Myeloma with Immunohistochemically Different Cell Populations: Implication for the Validity of Immunohistochemical Assessment of Lymphoma Cell Monoclonality

K. Metz and L.-D. Leder
West German Tumor Center, Institute of Pathology (Director: Prof. Dr. L.-D. Leder), University of Essen

Plasmocytoma cells and normal plasma cells are capable of forming immunoglobulins (Ig). Reactive plasma cells are polyclonal in contrast to myeloma cells which are usually monoclonal and, hence, produce monoclonal Ig. Possible repressions or derepressions of some of the respective genes during the course of the disease might result in production of different amounts of heavy and light chains. At least, it is well known that myelomas do not form normal proportions of heavy and light chains (Zolla et al. 1970; Bauml and Scharff 1973). Usually light chains predominate, and in about 20% of plasmocytoma cases solely light chains are found (Williams et al. 1966; Hobbs 1974).

Some few cases with biclonal paraproteinemias have been presented (Bihrer et al. 1974; Bouvet et al. 1975; Sarasombath et al. 1977) which event is in general considered as a fortuitous coincidence of two different myelomas.
Recently, it has become possible to demonstrate monotypical Ig within myeloma cells by means of the immunoperoxidase method using paraffin embedded material (Taylor and Mason 1974; Halliday et al. 1977; Pinkus and Said 1977). Monotypical cytoplasmic Ig, however, can also be found with other B-cell types of malignant lymphomas (Dutcher and Fahey 1959; Franklin et al. 1964; Child et al. 1977; Wendt et al. 1979), in benign monoclonal gammopathies (Waldenström 1964), and as reactive change secondary to a variety of diseases (Kühn 1978; Brubaker and Whiteside 1978).

Thus, different conditions may present with increased monoclonal Ig. Therefore, immunohistochemical results must be very cautiously interpreted. This is emphasized by the following case report which concerns a myeloma with unusual immunohistochemical findings. These will be discussed with special reference to the validity of immunohistochemical assessment of lymphoma cell monoclonality.

Case Report

In January 1976 a 72 years old male presented with a mediastinal tumor mass and several osteolytic lesions in ribs, humerus and skull. There was an extremely high sedimentation rate of 124 mm/hr and an increase of gamma globulins up to 38%. The total serum protein content amounted to 10.5 g/100 ml. Immuno-
electrophoresis revealed an IgG-kappa type paraproteinemia.

Cervical and mediastinal lymph node biopsies were done. Histologically, a mature myeloma was diagnosed (Fig. 1). 5 months after diagnosis the patient deceased. No autopsy was performed.

Histological and Immunohistochemical Investigations

Lymph node tissue was embedded in paraffin, and H & E, Giemsa, and PAS stained sections were prepared. Immunohistochemically, kappa, lambda, gamma, alpha, my, delta, epsilon, and lysozyme (muramidase) were demonstrated by means of the modified (Taylor 1974) peroxidase-anti peroxidase method of Sternberger et al. (1970) using routinely formalin fixed paraffin sections, air-dried for 5-7 days at 30°C, dewaxed with xylol, and brought into absolute methanol. Endogeneous peroxidases were destroyed by 0.3% H₂O₂ in absolute methanol for 30 min (Streefkerk 1972). Thereafter the slides were hydrated by tris-saline buffer 3 times for 10 min, pH 7.6 (Sternberger et al. 1970).

Nonspecific background staining was avoided by application of swine serum (GIBCO, Scotland) in tris-saline buffer (1:10). Excess of serum was removed with tris-saline buffer (3 times for 10 min). Thereupon, the slides were covered for 30 min with rabbit antisera against the various immunoglobulin chains and lysozyme. Anti-epsilon was obtained from Behring (Germany), anti-lysozyme from Dakopatts A/S (Denmark), all others from Nordic (Netherlands). Antisera were diluted as follows: Alpha and epsilon 1:50; kappa, lysozyme and my 1:75; delta 1:125; and gamma and lambda 1:150.

The slides were rinsed again, and diluted (1:20) swine-antirabbit IgG (Nordic, Netherlands) was applied (30 min) followed by another washing procedure. Thereafter, incubation with rabbit horseradish peroxidase-antihorseradish peroxidase complex (PAP; Dakopatts A/S, Denmark) was done.

Finally, the slides were washed again 2 times for 10 min in tris-saline buffer and 1 time for 10 min in tris-water (Sternberger et al. 1970). Following a fixation for 3 s at 20°C in formalin-methanol (1:10) the slides were rinsed in tap water, air-dried, subjected to the peroxidase-reaction (Leder 1970) with 3-amino-9-ethyl-carbazol (Sigma, USA), counterstained with hemalum and covered with Kaiser's glycerine gelatin (Merck, Germany).

Results

Histologically, there was the typical monotonous morphology of a mature myeloma (Fig. 1). Multinuclear myeloma cells and mitoses were rare. Some cells contained small PAS-positive cytoplasmic inclusions. Large PAS-positive inclusions could be often found