Inhibition of fibrin monomer polymerisation by myeloma immunoglobulin

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Abstract
Myelomatosis was diagnosed in a 64 year old man on the basis of a serum paraprotein band (type IgGλ, 42 g/l), plasma cell infiltration of bone marrow, and multiple lytic lesions evident on skull x ray picture. Blood specimens taken into plain glass tubes showed bulky gelatinous clot formation with minimal clot retraction. Coagulation tests were significantly abnormal with an increase in thrombin time, prothrombin time, and reptilase time. The possibility that the paraprotein was interfering with fibrin production was investigated. The rate of fibrin monomer polymerisation (measured turbidometrically) was reduced in patient plasma compared with control plasma. Although purified fibrin monomer prepared from the patient's fibrinogen polymerised normally, the addition of purified paraprotein caused a dose dependent reduction in the rate of polymerisation. These results suggest that the paraprotein was impeding fibrin formation by inhibiting fibrin monomer polymerisation. After chemotherapy the paraprotein concentration decreased and the coagulation results returned to normal.

Case report
A previously well 64 year old man presented with a four week history of bone pain. A diagnosis of multiple myeloma was made on the basis of multiple radiological lytic lesions in the pelvis, long bones, and skull; 70–80% plasma cell infiltration of bone marrow; and a type IgGλ paraprotein band in serum (42 g/l). Baseline laboratory investigations were as follows: haemoglobin 83 g/l, white cell count 3.9 × 10^9/l, platelet count 263 × 10^9/l, sodium 140 mmol/l, potassium 3.5 mmol/l, urea 10.0 mmol/l, Ca^2+ 2.5 mmol/l, albumin 28 g/l. Blood specimens taken into plain glass tubes without added anticoagulant showed bulky gelatinous clot formation with minimal clot retraction, making it difficult to separate the serum even after prolonged centrifugation. As a result there were problems in obtaining sufficient serum for biochemical analyses. In view of the abnormal clot formation coagulation tests were performed and were as follows: prothrombin time 17 seconds (control 14 seconds); activated (kaolin) partial thromboplastin time 41 seconds (control 42 seconds); thrombin time 50 seconds (control 17 seconds); the thrombin time on mixing equal volumes of patient and control plasma only partially corrected to 20 seconds; reptilase time 30 seconds (control 14 seconds); quantiative fibrinogen 6.4 g/l (reference range 2.4 g/l); D–dimer 0.5 µg/l (reference range < 0.5 µg/l). Cryoglobulins were not detected. The patient displayed no tendency towards bleeding or bruising. No fundal haemorrhages were noted and there was no excessive haemorrhage either after venepuncture or after the insertion of venous lines.

In view of the diagnosis of myeloma, the coagulation abnormalities, and the unusual gelatinous appearance of clotted blood specimens the possibility that the paraprotein was interfering with fibrin formation was investigated.

Methods and results
The gammaglobulin fraction of the patient's serum was precipitated with ammonium sulphate solution and resuspended in phosphate buffered saline (0.02 mol/l phosphate in 0.15 mol/l sodium chloride, pH 7.4 (PBS)).
Electrophoresis of the gammaglobulin preparation was performed in 1\% agarose. The paraprotein band was identified and eluted from the gel. The isolate was 98\% pure as indicated by agarose electrophoresis, staining with Coomassie brilliant blue and densitometric scanning. For use as a control in subsequent experiments paraprotein was also purified in an identical manner from another patient with an IgG λ myeloma but who showed no abnormalities of coagulation.

Fibrinogen was isolated from patient plasma by ammonium sulphate precipitation.\(^5\) Fibrin monomer was prepared both from this and commercially available human fibrinogen (Sigma Chemical, Poole, Dorset). Five units of thrombin were added to 100 mg fibrinogen dissolved in 2 ml PBS and incubated at 40°C for 45 minutes. The fibrin clot so formed was removed, washed in saline, and dissolved in 2 ml of a solution containing 2-5 mol/l urea in 0-05 mol/l acetate buffer, pH 4.8. The solution was dialysed at 4°C for 48 hours against 0-05 mol/l acetate buffer (pH 4.8). Fibrin is stable in the monomeric form at this pH, but readily polymerises to give a fibrin gel at higher pH.

Fibrin monomer polymerisation was followed spectrophotometrically by measuring the increase in absorbance at 350 nm (caused by increased turbidity due to gel formation) after the initiation of polymerisation.\(^5\)

For whole plasma, polymerisation was initiated by the addition of thrombin 0-2 units to 0-4 ml plasma and 0-8 ml PBS. Normal control plasma showed a characteristic polymerisation profile (fig 1A) with a short lag period, a rapid increase in absorbance, and subsequent plateau. In contrast, patient plasma showed a longer lag period and slower rate of absorbance increase with a lower plateau absorbance reading.

For purified fibrin monomer preparations, polymerisation was initiated by increasing the pH to 7-4. To 0-1 ml of monomer solution (concentration 1g/l) was added 0-9 ml of 0-15 mol/l imidazole containing 0-15 mol/l sodium chloride, pH 7-4. In some experiments purified paraprotein was added to the reaction cuvette (final concentration ranged from 0-5—7 g/l). Fibrin monomer prepared from both patient fibrinogen and commercial human fibrinogen showed identical polymerisation profiles (fig 1B). The addition of paraprotein to either of these preparations impaired polymerisation as indicated by longer lag period, slower initial rate of absorbance increase, and a lower plateau absorbance reading (fig 1C). In contrast, purified paraprotein from the control patient did not alter the polymerisation profile.

These results suggest an abnormality of fibrin formation in whole plasma. Purified patient fibrin monomer, however, can polymerise normally in vitro but polymerisation is inhibited in a dose dependent manner by the paraprotein.

Immunoelectrophoresis was used to investigate the immunoreactivity of the paraprotein towards fibrinogen. Agarose gels were prepared containing 1\% agarose in TRIS-barbital buffer (pH 8-6) and containing either 0-1 mg, 1 mg, or 10 mg purified paraprotein per ml of agarose.

Six microlitre samples of purified fibrinogen (prepared from both commercial and

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**Figure 1** (A) The time course of fibrin formation in patient and control plasma initiated by the addition of thrombin at time zero. (B) The time course of polymerisation of purified fibrin monomer prepared from patient and control fibrinogen. (C) The effect of control paraprotein and increasing concentrations of patient paraprotein on fibrin monomer polymerisation.

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**Figure 2** The relation between serum paraprotein concentration [- - - -] and thrombin time [———] after chemotherapy.

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human sources, concentration range 0.1—2 g/l) were applied to wells in the gel and electrophoresis was performed overnight at 4°C. The gels were pressed, washed, and stained with Coomassie Brilliant blue. No immunoprecipitin arcs were visible on any of the three gel plates, suggesting that there is no antigen-antibody reaction between the paraprotein and fibrinogen.

The patient was treated with adriamycin, vincristine, dexamethasone and radiation to the dorsal spine. Serial monitoring of coagulation tests showed that these returned to normal as the serum paraprotein concentration decreased (fig 2).

Discussion
We have described a patient with myelomatosis, abnormal coagulation tests, and unusual appearance of clotted blood specimens. We suggest that this was due to interference of the paraprotein in fibrin monomer polymerisation. There are three lines of evidence for this. Firstly, there was an abnormality of fibrin production in whole plasma, but fibrin monomer purified from the patient's fibrinogen polymerised normally in vitro, thereby excluding an inherited defect of monomer polymerisation. Secondly, purified paraprotein caused a dose dependent inhibition of polymerisation, and thirdly, the coagulation tests returned to normal as the serum paraprotein concentration decreased with treatment.

The exact mechanism by which the paraprotein interfered in monomer polymerisation remains unclear and in particular whether it results from a classic antigen-antibody reaction between fibrin monomer and the paraprotein or a more non-specific effect. The insolubility of fibrin monomer at a pH of more than 4.6 poses a difficulty in the elucidation of the mechanism as it effectively precludes investigation in the liquid phase. There was no immunoreactivity of the paraprotein towards fibrinogen so the proteolytic cleavage of fibrinopeptides from fibrinogen by the action of thrombin might expose epitopes on monomeric fibrin which are recognised by the paraprotein.

Despite having abnormal coagulation tests, this patient displayed no clinically important bleeding tendency. This is similar to the situation in patients with inherited defects of monomer polymerisation who in general do not have a bleeding tendency either, unless there is a concomitant problem such as hypofibrinogenemia. The defect, however, was not without its clinical relevance in this patient as the bulky gelatinous clots present in blood samples made it extremely difficult to harvest adequate serum for basic biochemical analyses. The unusual appearance of clotted blood specimens has been termed the "gelation phenomenon" and has been recognised in patients with myeloma for over 30 years. Coleman investigated a group of seven such patients displaying this phenomenon and his findings were identical with those described here: abnormal coagulation tests and in vitro evidence of antibody interference in fibrin monomer polymerisation.

5 Coleman M, Vigliano EM, Wehler ME, Nachman RL. Inhibition of fibrin monomer polymerisation by lambda myeloma globulins. Blood 1975;35:210-23
7 Frick PG. Inhibition of conversion of fibrinogen to fibrin by abnormal proteins in multiple myeloma. Am J Clin Pathol 1955;25:1263-73
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doi: 10.1136/jcp.47.3.266