Essential mixed cryoglobulinaemia with false-positive serological tests for syphilis

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SUMMARY Analysis of serum from a patient with cutaneous leukocytoclastic vasculitis showed a mixed cryoglobulin with a monoclonal IgM κ -antiglobulin component (6.5 mg/ml), strong rheumatoid factor activity (latex titre 1/5000), and positive serological tests for syphilis (fluorescent treponemal antibody-absorbed and *Treponema pallidum* haemagglutination assay). After removal of antiglobulin activity by immunoabsorption with heat-aggregated gammaglobulin all serological test results for treponemal infection became negative. Serological tests for syphilis and rheumatoid factor on the supernatant from whole serum (minus cryoglobulin) remained positive though at a lower titre (latex 1/1250). Cryoglobulin isolated from whole serum retained rheumatoid and TPHA reactivity but was negative in the FTA-ABS test. The IgM and IgG cryoglobulin components purified by gel filtration on Sepharose showed no antitreponemal reactivity even when tested individually. Reducing the concentration of cryoglobulin to 1.5 mg/ml by plasma exchange converted the test results for syphilis to doubtful-positive or negative. These results indicated that high concentrations of antiglobulin activity may be associated with falsely positive specific antitreponemal test results and that this phenomenon depends on the concentration of cryoglobulin in the test sample.

Introduction

The serological diagnosis of syphilis is usually based on the results of a non-treponemal cardiolipin flocculation test (Venereal Disease Research Laboratory, VDRL) and the detection of specific antitreponemal antibody¹ (fluorescent treponemal antibody-absorbed, FTA-ABS; and *Treponema pallidum* haemagglutination tests, TPHA). False reactivity in the cardiolipin tests, which may occur in a wide variety of disorders, is shown by the absence of specific antitreponemal antibody. False reactivity in the FTA-ABS test has, however, been recorded in collagen-vascular disorders such as lupus erythematosus,^{2,3} rheumatoid arthritis, scleroderma, and mixed connective tissue disease,⁴ as well as in several miscellaneous conditions such as genital herpes simplex, alcoholic cirrhosis, diabetes, autoimmune haemolytic anaemia, hypergammaglobulinaemia, pregnancy, and after smallpox vaccination.⁵⁻⁸ In two cases a positive rheumatoid factor was the only other

abnormal laboratory finding.⁹ In our patient the physical properties of the IgM rheumatoid factor allowed its isolation by the technique of cryoprecipitation and this provided some insight into the phenomenon of false syphilitic reactivity.

Case report

In April 1980 a 57-year-old man was admitted to St John's Hospital for Diseases of the Skin with a 14-year history of Raynaud's disease associated with a purpuric rash mainly of the lower limbs. In addition he had had pain and swelling of his feet and hands for seven years, particularly in the winter months. He had noticed that exposure to cold was an important factor affecting his cutaneous lesions and had therefore given up his previous employment as a brick layer.

His past history included four episodes of jaundice, and in 1966 he had evidence of mild hepatic dysfunction. A liver biopsy performed at that time showed no abnormality. He denied any previous history of venereal disease. Since becoming unemployed he had increased his consumption of alcohol to about 30 pints of beer a week and also smoked 20 cigarettes a day.

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He was well nourished with an urticarial eruption on his trunk and limbs, which faded within 48 hours of admission to hospital to leave purpuric staining. His legs were pigmented, consistent with previous deposition of haemosiderin. Examination showed clubbing of the fingers and enlargement of the liver to two fingers below the right costal margin. Otherwise there were no signs of chronic liver disease. Although he complained of intermittent hyperaesthesia of the lower extremities no notable neurological deficit was evident on examination.

INVESTIGATIONS

A skin biopsy specimen from an urticarial lesion of the thigh showed: "a heavy infiltrate of polymorphs surrounds blood vessels at all levels of the dermis. Although red cell extravasation is not a marked feature, there is considerable leukocytoclasia, fibrinoid change affecting vessel walls and phagocytosis of nuclear debris by tissue macrophages. The appearances are those of a leukocytoclastic vasculitis. No PAS-positive material was visualised within cutaneous blood vessels."

A liver biopsy was reported as: "the liver architecture is preserved; there is fibrosis in portal tracts with occasional bridging between tracts. Lymphocytes and plasma cells are present without piecemeal necrosis. Occasional small foci of liver cell necrosis are associated with mild lymphocyte reaction. There is a minor degree of centrilobular necrosis and the appearances favour a chronic hepatitis."

Routine haematological investigation showed a normal blood count and an erythrocyte sedimentation rate of 1 mm in the first hour. Bone marrow trephine biopsy showed a normocellular marrow with mild plasmacytosis and absent iron stores. Serum protein electrophoresis showed a narrow band provisionally identified as an IgM complex and subsequently shown to be a mixed cryoglobulin. Serum concentrations of IgA and IgG were within normal limits (2.6 and 9.8 g/l respectively). Biochemical investigations gave evidence of mild hepatic and renal dysfunction: bilirubin 20 $\mu\text{mol/l}$ (normal range 4-16 $\mu\text{mol/l}$), aspartate transaminase 52 IU/l (normal range 5-35 IU/l), alanine transaminase 61 IU/l (normal range 5-35 IU/l), alkaline phosphatase 84 IU/l (normal range 22-92 IU/l), urea 6.8 mmol/l (normal range 2.0-6.6 mmol/l), creatinine 146 $\mu\text{mol/l}$ (normal range 50-110 $\mu\text{mol/l}$), creatinine clearance 32 ml/min (normal range 80-120 ml/min), 24-hour protein excretion 0.5 g/l. Examination of a midstream urine specimen showed casts but no notable haematuria. Viral antibody titres for cytomegalovirus were normal, the Paul-Bunnell test was negative, and there was no evidence of infection with hepatitis B.

SPECIAL STUDIES

Cryoglobulin estimation and analysis

Cryoglobulin concentrations were determined by the method of Brouet *et al*¹⁰ on serum separated at 37°C. The cryoprecipitate was tested for the presence of IgG, IgM, IgA, C1q, C3, and C4 by double immunodiffusion using monospecific antisera. The two cryoglobulin components were purified by gel filtration on Sepharose 6B at pH 4 at 4°C. The IgM and IgG fractions were tested for the presence of κ and λ light chains by double immunodiffusion.

Tests for treponemal infection

Four serological tests were included in this study: the VDRL slide test, the FTA-ABS, the modified microhaemagglutination (TPHA), and *T pallidum* immobilisation (TPI) test, and recommended procedures were followed.¹¹ Serum samples and cryoglobulin fractions were absorbed with both sorbent and a sonicate of Reiter treponemes. Different batches of TPHA reagents were used to exclude positive results associated with a particular batch of reagents. Removal of antiglobulin rheumatoid factor activity by absorption of the serum with glutaraldehyde-polymerised heat-aggregated gammaglobulin has been shown to abolish false-positive reactions for treponemal antibodies.¹² Positive samples were therefore tested before and after removal of rheumatoid factor activity, as detected by a latex slide test. Initially specimens of whole serum before and after plasma exchange, cryoglobulin components of serum, and a sample of cerebrospinal fluid (CSF) were tested. Subsequently two specimens of whole serum were examined at six-monthly intervals within one year. Each serological test was repeated for reproducibility of results.

Results

CRYOGLOBULIN ESTIMATION AND ANALYSIS

Before plasma exchange the serum contained a mixed cryoglobulin present at a concentration of 6.5 g/l; the upper limit of normal for our laboratory is 0.04 g/l. Immunoglobulin analysis and light-chain typing showed that the cryoglobulin consisted of a monoclonal IgM antiglobulin component, polyclonal IgG, and trace amounts of C3. After three 4-litre plasma exchanges for plasma protein fraction on a discontinuous flow cell separator (Haemonetics model 30) the cryoglobulin concentration fell to 1.5 g/l.

TREPONEMAL SEROLOGY

Both the TPHA and FTA-ABS tests gave positive results in serum but not in the CSF; the TPI test was anticomplementary in serum and negative for the IgG and IgM components of the cryoglobulin. The latex

test was positive for serum and cryoglobulin fractions, indicating rheumatoid factor. The results of the latex, FTA-ABS, and TPHA tests for six samples before and after immunoabsorption with Cohn Fraction II are shown in the table.

A homogeneous reaction was obtained in the FTA-IgG and IgM tests without beading,²¹³ and a reactive TPHA result was found only at the initial serum dilution of 1/80. After absorption the FTA-IgG and IgM results were negative; in contrast the TPHA was weakly positive (dilution of 1/80) using sheep cell but negative with chick cell reagents. The TPI test result for serum after absorption was negative.

The degrees of reactivity and fluorescence were determined by the same laboratory technologists who compared the results with a wide range of standard quality control sera representing specific and non-specific sources of positive reactivity.

TABLE Serological test results on sera absorbed and unabsorbed with gammaglobulin to remove rheumatoid factor activity

Test	Absorption	Specimen No†					
		1	2	3	4	5	6
Latex	U	++	++	++	++	++	0
	A	0	0	0	0	0	0
TPHA (sheep)*	U	++	D	+	+	0	0
	A	D+	0	0	0	0	0
TPHA (chick)*	U	1/C	0	0	0	0	0
	A	0	0	0	0	0	0
FTA-ABS-IgG	U	++	D	D	0	0	0
	A	0	0	0	0	0	0
FTA-ABS-IgM	U	++	0	+	0	0	0
	A	0	0	0	0	0	0

+ = Positive, D = doubtful; 1/C = inconclusive; U = unabsorbed; A = absorbed with aggregated gammaglobulin; 0 = non-reactive
 *Sheep cell reagents (Fujizoki); chick cell reagents (Whitley)
 †Sample Nos: 1 = serum before three plasma exchanges (5.6 mg/ml); 2 = serum after three plasma exchanges (1.2 g/l); 3 = serum minus cryoglobulins-supernatant of (4); 4 = cryoglobulins (pellet of mixed cryoglobulins washed three times); 5 = purified IgM (0.56 g/l); 6 = purified IgG (0.97 g/l).

Discussion

Patients with cryoglobulinaemia can be classified into three classes depending on the composition of the cryoprecipitate.¹⁰¹⁶ Type I cryoglobulins are monoclonal immunoglobulins which lack antiglobulin activity. Type II cryoglobulins display antiglobulin activity and consist of a monoclonal immunoglobulin (usually IgM) complexed with IgG. In type III cryoglobulins both globulin and antiglobulin components are polyclonal. They occur in a wide variety of chronic inflammatory disorders and are usually present in small amounts (<1 g/l). Both

type I and type II cryoglobulins may arise from benign or malignant lymphoproliferative disorders affecting the B-cell series and are usually present in concentrations exceeding 1 g/l. Type II cryoglobulins have also been recorded in association with autoimmune disorders such as systemic lupus erythematosus and infectious processes such as infectious mononucleosis.¹⁷¹⁸ In the absence of any associated disorder the condition is labelled "essential". Essential mixed cryoglobulinaemia is a distinct clinicopathological entity characterised by Raynaud's phenomenon, cutaneous vasculitis, arthralgia, renal disease, and a high incidence of liver impairment.¹⁹ Evidence of hepatitis B infection has been identified in an appreciable number of cases,²⁰ but there still remain patients in whom no precipitating agent can be identified and in whom the term "essential mixed cryoglobulinaemia" is justified.

Our patient fulfilled the criteria for the diagnosis: he had Raynaud's disease, urticarial skin lesions with the histology of a leukocytoclastic vasculitis, arthralgia, and mild renal dysfunction. There was no evidence of hepatitis B infection despite evidence of hepatic pathology.

Although the serological test results for syphilis were positive there was no clinical evidence or history of venereal disease. Small amounts of cryoprecipitable immunoglobulin have been documented in a wide variety of chronic infectious disorders including established cases of syphilis.¹⁹ Furthermore, false-positive reactions for treponemal antibodies have previously been reported associated with high titres of antiglobulin factors.⁹ It was, therefore, important in this patient to establish whether the type II cryoglobulin was associated with genuine syphilitic infection. False-positive reactivity for antitreponemal antibodies associated with antiglobulin activity can be removed by absorption of the serum with glutaraldehyde polymerised heat-aggregated gamma-globulin. This procedure removed all antitreponemal activity from this patient's whole serum, suggesting that the high degree of latex activity (1/5000) was responsible for the false-positive antitreponemal test results. Antitreponemal reactivity was detected in both the supernatant and the cryoglobulin recovered from whole serum. The supernatant still showed considerable antiglobulin activity (latex titre 1250). Removal of latex reactivity by immunoabsorption also abolished antitreponemal activity in these samples. After the reduction of the cryoglobulin concentration to 1.5 g/l by plasma exchange the antitreponemal antibody tests became doubtful or negative. False reactivity did not occur with all the serological tests since the TPHA was positive with the Fujizoki sheep cell reagents but not with the Whitley reagents which use chicken cells²⁰ (table).

The FTA-ABS test result was positive using both anti-IgG and anti-IgM fluorescent conjugates. The most likely explanation for these observations is that large lattice-like complexes of IgM and IgG bind non-specifically to the killed treponeme. The absence of any antitreponemal activity in the purified cryoglobulin components when tested individually supports this interpretation of the findings.

Finally, it cannot be assumed that all positive test results for syphilis associated with the presence of cryoglobulins are false. Jobbagy and Kiraly²¹ reported a case with type II cryoglobulinaemia (2 mg/ml) who had previously received antisyphilitic treatment; they do not state the grounds for the original diagnosis. On analysis of these cryoglobulins it was found that rheumatoid factor activity was confined to the IgM fraction of the cryoglobulin whereas TPI and FTA-ABS reactivity occurred only in the IgG fraction. By contrast, in our case the TPI test result became negative after removal of antiglobulin activity and the purified IgG fraction from the cryoglobulin showed no antitreponemal activity; thus no evidence was found that the antitreponemal activity was separate from the antiglobulin activity.

On the basis of these results we conclude that the antitreponemal reactivity detected in our patient's serum was attributable to the high concentrations of circulating type II cryoglobulin.

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References

1. Sparling PF. The diagnosis and treatment of syphilis. *N Engl J Med* 1971;**284**:642-53.
2. Kraus SJ, Haserick JR, Lantz MA. Fluorescent treponemal antibody-absorption test reactions in lupus erythematosus. A typical beading pattern and probable false-positive reactions. *N Engl J Med* 1970;**282**:1287-90.
3. Monson RAM. Biologic false-positive FTA-ABS test in drug-induced lupus erythematosus. *JAMA* 1973;**224**:1028-30.
4. McKenna CH, Schroeter AL, Kierland RR, Stilwell GG, Pien FD. The fluorescent treponemal antibody absorbed (FTA-ABS) test beading phenomenon in connective tissue diseases. *Mayo Clin Proc* 1973;**48**:545-8.
5. Maskey DM, Price EV, Knox JM, Scotti A. Specificity of the FTA-ABS test for syphilis: an evaluation. *JAMA* 1969;**207**:1683-5.
6. Buchanan CS, Haserick JR. FTA-ABS test in pregnancy a probable false-positive reaction. *Arch Dermatol* 1970;**102**:322-5.
7. Jacobs M, Prothro GW, Carpenter RL. Apparent transient false-positive FTA-ABS test following smallpox vaccination. *J Okla State Med Assoc* 1971;**64**:372.
8. Wright JT, Cremer AW, Ridgway GL. False-positive FTA-ABS results in patients with genital herpes. *Br J Vener Dis* 1975;**51**:329-30.
9. Bradford LL, Tuffanelli DL, Puffer J, Bissett ML, Bodily HL, Wood RM. Fluorescent treponemal absorption and *Treponema pallidum* immobilisation tests in syphilitic patients and biological false-positive reactors. *Am J Clin Pathol* 1967;**47**:525-32.
10. Brovet JL, Clauvel JP, Danon F, Klein M, Seligmann MP. Biological and chemical significance of cryoglobulins — a report of 86 cases. *Am J Med* 1974;**57**:775-88.
11. *Manual of Tests for Syphilis*. US Public Health Service publication No 411. Washington DC: Department of Health, Education and Welfare, 1969.
12. Chantler S, Devries E, Allen PR, Hurn BAL. A rapid immunofluorescent procedure for the detection of specific IgG and IgM antibody in sera using *Staphylococcus aureus* and latex IgG as absorbents. *J Immunol Methods* 1976;**13**:367-80.
13. Shore RN, Faricelli JA. Borderline and reactive FTA-ABS results in lupus erythematosus. *Arch Dermatol* 1977;**113**:37-41.
14. Grey HM, Kohler PF. Cryoimmunoglobulins. *Seminars in Haematology* 1973;**10**:87-112.
15. Hanauer LB, Christian CL. Studies of cryoproteins in systemic lupus erythematosus. *J Clin Immunol* 1967;**46**:400.
16. Wands JR, Perrotto JL, Isselbacher KJ. Circulating immune-complexes and complement sequence activities in infectious mononucleosis. *Am J Med* 1976;**60**:269-72.
17. Meltzer M, Franklin EC, Elias K, McCluskey R, Couper N. Cryoglobulinaemia — a clinical and laboratory study: 11 cryoglobulins with rheumatoid factor activity. *Am J Med* 1966;**40**:837-56.
18. Levo Y, Gorevic PD, Kassab HJ, Zucker-Franklin D, Franklin EC. Association between hepatitis B virus and essential mixed cryoglobulinemia. *N Engl J Med* 1977;**296**:1501-4.
19. Mustakallio K, Lassus A, Wager O. Auto-immune phenomena in syphilitic infections. Rheumatoid factor and cryoglobulins in different stages of syphilis. *Int Arch All* 1967;**31**:417-26.
20. Sequeira PJ, Eleridge AE. Treponemal haemagglutination test. *Br J Vener Dis* 1973;**49**:242-8.
21. Jobbagy A, Kiraly K. Cryoglobulinaemia serum containing antibodies immobilising *T pallidum*. *Pathol Microbiol* 1974;**41**:65-74.