et al.* (1973), Lagios *et al.* (1974), Brunning *et al.* (1975), Cawley *et al.* (1975), and Stefani *et al.* (1977).

The peroxidase-labelled antibody method (Mason *et al.*, 1969; Taylor and Burns, 1974; Garvin *et al.*,

1976; Pinkus *et al.*, 1977) used in this study with immunofluorescence and electron microscopy is an original technique for the specific identification of the immunoglobulin in these crystalline inclusions. We have tried to fit our findings into the new classification of malignant lymphomas by Lennert *et al.* (1975).

Patients and methods

The relevant clinical and haematological findings in the three cases studied are shown in Tables 1 and 2.

Normocytic anaemia in the patient in case 1 was due to bone marrow infiltration with central thrombopenia, whereas microcytic anaemia in the patient in case 2 was related to blood loss from a duodenal ulcer. Later the latter patient had several incidents of haemolysis with a direct positive Coombs test (IgG specificity). The serum immunoglobulin levels were low in all three patients and urine immunoelectrophoresis did not show any immunoglobulin chain. All patients reacted to chlorambucil treatment with regression of lymph node enlargement and of lymphocytosis. The haemolytic anaemia in case 2 was cured with prednisolone. The three patients were still alive 22, 38, and 40 months respectively after their illness was diagnosed.

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Table 1 Clinical findings in three cases of immunocytoma

Case No.	Sex	Age (Yr)	Peripheral lymph nodes	Hepatomegaly	Splenomegaly	Hb (g/dl)	Platelets ($\times 10^9/l$)
1	M	47	+	—	+	5.8	14.2
2	M	61	+	—	+	5.8	202
3	F	65	+	—	+	12.4	206

Table 2 Haematological findings in three cases of immunocytoma

Case No.	WBC ($\times 10^9/l$)	Peripheral lymphocytosis ($\times 10^9/l$)	Peripheral lymphocytosis (%)	Bone marrow lymphocytosis (%)	IgG (mg/100 ml)	IgA (mg/100 ml)	IgM (mg/100 ml)	Urine Ig
1	13.8	8.8	63.8	93.8	460	85	85	—
2	100.8	98.8	98	82.2	700	112	72	—
3	33.5	29.1	86.9	96.2	500	65	30	—

LIGHT MICROSCOPY

Blood and bone-marrow smears were treated with May-Grünwald-Giemsa, PAS staining according to Hayhoe and Flemans (1969), methylgreen-pyronine (Bessis, 1972), and orange acridine (Forteza, 1964).

IMMUNOPEROXIDASE AND IMMUNO-FLUORESCENCE TECHNIQUES

The smears were fixed for five minutes in absolute ethanol and rinsed in 0.15 M pH 7.4 phosphate buffer at room temperature. The Dako rabbit antihuman immunosera IgA, IgM, IgG, kappa, and lambda were used first at a dilution of 1/40 at 37°C. Next the swine antirabbit immunoserum conjugated with horseradish peroxidase was used at 1/10 dilution. The peroxidase was processed with alpha-naphtholpyronine staining according to Lillie (1965). For the immunofluorescence (Pearse, 1960) the Dako rabbit antihuman fluorescein-conjugated antibodies IgA, IgM, IgG, kappa, and lambda were used.

TECHNIQUE FOR ELECTRON MICROSCOPY

Bone marrow obtained by sternal puncture was immediately fixed for 30 minutes at room temperature in 4% distilled glutaraldehyde (Sabatini *et al.*, 1963) in 0.1 M phosphate buffer (Millonig, 1962). Next, the cells were rinsed in the same buffer and postfixed with 2% osmium tetroxide. Dehydration took place in graded concentrations of ethanol with centrifugation for 10 minutes at 1000 rpm at each step of the manipulation.

Finally, the material was embedded in Epon (Luft, 1961), sectioned with a Diatome diamond knife on a LKB Ultratome III microtome. Ultra-thin sections were stained with uranyl acetate and lead and observed with a Siemens Elmiskop I electron microscope.

Results

LIGHT AND FLUORESCENT MICROSCOPE FINDINGS

The morphology was similar in each of the cases. The average diameter of the lymphocytes was 11.3 μ . The nucleus showed a normal chromatin and often contained a nucleolus. With May-Grünwald-Giemsa stain a varying number of colourless crystals were visible in the basophilic cytoplasm of 1 to 10% of the lymphocytes (Fig. 1). They were PAS-negative, although this procedure showed glycogen inside the cytoplasm (becoming negative-stained after adding saliva amylase). The treatment with ribonuclease suppressed the cytoplasmic pyroninophilic staining which did not involve the crystals. Only the crystalline bodies became fluorescent after treatment with immunosera anti-IgM and anti-lambda (Figs. 2, 3). Fluorescence remained negative after exposure to antisera anti-IgA, anti-IgG, anti-kappa and also after staining with orange acridine.

The processing of the exogenous peroxidase by the immunoperoxidase method confirmed the results obtained with immunofluorescence. The crystals appeared brownish (Fig. 4) for anti-IgM and anti-lambda.

ULTRASTRUCTURAL FINDINGS

The ultrastructural features were identified in the three cases. In case 1 similar anomalies were found in four different specimens of marrow obtained at four-month intervals.

Usually rounded cells (Fig. 5) sometimes had long, thin cytoplasmic protrusions. Nucleoli were often encountered. The cytoplasm contained numerous ribosomes and mitochondria and in some places the rough endoplasmic cisternae appeared annular.

Fig. 1 *May-Grünwald-Giemsa staining of lymphocyte with crystalline bodies (arrowed). ($\times 1300$)*

Fig. 2 *Fluorescence of crystals with fluorescein-conjugated rabbit antihuman IgM serum (arrowed). ($\times 870$)*

Fig. 3 Fluorescence of crystals with fluorescein-conjugated rabbit antihuman lambda serum (arrowed). ($\times 870$)

Fig. 4 Crystalline bodies coloured by the immunoperoxidase method (arrowed). ($\times 1300$)

Fig. 5 Electron micrograph of lymphocyte with eight crystals. C = crystals; N = nuclear; M = mitochondria; R = rough endoplasmic reticulum. ($\times 24\,000$)

The Golgi apparatus did not seem to be very active. Some acanthosomes, microtubules, and multivesicular bodies were also seen (Fig. 6). The most important ultrastructural feature was the intracytoplasmic crystals, which were square in transverse section with a periodic structure and bordered by membranes of rough cytoplasmic reticulum (Figs. 7, 8). On longitudinal section the crystalline body had a rectangular or trapeze profile often with two open ends (Fig. 6). The periodic structure was then oriented parallel to the longitudinal axis of the crystal.

The number of crystals varied and could be eight on a cellular section. Moreover, lymphocytes in case 3 showed many centriolar complexes.

Discussion

According to the classification of Lennert *et al.* (1975) our three cases were 'immunocytomas' like the 15 analogous cases reported since 1950. This terminology, initially used by Heremans (1960) and Hobbs (1971), refers to malignant lymphoma of low-grade malignancy. The clinical pattern is similar to chronic lymphocytic leukaemia but the lymphoid infiltration is characterised by signs of immunoglobulin synthesis. With light microscopy increased protein synthesis is suggested by the basophilia, distinct cytoplasmic coloration with methyl green-pyronine, and the frequent occurrence of nucleoli. However, the common morphological peculiarity

Fig. 6 Electron micrograph of a lymphocyte showing crystals (C), multivesicular bodies (A), annular cistern of rough endoplasmic reticulum (R), acanthosomes (X), nucleolus (N). ($\times 20\,800$)

of these 18 cases is the presence of crystalline bodies whose periodic structure and the location inside the rough endoplasmic reticulum are demonstrated by transmission electron microscopy.

Cawley *et al.* (1976) have also observed such a crystal in the perinuclear cistern. Goldberg and Deane (1960) have demonstrated the presence of immunoglobulins inside those crystals by comparing the isoelectric point with a reference gamma-globulin. Until now immunoglobulin has been identified by immunofluorescence in 13 cases. IgM lambda was found in 11 and IgA lambda in two cases. One case with IgG kappa crystals has been reported but with a IgG kappa monoclonal seric peak (Preud'homme and Seligmann, 1972a).

The immunoperoxidase technique provided additional confirmation of the results obtained by immunofluorescence in our three cases. The prevalence of surface-bound monoclonal IgM in the other immunoglobulin classes is well established in chronic lymphocytic leukaemia and other B-lymphomas. However, it is more difficult to demonstrate the IgM in the cytoplasm of the B-lymphocytes with the immunofluorescence method (Brouet and Seligmann, 1977; Preud'Homme and Seligmann, 1972b). The nature of the defect in the leukaemic lymphocytes leading to intracytoplasmic accumulation of immunoglobulin crystals remains hypothetical.

A lack of balance between immunoglobulin synthesis and secretions might be considered.

Fig. 7 High magnification electron micrograph of crystal inside rough endoplasmic cistern. ($\times 67\,000$)

Fig. 8 High magnification electron micrograph showing periodic structure. ($\times 84\,000$)

Either synthesis is increased but secretion does not follow at the same rate or some delay in secretion occurs in the presence of a usual level of synthesis. There is no ultrastructural feature that points to increased protein production. Indeed, the ergastoplasm of the lymphocyte is less abundant than in the plasmocyte and there is no hypertrophy of the Golgi apparatus. Nevertheless, the accumulation of the crystals inside the rough endoplasmic reticulum

might correspond to a block before the Golgi apparatus. The PAS-negative staining of the crystalline bodies (except for the case of Goldberg) suggests that these immunoglobulins are lacking their carbohydrate chain. This lack might perhaps correspond to a defect at the level of the glycosyltransferases (Hurez *et al.*, 1969; Sherr and Uhr, 1971; Schachter, 1974; Louisot *et al.*, 1976).

The suggested form of the crystals is described by

De Man and Meiners (1962). The mechanism of the crystallization might be explained by a surpassing of the solubility process with modification of the tertiary structure of the immunoglobulin.

Flandrin *et al.* (1971) tended to suppose that the immunoglobulin release from the plasma membrane was impeded. Indeed, they found surface-bound immunoglobulins identical with those of the crystalline bodies. But these findings tend to be divergent. For example, Cawley *et al.* (1976) did not find an alpha heavy chain on the plasma membrane of cells that contain IgA lambda crystals, whereas they demonstrated the μ heavy chain in the cases with IgM lambda crystals. Moreover, only 1 to 2% of the circulating lymphocytes in cases 1 and 3 showed membrane-bound immunoglobulins by immunofluorescence (case 2 was not tested).

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