IMMUNOLOGICAL STUDIES OF AN ANTIVIRAL MONOCLONAL
IgG CRYOGLOBULIN

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Cryoglobulinemia is characterized by the presence in serum of cold precipitable immunoglobulins which redissolve at 37 °C. Cryoglobulins (cryo) are a heterogeneous group that occur in various forms; one of these is the single cryo containing one monoclonal component.

Antibody activities of monoclonal immunoglobulins are reported in comprehensive reviews; the only demonstrated antiviral activity is against rubella virus. Cytomegalovirus (CMV) infections associated with monoclonal gammapathies occur especially in the course of immunodeficiencies produced by the malignant disease or the immunosuppressive therapy.

We describe a case of monoclonal cryo IgG which appeared in a patient with a chronic lymphocytic leukemia of T cell nature; anti-CMV antibody activity was found to be associated with the abnormal globulin.

MATERIALS AND METHODS

Case report

A 65-year-old man was found to have chronic lymphocytic leukemia in March 1978 (220,000 leukocytes/μl). More than 95% of the patient’s leukocytes were

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T cells, although expressing C3b and Epstein-Barr virus receptors. He was given chlorambucil and cortisone until the end of May. Splenectomy was performed in July because of further enlargement of the spleen. A monoclonal component appeared in the serum. The patient died in November of the same year.

Serum protein analysis

Electrophoresis was done on cellulose acetate strips in Veronal buffer, pH 9.8. Agar gel immunoelectrophoresis was carried out using anti-human serum and the specific anti-γ, α, μ, δ, ε, Fab γ, Fe γ, κ, λ antisera (Behring Institut). Immunoglobulins were quantified by immunoturbidimetry, but the serum paraprotein level was estimated from the electrophoregram.

Isolation and analysis of cryoglobulin

Fifty ml blood were kept at 37 °C until complete clot retraction, thereafter the serum was stored at 4 °C for 7 days. A series of centrifugations and sterile washes were used to separate the cryoprecipitate. Further identifications were carried out as previously described. Separation of the constituents was done by ultracentrifugation in a stabilized density gradient.

Preparation of (Fab')2 fragments

Isolation of the non-cryoprecipitable monoclonal IgG was accomplished from the supernatant serum after cryoprecipitation using ammonium sulfate precipitation and ion exchange chromatography. Digests of the cryo and the non-cryoprecipitable IgG were prepared using pepsin and purified by gel filtration. Fractions collected at each purification step were immunochemically tested after concentration to a small volume.

Electron microscopy of the cryoglobulin

The cryo was fixed 3 h with 4% paraformaldehyde followed by a 30 min osmium post-fixation, dehydrated in alcohol and embedded in epoxy. Ultrathin sections were examined as previously described under a Philips EM 201 electron microscope.

Viral antibody activity

The indirect immunofluorescence (IF) test was performed using the smears of CMV infected cells stored at -70 °C. Anti-human IgM, IgG, κ, λ antisera conjugated with fluorescein (Behring Institut) were used. All manipulations concerning the cryo were done at 37 °C. Preparations were examined by a Leitz Orthoplan microscope.

RESULTS

Serial analyses of the serum electrophoretic pattern showed the appearance of a monoclonal component in the γ fraction, in September (fig. 1). Immunoelectro-