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IgM–IgG CRYOGLOBULINAEMIA WITH IgM PARAPROTEIN COMPONENT

F. KLEIN, J. J. VAN ROOD, R. VAN FURTH AND H. RADEMA

Institute for Rheumatism Research, Department of Immunohaematology and Department of Microbial Diseases, University Hospital, Leiden, The Netherlands

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SUMMARY

Four patients with mixed IgM–IgG cryoglobulinaemia are described. Clinically they all had some features of an autoimmune disease, while two of them had a lympho-epithelial tumour in the parotid gland. The mixed cryoglobulins of all patients contained an IgM paraprotein with the properties of a rheumatoid factor. They can be regarded as cryoprecipitates of a rheumatoid factor with autologous IgG. In one case the parotid tumour, and not the bone marrow, produced the IgM paraprotein. The clinical significance of the cryoglobulins is discussed. The IgM paraproteins with rheumatoid factor activity may be an expression of an underlying abnormality of the immunological system of these patients.

INTRODUCTION

In 1962, Lospalluto *et al.* described a patient with a cryoglobulin consisting of IgG and IgM. Since that time a number of papers have appeared describing such mixed cryoglobulins in patients with a variety of diseases (Peetoom & van Loghem-Langereis, 1965; Costanzi *et al.*, 1965; Curtain, Baumgarten & Pye, 1965; van der Geld *et al.*, 1966; Meltzer *et al.*, 1966). Many of these patients had symptoms of an autoimmune disease. The components of the cryoglobulins had the characteristic heterogeneity of the immunoglobulins.

Recently a few cases have been described (Kushner, 1966; Johnson, Waldmann & Talal, 1967; Metzger, 1967) in which the IgM part of the complex was a paraprotein but detailed descriptions of the clinical as well as the biochemical aspects of this exceptional phenomenon are rare. The term paraprotein is used in this article for electrophoretically homogeneous immunoglobulins, which belong to only one light chain type. It implies no further assumptions about their nature and origin. It is the intention of this paper to report in detail the clinical and biochemical findings in four patients with IgM–IgG cryoglobulins, the IgM part of which had the characteristics of a paraprotein with anti-IgG activity. All four

Correspondence: Dr F. Klein, Institute for Rheumatism Research, University Hospital, Leiden, The Netherlands.

patients showed signs of autoimmune disease, while two of them were suffering from a lympho-epithelial tumour. The possible relationship between the autoimmune phenomena and the formation of paraproteins will be discussed.

Case Reports

Patient Ke.

A 46-year-old male butcher was admitted to the University Hospital in February 1965 because of nocturnal dyspnoea and auricular fibrillation of about 1 year's duration. He had been healthy until 1954 when he suddenly lost hair without becoming completely bald. The past history included a purulent arthritis of the ankle (haemolytic streptococci) in 1966. Since that time he had suffered from Raynaud's phenomenon and frequent nosebleeds. In 1966 he underwent surgery because of an enlargement of the left parotid gland, which proved to be a benign lympho-epithelial tumour.

Physical examination revealed an ill patient with a pulse rate of 80/min, blood pressure 200/110 mmHg and a temperature of 37°C. The right-sided parotid gland was enlarged. He had a purpura on the legs and a 2+ pitting oedema bilaterally. The left ventricle of the heart was enlarged and a gallop rhythm was present. There were signs of left-sided heart failure, pleuritis and myopericarditis. Rhonchi were heard over both lungfields. The liver and spleen were not palpable.

Relevant laboratory tests are summarized in Table 1. A Schirmer test was pathological, which demonstrated that a keratoconjunctivitis sicca and xerostomia was present, as seen in Sjögren's disease. Bone marrow biopsy showed a slight increase of plasma cells.

The diagnoses were: benign lympho-epithelial tumour with cryoglobulinaemia, haemolytic anaemia, myocarditis, nephritis, and Sjögren's disease.

The patient was treated with methyldopa, low-protein diet, prednisone (first 40 mg/day, later 15 mg/day) and blood transfusions. The clinical condition of the patient improved. During the time of writing he was ambulatory and able to work part time. The cryoglobulins were not present any more, the parotid tumour diminished in size, and the Raynaud's phenomenon improved. The improvement was reflected in other laboratory data; the erythrocyte sedimentation rate was 4 mm, haemoglobin 13·9 g/100 ml, blood urea nitrogen 37 mg/100 ml, and creatinine 1·3 mg/100 ml.

During this remission and while still under prednisone therapy, he developed typical sunlight-induced butterfly lesions of systemic lupus erythematosus. A basal membrane fluorescence test was positive. The direct Coombs test, originally negative, became positive.

Patient Ge.

A 77-year-old carpenter was admitted to the University Hospital in October 1964, because of a 2-month history of swollen and painful legs, severe intermittant claudication and Raynaud's syndrome, chiefly affecting his hands. In 1961 a left-sided parotidectomy was performed because of a benign lympho-epithelial tumour. In the same year the left lacrimal gland was removed because of a tumour which had the same histological characteristics as that in the parotid gland. The tumour was not completely removed but X-ray therapy was applied (1500 r). Some tumour tissue remained palpable during the rest of his life. An enlargement of the right parotid gland was noticed in 1963. This tumour was not removed surgically and disappeared spontaneously. From 1953 on he was seen regularly in the out-patient department of the University Hospital in Leiden for chronic asthmatic bronchitis, myocardial fibrosis and auricular fibrillation. In 1962 gastrectomy was performed because of a gastric and duodenal ulcer.

On admission to the hospital the pulse rate was 62/min and totally irregular; blood pressure was 125/80 mmHg; temperature was 37°C. Over the skin of both lower extremities there was a purpura. There was a haemorrhagic eczema on pressure points, especially the buttocks and shoulders. Arterial pulsations in both legs were absent. The patient had trophic lesions of the hands and toes. The legs were oedematous, painful and red violet in addition to the purpura. The liver and spleen were not palpable and no pathological lymph nodes were found.

The relevant laboratory data are summarized in Table 1. The electrocardiogram showed auricular fibrillation and slight right ventrical hypertrophy. The histology of a skin biopsy showed a pattern which resembled vasculitis allergica. The bone marrow biopsy showed a slight increase of plasma cells and reticulocytes.

The diagnoses were: lympho-epithelial tumour accompanied by cryoglobulins, Raynaud's syndrome, myocardial fibrosis and chronic bronchitis.

The patient was first treated with chlorambucil (10 mg/day for 4 months) and when he made no improvement, he was treated with prednisone (40–20 mg/day). With this treatment there was a significant improvement of the clinical condition. However, the patient contracted a pneumococcal pneumonia in 1965 and a coronary infarction later in that year. He recovered from both, but his general condition deteriorated slowly in 1966. He died in July 1966 while at home. A post-mortem examination was not performed.

Patient St.

A 38-year-old housewife, mother of four children, was admitted to the Hospital Bronovo in The Hague on February 1967 because of fever, light sensitivity, facial and pretibial oedema, and oliguria. For 1 month prior to admission she had been suffering from a Raynaud's syndrome. Since 1959 she had had skin lesions which had been diagnosed as lupus erythematosus, and in 1961 she developed rheumatoid arthritis. Initially she was treated with chloroquine and gold salts. The latter were discontinued because of dermatitis and albuminuria. Between 1961 and 1967 she had few complaints about her joints but was regularly seen because of her skin lesions.

On admission her temperature was 37·6°C, pulse rate 100/min, and blood pressure 170/110 mmHg. She had facial and pretibial oedema. The heart was enlarged with a gallop rhythm at the apex and a pericardial friction rub was heard. The liver was enlarged 3 cm below the right costal margin and the spleen was just palpable below the left costal margin. The sensibility and strength of the right arm were slightly diminished. A roentgenogram of the chest showed pulmonary vascular engorgement with a right pleural effusion.

The laboratory data are summarized in Table 1. The urobilinogen excretion in the faeces was increased (haemolytic index = 75). Bone marrow biopsy showed increased megakariopoiesis with hypersegmentation, and slight plasmocytosis. A biopsy of skin, fascia and muscle demonstrated the presence of polymyositis with possibly a slight fibrosis of subcutaneous tissue. This can be seen in systemic lupus erythematosus but is not pathognomonic of it. A skin biopsy, however, showed basal membrane fluorescence.

The diagnosis of systemic lupus erythematosus with cardiac insufficiency, pericarditis, pleuritis and nephritis was sustained.

The patient was put on a low protein and low sodium diet, diuretics, digitalis, and prednisone. Also two blood transfusions were given. After initial improvement the cardiac insufficiency reappeared. The patient was then admitted to the University Hospital in Leiden, where essentially the same physical and laboratory findings as those mentioned above were obtained. A kidney biopsy showed a picture consistent with a severe glomerulonephritis due to systemic lupus erythematosus. The electrocardiogram showed signs of coronary insufficiency.

Therapeutic regime consisting of more intensive digitalis therapy, diuretics and sodium free diet improved the cardiac situation. The patient was taking an oral contraceptive and because of the possibility that this had an adverse effect (Pimstone, 1966) it was discontinued. The hypertension was treated with methyldopa which was replaced by guanethidine because of the possibility that methyldopa would aggravate the already existing haemolytic anaemia. The blood pressure stabilized at 160/100 mmHg. Prednisone (30 mg/day) was administered to treat the autoimmune process. When the general condition did not improve (hypertension, nephritis and cryoglobulinaemia), 6-mercaptopurine (100 mg/day) was given. Also an intensive plasmaphoresis was performed (11·5 litres in 6 days) which diminished the amount of cryoglobulin significantly, reduced the erythrocyte sedimentation rate to normal, and the serum protein electropherogram also became normal. In the first two months after hospitalization the clinical condition was rather labile. As long as the patient did not work, rested regularly, and followed her diet strictly, her condition was reasonable. Although these conservative measures appeared effective, it was also evident that the basic process was almost unchanged.

Patient Ul.

A 68-year old housewife and mother of five children was admitted to the Hospital Bronovo in The Hague on March 1966 because of malaise, fever of unknown origin which had started as a 'flu', and coughing for 5 months. Since 1922 the patient had suffered from shaking chills three to four times a

year, especially after going out in the cold. Three to 4 days after these shaking chills, reddish efflorescences appeared on her arms and legs but disappeared spontaneously after about 3 days. According to the patient's husband these efflorescences looked like scarlet fever. During the last 4 years the chills were followed more often by dyspnoea, paleness and sweating than by the efflorescences. A hot water bottle or hot tea gave relief. The past history indicated that the patient also had chronic asthmatic bronchitis and had had pneumonia five times since 1927.

In 1962 she was treated surgically for a fracture of the femur. This treatment was complicated by a thrombosis.

On physical examination her temperature was 38·6°C, pulse 98/min, blood pressure 220/110 mmHg. Petechiae were visible on the arms. The heart, liver and spleen were enlarged. (The liver, 3 cm under the right costal margin, and the spleen, 3 cm under the left costal margin.) No abnormal lymph nodes were found. A bone marrow biopsy showed many lymphocytes with formation of germinal centres.

The relevant laboratory data are summarized in Table 1. The electrocardiogram showed signs of a diffuse myocardial lesion. Roentgenograms of the thorax showed cardiac enlargement and calcified lymph nodes. A muscle biopsy showed necrotizing arteritis consistent with the diagnosis periarteritis nodosa, although other types of necrotizing arteritis could not be excluded. No immunofluorescence studies were performed to decide between these possibilities.

The diagnosis was made of connective tissue disease, possibly periarteritis nodosa.

The patient was treated with prednisone (40 mg/day in the beginning; 15 mg/day later) after penicillin therapy had failed to bring her temperature within normal limits. To remove the cryoglobulin, plasmaphoresis was performed (twelve times 250 ml plasma). Hereafter the clinical condition improved and the patient was discharged. Three weeks later she was readmitted because of a Raynaud's syndrome and neurological disturbances, possibly on the basis of lesions of the vascular elements of the nerves. Treatment consisted of prednisone and cyclophosphamide (total dose 1100 mg). The temperature rose again 3 weeks after admission and on the basis of chest roentgenograms and sputum culture a staphylococcal pneumonia was diagnosed. Methicillin was given, but the general condition deteriorated and the patient died 2 weeks later.

A post mortem did not sustain the diagnosis periarteritis nodosa, in view of the scanty occurrence of arterial lesions. Kidney lesions were consistent with the diagnosis of glomerulonephritis. In the lungs there was a bronchopneumonial abscess formation in the lower lobe of the right lung and a lung embolus.

In summary all patients had a clinical history of symptoms of a Raynaud's syndrome, signs of an autoimmune disease, and myocardial lesions. Two patients had a lympho-epithelial tumour. Cryoglobulins were found in the serum of all patients.

MATERIALS AND METHODS

The cryoglobulins were isolated and purified by centrifugation from the serum after standing overnight at 4°C, redissolution in buffered saline (pH = 7·2) at 37°C and reprecipitation by keeping the solution overnight at 4°C. This procedure was repeated four times with fresh saline, after which the insoluble residues were centrifuged off at 37°C. In order to avoid losses the four washings were done in one case with cold buffered saline without redissolving. Before and after these washings one redissolution and precipitation cycle was performed to ensure that an actual cryoglobulin was isolated. The isolation procedure entailed considerable losses, because of the formation of insoluble complexes, probably due to denaturation of the IgG part of the cryoglobulin.

Ultracentrifugal analyses were performed in the Spinco model E ultracentrifuge with buffered saline as a solvent.

Gel filtration through Sephadex G-200 was performed using columns of 70 cm length and 3 cm diameter. About 40 mg of purified cryoglobulin was applied to the column. In one instance the medium was 0·05 M-phosphate buffer of pH = 8·0 containing 0·1 M-NaCl and kept at 37°C by a water mantle around the column. In another instance 0·1 M-acetate buffer of pH = 5·0 containing 0·5 M-NaCl was used, in which the cryoglobulin was soluble at

room temperature. Ultracentrifugal analysis showed that at this pH no aggregation or dissociation took place. A similar separation proved possible at pH = 4·0 as well.

Agar electrophoresis was carried out according to Wieme (1959) and immunoelectrophoresis was done on standard microscope slides in agar gel containing barbiturate buffer of pH = 8·6.

The latex fixation test was performed as described by Valkenburg (1963). The Waaler–Rose test was carried out with human O Rh+erythrocytes sensitized with rabbit antibody.

TABLE 1. Laboratory data of the patients

	Patient Ke.	Patient Ge.	Patient St.	Patient Ul.
Haemoglobin (g/100ml)	7·5	12·7	10·6	9·6
Reticulocytes (‰)	65	62	60	
Leucocytes/mm^3	8,800	5,800	11,600	5,300
Neutrophils/mm^3	7,000	4,600	10,500	3,300
Platelets/mm^3	360,000	230,000	110,000	200,000
Sedimentation rate/hr (at room temperature)	61	32	73	53
Blood urea N(mg/ml)	135	33	67	88
Urine protein	±	±	±	+
Sediment				
Red cells	+	−	2+	3+
White cells	+	−	2+	+
Direct Coombs test	−→+	−	−	−
Complement activity	Diminished	Absent	Absent	Absent
Cold agglutinins	−	−	−	−
Cryoglobulins	+	+	+	+
Rose test	−	−	+	+
Latex test	+	+	+	+
Antinuclear factor (ANF)	−	−	+	−
LE cells			−	
Albumin (g/100 ml)	2·6	4·2	4·0	2·5
γ-Globulin (g/100 ml)	1·0	1·6	0·7	0·3

For *in vitro* culture studies, a biopsy from the tumour (619 mg) and a bone marrow aspiration (144 mg) of patient Ge were incubated in a culture medium containing ^{14}C-lysine and ^{14}C-isoleucine. A detailed description of the method has been published elsewhere (van Furth, 1967). After an incubation period of 48 hr. the culture fluid was centrifuged and dialysed at 37°C. The immunoglobulins synthesized *in vitro* were detected and characterized by autoradiograpy of the electropherograms on agar and cellulose acetate (Beckman Microzone Apparatus) and of the immunoelectrophoretic patterns.

RESULTS

All four cryoglobulins contained two components with sedimentation coefficients of 7S and 19S, which precipitated together in the cold. The ultracentrifuge diagram of Fig. 1 gives an example. Immunoelectrophoretic analysis revealed only two components, IgM and IgG,

without further impurities (see Fig. 2). In three cases the 7S and 19S components were separated by gel filtration (see Fig. 3). Table 2 gives a survey of the properties of fractions thus obtained from the three patients Ge., Ul. and St. Material from the fourth patient Ke. was insufficient for fractionation. The paraprotein character of the macroglobulin fraction and the heterogeneous appearance of the lighter fraction are illustrated by the agar electrophoresis shown in Fig. 4. It is noteworthy that fractions I of samples Ge. and Ul. penetrated into the agar only after previous reduction by 0·2 M-2-mercaptoethanol. A confirmation was given by reaction of the separated fractions with anti-κ and anti-λ chain antisera. As

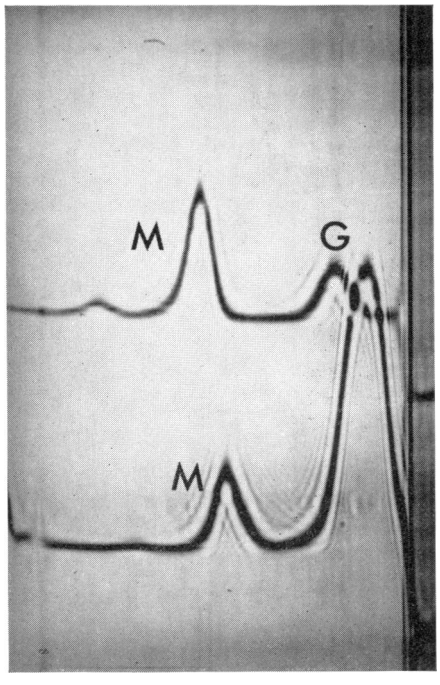

FIG. 1. Ultracentrifuge diagrams of serum Ge. (below) and purified cryoglobulin Ge. (above). Sedimentation from right to left. M, Macroglobulin fraction; G, 7S-globulin fraction.

Fig. 5 shows, the 7S fraction contained both κ and λ chains while the homogeneous 19S fraction was only κ. In the serum of patient Ke. the agar electrophoresis showed the presence of two paraproteins. The shape of the precipitation line with anti IgM serum in immunoelectrophoresis made it very probable that these were IgM components.

All four cryoglobulins had a very strongly positive latex fixation test. In two of them (Ge. and Ke.) the Waaler–Rose test was only very weakly positive. Sample Ul. had a strongly positive Rose test as well and sample St. a moderately strong one. As Table 2 shows, the rheumatoid factor activity was found only in the paraprotein fraction in the cases of Ge., Ul. and St. For Ke. no further characterization was possible. However, here the latex fixation test was negative after reduction of the cryoglobulin with 0·1 M-2-mercaptoethanol, so that it can be stated that in all four cases we are dealing with IgM paraproteins with rheumatoid factor activity. The activity in the Waaler–Rose test could not be found back in

any of the fractions of cryoglobulin St. Possibly the Waaler–Rose activity of the IgM paraprotein was lost by partial denaturation of the molecule during the isolation procedure.

The rheumatoid factor properties of these IgM paraproteins appear particularly clearly in the case of Ge., where soluble complexes of IgG and IgM were formed at 20°C (Fig. 6).

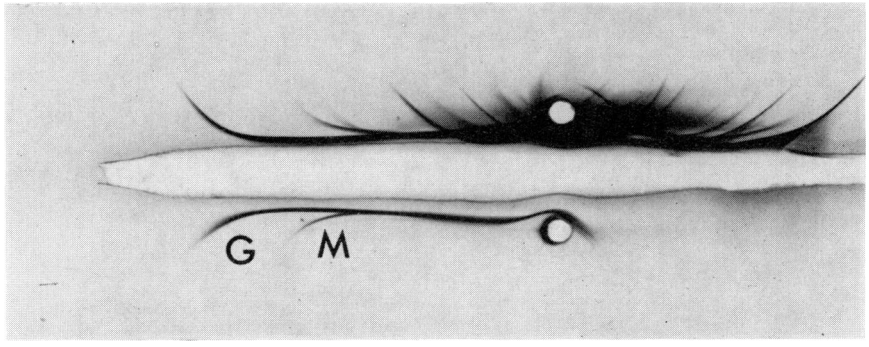

FIG. 2 Immunoelectrophoresis of serum Ge. (above) and purified cryoglobulin Ge. (below) against anti-total human serum. M, IgM line; G, IgG line. Cathode to the left.

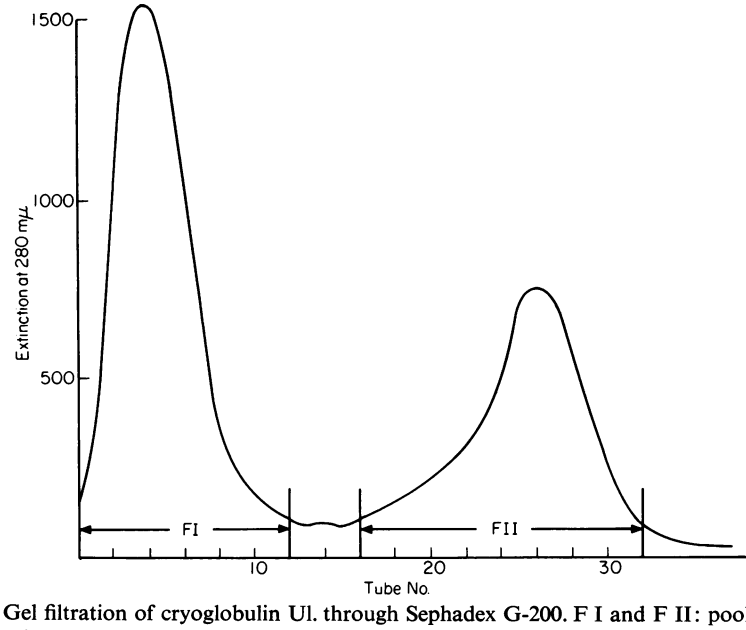

FIG. 3. Gel filtration of cryoglobulin Ul. through Sephadex G-200. F I and F II: pooled IgM and IgG fractions.

These heterogeneous complexes with sedimentation velocities >19S can be compared with the 22S components present in some rheumatoid sera. Like the latter they dissociated at pH = 3·5 as demonstrated by ultracentrifugation. Here the 7S fraction increased after bringing the pH to 3·5. Unlike the classical 22S complexes this complex also dissociated at 37°C and neutral pH. Addition of normal human IgG at 20°C resulted in an increase of the

complex at the expense of the 7S fraction. This also happened at 37°C, proving that at this temperature the dissociation is not complete. In accordance with this the St. values were slightly higher than is usually found for the IgM fraction. Fig. 6 also shows that the equili-

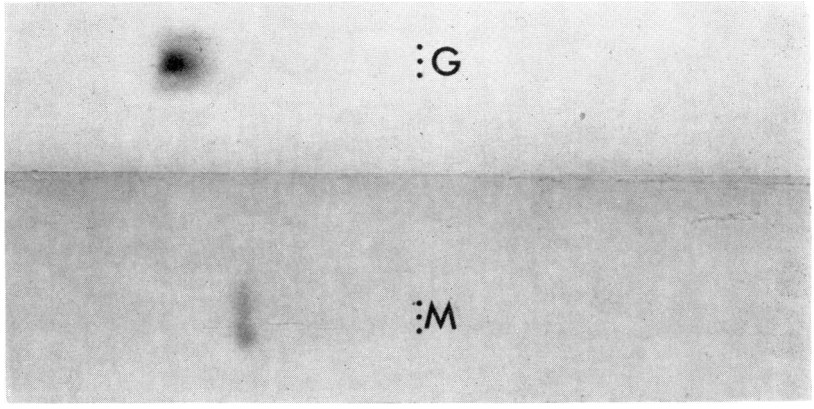

FIG. 4. Agar gel electrophoresis of IgM and IgG fractions obtained by gel filtration of cryoglobulin Ge. Cathode to the left.

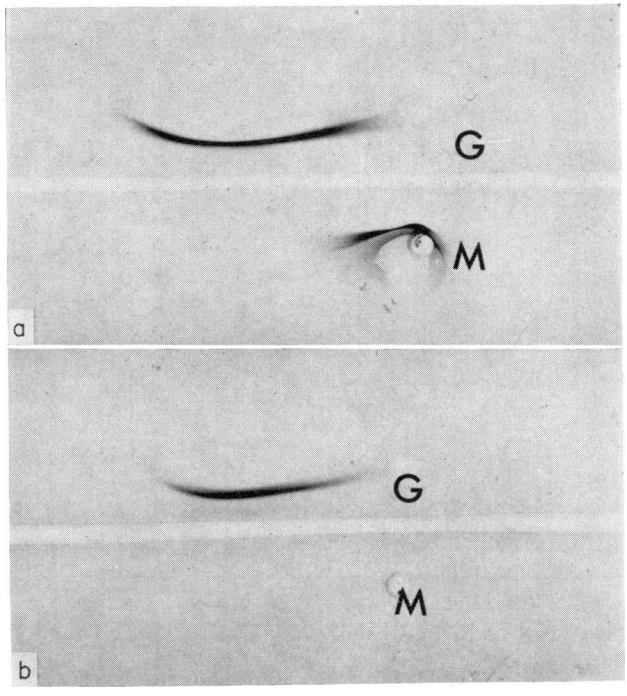

FIG. 5. Immunoelectrophoresis of IgM and IgG fractions from cryoglobulin Ge. with anti-κ (a) and anti-λ serum (b). Cathode to the left.

brium is dependent on the total concentration of the components, since the shape of the ultracentrifuge diagram was changed after dilution. Cryoglublin Ul. also showed evidence of the formation of soluble complexes at 20°C.

As in the case described by Lospalluto *et al.* (1962), the isolated components of the cryoglobulins Ge., Ul. and St. did not precipitate in the cold separately. Recombination again produced a cryoprecipitate. Recombination of the IgM part of Ge. with an isolated

TABLE 2. Investigation of cryoglobulin fractions

Fractions	$S°_{20}$	Electrophoresis	Immuno-electrophoresis	Latex fixation test	Waaler–Rose test
F I: Ge.	20	β–γ; paraprotein	IgM type-κ	Positive	Weakly positive
F I: Ul.	20	β–γ; paraprotein	IgM type-κ	Positive	Positive
F I: St.	19	β–γ; paraprotein	IgM type-κ	Positive	Negative
F II: Ge.	7	γ; heterogeneous	IgG	Negative	Negative
F II: Ul.	7	γ; heterogeneous	IgG	Negative	Negative
F II: St.	7	γ; heterogeneous	IgG	Negative	Negative

F I and II: first and second fractions of gel filtration. Ge. Ul. and St. refer to the patients.

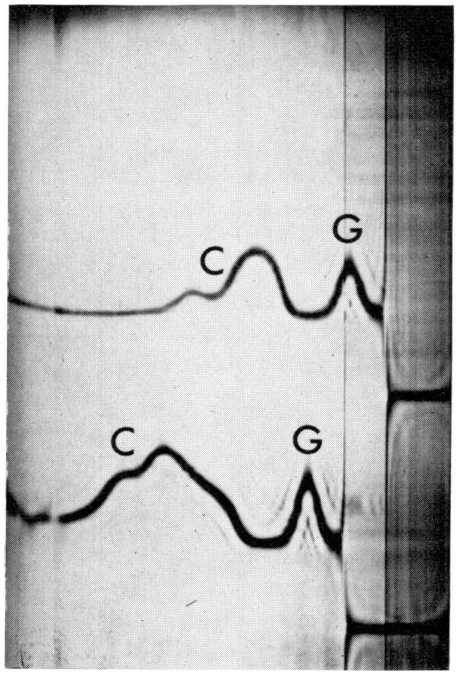

FIG. 6. Ultracentrifuge diagram of soluble fraction of cryoglobulin Ge. at 20°C. Concentration below 2 × concentration above. Sedimentation from right to left. C, IgM–IgG complex; G, IgG fraction.

IgG fraction from normal human sera (Cohn Fraction II) also produced a precipitate. This precipitate, however, did not dissolve well on heating at 37°C. The reason for this may be that the isolated IgG cannot be regarded as completely undenatured, the native state being essential for the reversibility of the phenomenon.

In vitro culture studies of bone marrow and parotid tumour were performed on the patient Ge. For the culture fluid of the bone marrow the autoradiographs of the electrophoretic patterns on agar or cellulose acetate showed a diffuse labelling in the γ region but no homogeneous band. Clearly and uniformly labelled IgG, IgA and IgM lines were visible on the autoradiograph of the immunoelectrophoretic pattern. For the tumour the autoradiograph of the cellulose acetate membrane showed a weakly labelled homogeneous band in the γ-globulin region. The autoradiograph of the immunoelectrophoresis against anti-human sera showed a radioactive precipitate at the cathodic side of the antigen well, caused by difficult penetration of protein, and a labelled IgM line. The fast moving part of this IgM line near the antigen well, was strongly labelled and the slow moving part weakly labelled (Fig. 7). The IgG line was also weakly labelled but no labelling of the IgA line could be

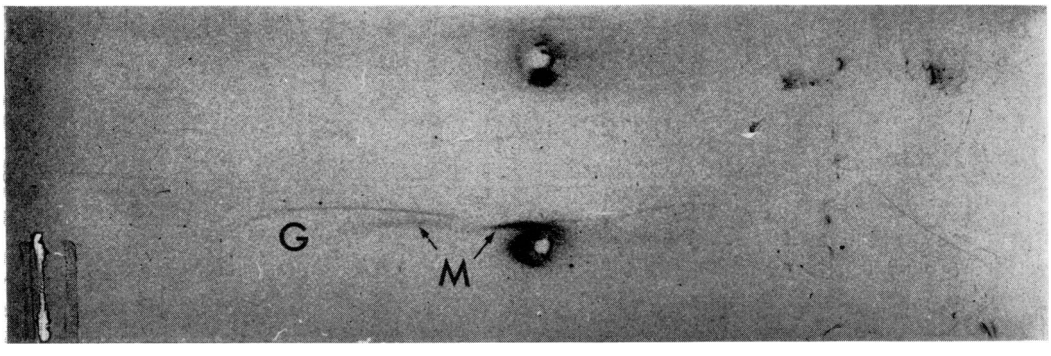

FIG. 7. Autoradiograph of an immunoelectrophoresis of a culture of parotid tumour tissue of patient Ge. In the trough anti-total human serum. Cathode to the left.

found even with anti-IgA serum. When anti-κ and anti-λ sera were used with the IgM-κ fraction (F I) added to the culture as a carrier, a strongly labelled precipitation arc was observed with the anti-κ serum, but only a very weakly labelled line with the anti-λ serum. The strongly labelled arc corresponded in size and position with the strongly labelled IgM arc of Fig. 7, while the faintly labelled line with anti-λ serum may indicate a small synthesis of heterogeneous IgM, represented by the slow moving part of the IgM line of Fig. 7. The identity of the strongly labelled IgM line with the paraprotein follows from the difficult penetration and the identical light chain type of both. Synthesis of IgM-L paraprotein can be excluded because this would have resulted in strong labelling with anti-λ serum even without a carrier. These observations therefore indicate that the tumour tissue synthesized IgM, at least part of which had a paraprotein character.

DISCUSSION

The IgM–IgG cryoglobulins described in this article, like those known in the literature, cannot be considered as actual cryoglobulins, since the individual components lack the property of cold-induced precipitability. They represent a less soluble modification of the 22S complexes between rheumatoid factors and autologous IgG. Soluble 22S complexes are found in many latex-positive rheumatoid sera (Franklin *et al.*, 1957). The solubility of our complexes is lower and they dissociate at least partly at higher temperatures. Accord-

ing to Peetoom & van Loghem-Langereis (1965) the former property is also shown by human IgG–horse anti-IgG complexes under conditions of great antigen excess. In all these instances one might speak of immune cryoprecipitates, rather than of cryoglobulins. It is not certain whether the mixed cryoglobulins described by Christian, Hatfield & Chase (1963) and by Hanauer & Christian (1967) also belong to this particular group, since they contain α_2-macroglobulin, and are not always positive in the rheumatoid serological tests.

The rheumatoid factor properties of the IgM part appear from the classical serological tests as well as from the formation of soluble complexes in the cases Ge. and Ul. Most of the IgM–IgG complexes described in the literature react only in the latex fixation test. Two of our cryoglobulins (Ul. and St.) also gave a reaction in the Waaler–Rose test.

It is remarkable that in all mixed cryoglobulins investigated by us, the IgM part was a paraprotein contrary to most of the cases described by other authors, where the IgM as well as the IgG part of the cryoglublin complexes was heterogeneous with respect to electrophoretic mobility and light chain type. The reason for this apparent selection is not clear, since in our material as well as in that described in the literature autoimmune phenomena are an important feature, although mixed cryoglobulins have also been found in infectious diseases like syphilis and mononucleosis (Wager *et al.*, 1967). It is possible that in some instances an IgM paraprotein may have escaped the attention since some of these proteins do not migrate as such in agar electrophoresis but show their homogeneous character only after reductive cleavage.

The four IgM globulins of our cases can therefore be described as rheumatoid factors with a paraprotein character, an exceptional property for such factors which usually show the same heterogeneity as the classical antibodies, although some exceptions to this general rule are known (Kritzmann *et al.*, 1961; Franklin & Fudenberg, 1964). The IgM part of the cryoprecipitates described here bears a certain resemblance to the cold agglutinins, since they too are paraproteins, which react with a substrate preferentially at low temperature.

Although the paraproteins have always been regarded as functionless immunoglobulins, several phenomena have been described in recent years which seem to bridge the gap between the classical paraproteins resulting from malignant processes and the classical heterogeneous antibodies. Some myeloma proteins have been found to react with bacterial antigens (Mansa & Kjems, 1965; Zettervall *et al.*, 1966) and monoclonal immunoglobulins have frequently been found without accompanying malignancy (Waldenström, 1964; Zawadski & Edwards, 1967). On the other hand paraprotein-like antibodies have been produced by Osterland *et al.* (1966) in rabbits by classical immunization with certain streptococcal antigens. One of the authors recently studied paraprotein components in two LE sera which could be shown to have no antinuclear activity since the homogeneous fractions remained after absorption of all the ANF activity by calf thymus nucleoprotein (Klein, Schuit & Hijmans, unpublished results). The possibility that these components have another antibody activity was not excluded.

Tissue culture studies of the tumour of patient Ge. demonstrated that an IgM paraprotein was synthesized locally. The bone marrow, however, did not synthesize this protein, but only the three immunoglobulins usually found in normal bone marrow cultures (van Furth *et al.*, 1966). Although a parotid tumour is not a prerequisite for the presence of this type of cryoglobulinaemia, two of the patients had such a tumour and the *in vitro* culture experiments with one of these demonstrated that at least a part of this protein was synthesized locally. In a normal parotid gland usually the synthesis of IgA can be demonstrated

(van Furth, unpublished observations). No IgA, however, seems to be formed in the tumour culture. The immunoglobulin synthesizing cells probably have infiltrated the tumour locally. It is very probable that other lymphoid organs, for example spleen or lymph nodes, also synthesize this abnormal protein, because it seems very unlikely that the tumour alone can produce such a large quantity of IgM (about 15 mg/ml).

Although the biochemical findings are very consistent, the clinical findings are not. Still it should be noted that all four patients probably suffered from Raynaud's phenomenon and myocardial lesions, and three from glomerulonephritis. An autoimmune disease other than the cryoglobulinaemia itself appears likely in patient Ul. with a biopsy which was highly suggestive for periarteritis nodosa and signs of glomerulonephritis, and is certain in patient Ke. with a positive basal membrane fluorescence test, positive Schirmer test, xerostomia and conjunctivitis sicca, and patient St. with positive ANF test, positive basal membrane fluorescence test and haemolytic anaemia. In the case of Ke. the diagnosis of Sjögren's disease is almost certain. Whether or not these findings are part of systemic lupus erythematosus (SLE) is uncertain. The fact that the patients had glomerulonephritis could be compatible with the diagnosis of SLE. Two patients also had many symptoms compatible with the diagnosis of SLE, among them a positive ANF test and a positive basal membrane fluorescence test. On the other hand patient Ge. showed no clearcut signs of autoimmune disease apart from the cryoglobulins. It is, however, of interest to note that three of the four patients reacted well on prednisone.

The purpura and the Raynaud's syndrome found in all four patients could be a consequence of the cryoglobulins, which obviously are responsible for the positive latex fixation test. The low complement level might be a consequence of diminished production as in liver cirrhosis, but there are no indications that this is the case. It appears more likely that either the complement is adsorbed onto the IgG globulin part of the cryoglobulin and/or that the complement is low as it is so often in SLE. In this connection it should be noted that Riethmüller *et al.* (1966) did not find any complement fixation by this type of cryoglobulins.

In conclusion it can be stated that three of the four patients showed signs of possible autoimmune disease other than the cryoglobulin. These findings pose a number of questions which are all hard to answer:

(1) Is the combination of the cryoglobulins and the lympho-epithelial tumour a coincidence, and if not, what is their relation?

Lymphatic malignancy has also been experimentally induced in situations analogous to autoimmune disease, i.e. graft-*versus*-host disease. Thus, although a relation between these cryoglobulins and a lympho-epithelial tumour has been demonstrated in one of these cases, the fact that two of the four patients did not suffer from such a tumour shows that this is not a necessary condition for the formation of these abnormal immunoglobulins. It will be interesting to study the site of formation in future patients.

(2) To which extent are the other clinical and laboratory findings caused by the cryoglobulins? Precipitation of these cryoglobulins is slow, even at room temperature, which makes a direct temperature effect improbable. As already has been discussed the low complement level and positive latex test can be directly ascribed to the cryoglobulins. This might be also the case for the purpura and Raynaud's syndrome. It is possible but certainly not proven that the nephritis in patients Ke. and Ul. was caused by the cryoglobulin. As mentioned before, the fact that two patients suffered from bronchitis might be a coincidence.

If it is not, it is still hard to see how the cryoglobulin would cause these symptoms (influence of cold?) The same goes for the myocardial lesions found in the patients. Or should one assume that this was caused by a vasculitis on the basis of the cryoglobulins? It is remarkable that in the cases of Meltzer *et al.* (1966) heart disease was not frequent, and chronic bronchitis was not mentioned at all.

(3) Finally, why are these special types of rheumatoid factors, here as in other cases, associated with rare forms of autoimmune disease which are difficult to classify? The unusual rheumatoid factors might be one of the expressions of an underlying fundamental abnormality of the immunological system of these patients, in contrast to the more common rheumatoid factors which in small quantities also occur in normal human sera and may be of a reactive nature (Klein *et al.*, 1966). The question why some cases of autoimmune disease form mixed cryoglobulins with a heterogeneous IgM, and others the same type of cryoprecipitate with an IgM paraprotein, also cannot be answered at present.

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