Artefacts associated with a cryoglobulin

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Summary: A 70 year old man is described in whom the presence of a cryoglobulin caused a number of unusual laboratory features which were not recognized as being due to a cryoglobulin initially. Pseudoleucocytosis has been recognized in the past. However, the intracytoplasmic inclusion bodies have only rarely been described. The curious appearance of the ESR has not previously been described.

Introduction

The term 'cryoglobulin' was first applied by Lerner and Watson¹ to plasma proteins characterized by reversible precipitation induced by cold. The cryoglobulins are, in the main, immunoglobulins but other plasma proteins are well recognized to be cold precipitable. These include fibrinogen, fibronectin, factor VIII and C-reactive protein.²

The physicochemical basis of cryoprecipitation is not well understood though differences in carbohydrate content of the cryoglobulin (compared with non-cryoprecipitable globulins) or electrostatic interactions may be important.^{3–5} Classification of cryoglobulins depends on the clonality of the cryoglobulin and whether one or several immunoglobulin classes are present in the cryoglobulin.⁶ The clinical features of these disorders depend on the cryoglobulin and have been extensively reviewed.^{6,7} The *in vitro* effects of cryoglobulins are less well documented: several reports exist documenting the effect of cryoglobulins on leucocyte, erythrocyte and platelet counts as ascertained by automated cell counters.^{8–10} Abnormal neutrophil inclusion bodies have occasionally been described¹⁰ (Jackson, J.M., personal communication). A patient, in whom a number of these artefacts occurred, is now described.

Case report

A 70 year old man attended a physician in June 1986 complaining of pain in his left third finger and great toe. He had noticed an area of bluish discolouration, about 3 cm in diameter on the anterior abdominal wall

and some 'red spots under the fingernails', which latter had occurred 6 months previously. Four weeks after his initial presentation, he noticed some improvement in the painful finger but not the toe. He had an area of ulceration on the abdominal wall at the site of the original discolouration and of the skin over the left elbow. He was otherwise well apart from feeling generally lethargic.

On admission, full blood count (Coulter S Plus, Coulter Electronics, Inc., Hialeah, Florida.) was normal apart from a slightly low haemoglobin (Hb 11.9 g/dl). The erythrocyte sedimentation rate (ESR) was elevated (32 mm/h, Westergren Method, ICSH, 1973). Biochemical profile (SMAC 20) was normal apart from an elevated serum triglyceride of 2.91 $\mu\text{mol/l}$ (normal range, 0.35–2.00 $\mu\text{mol/l}$). A presumptive diagnosis of vasculitis was made and subsequently confirmed on skin biopsy histology. Prednisone (40 mg/day) was started 3 days after admission. Three weeks later the white cell count (WBC) suddenly rose to $35.5 \times 10^9/l$, though on examination of the blood film, the WBC count appeared to be around $15.0 \times 10^9/l$, i.e. consistent with the previous 2–3 weeks. On the peripheral blood film numerous intracytoplasmic and extracellular bodies were noted (Figure 1). An ESR done at the same time showed a curious ragged appearance at the interface between cells and plasma (Figure 2). Films made from this sample were stained Gram's stain, Sudan Black, Oil Red O and PAS with negative results. A sample taken next morning showed no inclusion bodies on the blood film and the WBC as assessed from the blood film was consistent with the values obtained from the Coulter S Plus. Five days later a similar pattern was seen: numerous intra- and extracytoplasmic bodies were seen on the blood film and the leucocyte count from the Coulter S Plus was grossly excessive in comparison with the appearance of the peripheral blood film. By

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Figure 1 Blood film of patient showing intra- and extracytoplasmic inclusions (x800).

Figure 2 The ESR plasma – RBC interface.

this time, it was known that the patient had a serum paraprotein band typed as IgG, lambda on immunoelectrophoresis. A fresh sample was obtained and transported to the laboratory at 37°C. No inclusion bodies were seen on the blood film and the WBC was consistent with that obtained by the Coulter

S Plus. After cooling to room temperature, numerous inclusion bodies were seen. The presence of a cryoglobulin was confirmed and consisted only of IgG, lambda. Serum immunoglobulins were within normal range. In the urine, free light chains (lambda only) were present. Bone marrow aspiration showed a slight

plasmacytosis of 8% of the total cellularity. Skeletal survey was normal. Tests for rheumatoid factor, antinuclear factor and hepatitis B surface antigen were negative.

Discussion

The diagnosis was type I cryoglobulin in association with a monoclonal gammopathy of undetermined significance, there being insufficient criteria to support the diagnosis of myeloma.¹¹ The major clinical manifestation was that of a vasculitis which responded well to oral prednisolone. The haematological features were curiosities which caused great difficulty because of the intermittent nature of their appearance. Automated cell counters are recognized to be able to count the precipitated cryoglobulin droplets particularly if the size falls within the range of the counter.⁹ In this case, the leucocyte count was 'elevated' but no

effect on red cell or platelet counts was seen. The intracytoplasmic inclusion bodies were more puzzling: the usual neutrophil cytoplasmic inclusions such as May-Hegglin, Dohle bodies, Alder Reilly anomaly, Chediak-Higashi syndrome, are morphologically distinct.¹⁰ Immunoperoxidase staining confirmed that the inclusions consisted only of IgG, lambda. Artefacts such as reported here are relatively rare: the intracytoplasmic inclusions have been reported twice¹⁰ (Jackson, J.M., personal communication) and as far as can be ascertained, the strange appearance of the ESR has not been reported previously. The unravelling of the aetiology of these artefacts may be difficult and the importance of obtaining fresh samples which can be kept at 37°C before processing is stressed.

One final enigma remains: the patient became symptomatic during summer when the average daytime temperature is 27°C.¹² However, he remained asymptomatic during winter in Saskatchewan when temperatures vary from +7°C to -40°C.¹²

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