The Cryoglobulin Inclusion Cell

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THE PURPOSE OF THIS REPORT is to describe an in vitro phenomenon which was observed in the peripheral blood of a patient with essential cryoglobulinemia. The case history and response to treatment will be presented in greater detail in a separate publication.

The patient’s serum contained a cryoglobulin (cold precipitable globulin) in amounts up to 1.6 grams per 100 ml. This protein precipitated out slowly at room temperature (21 C.) or lower, and redissolved rapidly when the serum was warmed to body temperature (37 C.). Its concentration was not substantially altered by 20 days of intravenous ACTH therapy, or during 6 weeks of intramuscular ACTH (Duracon) therapy, although both of these courses of treatment improved the clinical status of the patient very greatly.

Since the course of the illness and the physical findings were somewhat suggestive of a “collagen disorder”, an attempt was made to demonstrate the presence of L.E. cells in the following manner. Specimens of venous blood were allowed to clot and stand for 2 hours, one at 21 C. and one at 5 C. The clotted portions of the blood were then macerated with applicator sticks, and the expressed cells placed in Wintrobe hematocrit tubes and centrifuged at 3000 r.p.m. for five minutes. Specimens of buffy coat were carefully removed, smeared on glass slides, and stained with Wright’s blood stain. In these smears, one could see many large, homogeneous, light blue masses of precipitated protein-like material (figs. 1 and 2) lying free amongst the cells. This material presumably represented the precipitated cryoglobulin. In almost every high power field, there were numerous polymorphonuclear neutrophils containing one or more inclusion bodies within vacuoles of the cytoplasm, frequently displacing the segmented neutrophil nucleus to one side. Some of the vacuoles appeared empty while others contained material which stained a light blue, identical with the staining of the precipitated protein lying free among the cells (figs. 2 and 3). While these cells superficially resembled L.E. cells, they differed in that the inclusion bodies were not derived from lysed nuclear material such as occurs in the characteristic L.E. reaction. When the patient’s serum was mixed with leukocytes from a normal subject and then cooled to room temperature or lower, an identical picture was obtained. When, however, the serum was first chilled and the precipitated globulin removed, the remaining serum plus leukocytes did not yield these peculiar cells under the test conditions described above. ACTH therapy resulted in no demonstrable decrease in the number of these cells, despite marked clinical improvement. When the test was performed on blood maintained at 37 C. throughout, (either the patient’s clotted blood or the pa-

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Fig. 1.—Extracellular masses of precipitated cryoglobulin

Fig. 2.—Buffy coat smear showing precipitated cryoglobulin and several vacuolated cells containing the same protein.
Fig. 3.—Polymorphonuclear neutrophil containing a large mass of cryoglobulin which has displaced the segmented nucleus to one side. Two other cells contain several smaller inclusion masses.

tient's whole serum plus donor's leukocytes), neither the homogeneous, protein-like material nor the inclusion bodies were observed. Slides prepared at room temperature rather than at 5 C. contained more leukocytes with empty vacuoles, suggesting that part of the cryoglobulin may have been redissolved at the warmer temperature.

This evidence suggested that the polymorphonuclear neutrophils had ingested the cold-precipitable globulin, an observation which was later confirmed by actual observation of wet film preparations with a phase contrast microscope. It was not possible to demonstrate these cells in smears of peripheral blood or bone marrow, when these smears were made immediately upon aspiration, but only after the specimen had been allowed to stand for some time at temperatures below 37 C. They have not been demonstrated in vivo.

Intracellular inclusions comparable to those described above have previously been encountered by other observers. Hutchison and Howell2 demonstrated similar cells in preparations of peripheral blood and bone marrow from a patient with cryoglobulinemia and gangrene of digits. In smears of bone marrow from a patient with multiple myeloma, Trubowitz3 observed neutrophils containing eosinophilic, amorphous material and similarly stained extracellular globules. However, this author speculated that the cells contained amyloid, although no mention was made of the presence or absence of cryoglobulin. Barr, Reader and Wheeler4 described myeloma cells containing multiple vacuoles in their cytoplasm, in bone marrow preparations taken from necropsy material of
a patient with multiple myeloma manifesting cryoglobulinemia. There were, additionally, masses of eosinophilic, colloid-like material lying free among the cells. These authors inferred that the myeloma cells were the site of production of the cryoglobulin, but it would be worth bearing in mind, in future studies, that it may have represented in vitro phagocytosis of the precipitated cryoglobulin by the myeloma cells. In support of this suggestion is the recent demonstration that plasma cells are capable of active phagocytosis.

Since this initial observation was made, identical intracellular inclusions of cryoglobulin have been observed in preparations of peripheral blood taken from two other patients with cryoglobulinemia. It is suggested that the demonstration of such cells in vitro, may be of some diagnostic value in drawing attention to the presence of cryoglobulin in the blood. Attention is also drawn to the possible misinterpretation of the phenomenon for a positive L.E. test. The term “Cryoglobulin Inclusion Cell” is advanced to describe the leukocytes containing phagocytized masses of cryoglobulin within vacuoles of the cytoplasm.

SUMMARIO IN INTERLINGUA

Es reportate le observation in vitro, in le sanguine peripheric de un patiente con cryoglobulinemia, de leucocytos continente intra le vacuolos del cytoplasma massas phagocytisate de cryoglobulina. Le technica usate in le observation es detaliate, e le termino “cellulas a inclusiones cryoglobulinic” es proponite pro le typo de leucocytos sub consideration. Le autores exprime br conviction que le demonstration in vivo de “cellulas a inclusiones cryoglobulinic” poterea esser de valor diagnostic in signalar le presentia de cryoglobulina in le sanguine.

REFERENCES

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