Kidney International, Vol. 16 (1979), pp. 522–530

Lymphoma, cryoglobulinemia, and renal disease

Principal Discussant: C. M. LOCKWOOD

Hammersmith Hospital, London, England

Case presentation

A 50-year-old white male was admitted to New England Medical Center Hospital (NEMCH) for evaluation of adenopathy and hypertension.

The patient was in good health until 2 months earlier when he developed anterior cervical adenopathy and fatigue. Over the 2 months prior to admission he suffered from drenching night sweats and debilitating arthralgias and lost 9 kg of weight. His local physician found hypertension (blood pressure, 208/110 mm Hg), hepatosplenomegaly, and diffuse adenopathy, and referred the patient for admission to NEMCH.

On admission to the hospital, the physical examination revealed the blood pressure to be 195/102 mm Hg. There was nontender symmetrical adenopathy (submandibular, posterior cervical, axillary, and supraclavicular nodes) and hepatosplenomegaly. No hemorrhages, exudates, or other abnormalities were found on funduscopic examination.

Laboratory findings revealed the following data: hemoglobin, 10.1 g/100 ml; hematocrit, 29%; white blood cell (WBC) count, 6300/cu mm with 43% polymorphonuclear leukocytes, 53% lymphocytes, and 4% monocytes; serum creatinine, 2 mg; blood urea nitrogen, 39 mg; serum albumin, 4.8 g; total serum protein, 7 g/100 ml. Results of urinalysis revealed: protein, 3+; urine sediment, numerous red blood cells (RBC); 4 to 7 WBC and 3 to 5 hyaline casts per high power field (HPF); no RBC casts on multiple urinalyses; 24-hr urine protein excretion, 3 g. An i.v. urogram revealed the right kidney to be 14.8 cm long and the left kidney to be 15.5 cm long. The ureters were not displaced. Additional serum findings were: total hemolytic complement, 7 U (normal, 190 to 250 U); Clq, 0 mg (normal, 0.16 to 0.2 mg); C3, 0.48 mg (normal, 0.87 to 2.2 mg); C4, 0.006 mg (normal, 0.15 to 0.54 mg/ml); cold agglutinins, negative; cryoglobulins were present in large amounts in the serum but could not be quantified because of their solubility characteristics. Serum concentrations of immunoglobulins were: IgM, 19.6 mg; IgA, 0.31 mg; IgG, 5.7 mg/ml. Protein electrophoresis and immunoelectrophoresis of serum warmed to 37° C showed an IgM kappa monoclonal spike that was almost entirely removed by cryoprecipitation; free kappa light chains were found in the urine. An IgM kappa monoclonal protein was present in the cryoprecipitate along with traces of IgG and IgM, however, precise characterization was not possible; no rheumatoid factor activity was detectable in the cryoprecipitate. Assays for antinuclear antibody, anti-RNA protein, and hepatitis B antigen were all negative.

A biopsy of the left axillary lymph node revealed a well-differentiated lymphocytic malignant lymphoma. T and B cell marker studies revealed: neutral red <1%; E-rosettes, 41%; surface markers for IgA, IgD, IgG, and lambda light chains <1%; IgM, 25%; kappa light chains, 22%. These surface markers on peripheral blood lymphocytes exhibit restriction of surface membrane immunoglobulins to IgM type kappa, a finding that corresponds to the monoclonal protein found in the serum. Bone marrow biopsy revealed nodular and diffuse well-differentiated lymphocytic infiltrates.

Hypertension was controlled with small doses of propranolol, and cyclophosphamide (100 mg daily) and prednisone (80 mg daily) were given. Two large volume (4000 cc) plasmaphereses were performed on successive days. Four days after the second plasmapheresis, the patient noted a marked decrease in arthralgias. One week later, the serum creatinine concentration was 1.5 mg/100 ml and the serum cryoglobulin concentration, measured on two occasions, was normal. Over the next 4 months, cyclophosphamide was continued and the prednisone dose was tapered. Follow-up studies performed at the end of that time period showed the serum protein electrophoresis to be normal and IgM and C3 concentrations had also returned to normal; however, C4 and Clq concentrations and hemolytic complement level remained markedly depressed.

One year after therapy was initiated the patient was working full time and feeling well. Serum creatinine concentration was 1.1 mg/100 ml and results of urinalysis revealed 2 to 4 RBC/HPF and no protein.

Discussion

DR. C. M. LOCKWOOD *(Research Fellow, Hammersmith Hospital, London, England):* It is a plea-

Presentation of the Forum is made possible by grants from Hoechst-Roussel Pharmaceuticals Inc., Smith Kline & French Laboratories, G. D. Searle & Co., Warner-Lambert Pharmaceutical Division, Burroughs Wellcome Company, Geigy Pharmaceuticals Inc., Ciba Pharmaceuticals Inc., and Boehringer Ingelheim Ltd.

0085-2538/79/0016-0522 $01.80

sure to be here to discuss the course of this patient with lymphocytic lymphoma, cryoglobulinemia, and presumed glomerulonephritis, in whom there proved to be novel aspects both to his disease and to the mode of therapy. In this presentation, I want *first* to discuss some of the features of cryoglobulinemia; *second,* to report our experience with the use of plasma exchange in the management of immune complex nephritis and cryoglobulinemia; and *third*, to examine the features of the cryoglobulinemia and glomerulonephritis in this patient.

Classification of cryoglobulins. The term cryoglobulin was introduced in 1947 by Lerner, Barnum, and Watson to describe a group of serum proteins that showed reversible precipitation in the cold [1]. Today we know that, apart from cryofibrinogen and C-reactive protein-albumin complexes, most cryoproteins are immunoglobulins in one form or another and that any of the major classes—IgG, IgA, or IgM—may take part in cryoglobulin formation. Cryoglobulins are classified in the following way: *type 1* is a single monoclonal immunoglobulin; *type 2* are mixed cryoglobulins, consisting of two or more immunoglobulins, one of which is monoclonal; *type 3* is polyclonal, consisting of one or more polyclonal immunoglobulins [2].

Isolation of cryoglobulins and identification of diseases associated with cryoglobulinemia. The optimal method for cryoglobulin isolation necessitates use of warm instruments for the collection of blood, and of prewarmed containers for the initial incubation. After exposure to cold, the serum should be observed for some time to allow for the emergence of a cryoprecipitate. Monoclonal cryoglobulins may precipitate within 24 hours at 4° C, whereas mixed cryoglobulins may take as long as 72 hours to precipitate at this temperature.

In what context do cryoglobulins occur? Relevant to the patient presented today is the presence of these proteins in hematologic malignancies and lymphoproliferative disorders. In these disorders, the monoclonal cryoglobulins are characteristic, particularly IgG cryoprecipitates with multiple myeloma, and IgM cryoprecipitates with Waldenstrom's macroglobulinemia. Cryoglobulins also occur in association with acute or chronic infections and collagen vascular diseases; most often these are mixed cryoglobulins. In 1966, Meltzer et al described mixed essential cryoglobulinemia, a syndrome consisting of purpura, arthralgia, lymphadenopathy, and hepatosplenomegaly associated with large concentrations of IgM and IgG cryoprecipitates [3]. This syndrome is of particular importance because some of the patients develop rapidly progressive glomerulonephritis (RPGN) as a consequence of the cryoglobulinemia. Cryoglobulins may also be found in patients who later develop a myeloproliferative disorder. As a final complicating matter, it should be remembered that concentrations of cryoglobulins as high as 80 μg/ml have been described in apparently normal individuals [4].

Physical properties. Although these proteins are defined by their cold insolubility, certain features seem to predispose to cold precipitation. Of these, I think protein concentration is the most important. The higher the concentration of the cryoprotein, the higher the temperature at which precipitation occurs. Stated another way, the same protein that precipitates at high temperature and high concentration, upon dilution requires a progressively lower temperature to effect a similar degree of precipitation [1, 3]. This phenomenon applies nicely to our discussion today of the efficacy of plasma exchange in reducing the concentration of circulating cryoglobulins: later I will describe one of our patients who well illustrates this therapeutic effect.

The optimal pH range for precipitation of cryoglobulins lies between pH 5.5 and pH 8, and progressively lower salt concentration produces less solubility for most cryoglobulins, but not all [3].

The mechanisms of cold precipitability of these proteins is not entirely explained. Ultracentrifugal studies indicate that two types of cryoglobulins exist, with sedimentation coefficients of 19S and 7S [2]. Various studies suggest that cryoglobulins are immunoglobulins that are not different from other immunoglobulins in molecular weight, viscosity, and diffusion coefficients. Chemical analysis either by amino acid configuration or N-terminal composition has also failed to disclose any unique structure that accounts for cryoprecipitability [5]. Studies of the chemical forces of intermolecular interactions that produce precipitation have shown these forces to be weak, and our best current hypothesis is that cryoprecipitability results from a variety of noncovalent interactions such as hydrogen bonding and hydrophobic forces.

Immunochemical characterization of cryoglobulins. In considering the immunochemical characterization of cryoglobulins, Brouet et al have reported the frequency of various types of cryoglobulins that occurred in 86 consecutive patients [6]. In Brouet's series, 50% of the cryoglobulins were polyclonal; 25% were monoclonal (IgM was more frequent than IgG); and 25% were mixed. In the last group, 19 of 22 cryoglobulins showed a monoclonal IgM component.

Studies of light chain classes show that approximately 95% of the IgM cryoglobulins have light chains of the kappa type but that this high proportion does not obtain for the IgG cryoglobulins [2, 6]. Studies of the variable region have not been extensive enough to permit understanding of the role it might play, but some data are available on heavy chain subclasses: it appears that among IgG cryoglobulins, IgG3 is the predominant subclass. This finding may provide insight for our understanding of relative solubility because this subclass has a much higher molecular weight than the other subclasses [7].

I want to mention briefly the characteristics of cryoglobulins that have rheumatoid factor activity—that is, those that test positive for rheumatoid factor. All cryoglobulins that have rheumatoid factor activity must, of course, be of the mixed variety because the rheumatoid factor is combined with IgG. For all instances in which the cryoglobulin exhibits rheumatoid factor activity, the antigen is IgG. While the noncryoglobulin rheumatoid factors have a wide spectrum of reactivity, the cryoglobulin rheumatoid factors have a more restricted spectrum, reacting most commonly with IgG 1, 2, and 4 [7-9]. The significance of this particular feature, however, is not certain.

Cryoglobulins as immune complexes. The evidence that mixed cryoglobulins may behave as immune complexes can be summarized as follows: *First,* analytical ultracentrifugation of the isolated cryoglobulin shows two distinct peaks, a 7S and a 19S [10]; *second,* many patients with mixed cryoglobulinemia show activation of the complement system, as evidenced by low levels of complement components [11]; *third,* immunofluorescence of tissue from patients with cryoglobulinemia complicated by glomerulonephritis or cutaneous vasculitis show deposits of IgG, IgM, and occasionally complement [3, 12].

Attempts to identify the antigen responsible for generation of a cryoglobulin, or to characterize the antibody specificity of the components of the cryoglobulin have not been particularly successful. In one series of patients with poststreptococcal glomerulonephritis, the cryoprecipitates were found to contain specific antigen or specific antibody, but not both [13]. A search for DNA in the cryoprecipitate of patients with systemic lupus erythematosus has shown that only a very small proportion of total DNA in the serum is contained in the cryoprecipitate, and thus it is unlikely that DNA is involved as the antigen [14]. In patients with glomerulitis and vasculitis associated with hepatitis B, however, cryoprecipitable complexes of hepatitis B antigen, specific antibody, and IgM rheumatoid factor have been isolated. The localization of these proteins in the glomeruli or in the vessels has suggested an immunopathogenetic role for the cryoprecipitates, representing the best evidence that cryoglobulins appearing in association with a specific antigen behave as immune complexes [13, 15].

Treatment. The therapeutic approach should be directed toward the condition associated with the cryoglobulinemia—for example, chemotherapy for lymphomas. Steroid and immunosuppressive agents have been effective in the management of some patients with monoclonal cryoglobulinemia [16, 17], but these agents are not often effective in the management of essential mixed cryoglobulinemia [3]. Dissociation of the cryoglobulins by reduction of the immunoglobulin disulphide bridges has been attempted using penicillamine—which also inhibits the formation of IgM cryoprecipitate—but the effect is delayed for 2 to 3 weeks after therapy is begun [18, 19].

Plasmapheresis is the newest approach to therapy of cryoglobulinemia. We have used plasma exchange to effect immediate removal of cryoglobulins in two patients with mixed cryoglobulinemia whose relevant clinical and laboratory features are shown in Table 1. All of the work I refer to here was carried out jointly with Dr. B. Pussell. Our approach was based on procedures that we developed for the management of patients with RPGN [20, 21]. In such patients we were able to remove pathogenetic antibody or immune complexes by plasma exchange and in addition we were able to demonstrate a significant effect on the splenic component of reticuloendothelial system (RES) function [22]. In patient 1 (see Table 1), successful removal of the cryoglobulins by a series of four plasma exchanges did not alter the clinical course because massive cerebral infarction, found at autopsy, probably occurred before starting plasma exchange. In patient 2, however, it was possible to measure cryoglobulin concentrations and to test RES function before and after a series of plasma exchanges; strikingly, impaired RES function improved after plasma exchange despite the continuing presence of circulating cryoglobulins, albeit at a lower level. Figure 1 shows the quantitative heat-damaged red cell scans before and after four plasma exchanges. Another feature of interest in this patient—and the one I alluded to earlier—was the alteration in the temperature required to bring about cryoprecipitation after

Table 1. Clinical and laboratory data for two patients with mixed cryoglobulinemia who underwent plasma exchange for removal of cryoglobulins at Hammersmith Hospital.

	Clinical data	Laboratory data	Response to therapy
Patient 1			
Male, age 54	Purpura and arthralgia, 4 years Fluctuating neurological signs Glomerulonephritis, 9 days Coma at presentation	*Serum studies* C3, 60%; C4, 5%[a] Rheumatoid factor positive IgM/IgG cryoglobulin (type 2) Monoclonal IgM component	Received 4 daily 4-liter plasma exchanges but neurological status deteriorated; death occurred 5 days after last exchange. Autopsy showed massive cerebral infarction and mesangiocapillary glomerulonephritis with necrotizing vasculitis. Immunofluorescence of kidney specimen showed deposits of IgM, IgG, and C3 in capillary loops.
Patient 2			
Female, age 75	Purpura, 9 months Distal neuropathy and paraesthesia, 7 months Vasculitic skin lesions with extensive ulceration, 6 months Disease unresponsive to prednisone, 50 mg/day and azathioprine, 2 mg/kg/day.	*Serum studies* C3, 50%; C4, 8% Rheumatoid factor positive IgM/IgG cryoglobulin (type 2) Monoclonal IgM component with kappa light chains. *Skin biopsy* Immunofluorescent examination showed deposits of IgG and IgM	Received 10 4-liter plasma exchanges and cryoglobulin levels fell (see Fig. 2). Cutaneous lesions healed rapidly. At follow-up 6 months later, C3 was 98% C4 was 62%, cryoglobulins were 1.4 mg/ml, and disease was in clinical remission.

[a] Complement values are expressed as % of normal human serum.

the concentration of cryoglobulins was lowered; the temperature at which cryoprecipitation occurred was 35° C before, and 29° C after exchange (Fig. 2).

Particular features of the patient presented today. In considering the patient who is the subject of this Forum, several interesting features emerge. The abnormal M band was almost entirely removed by cryoprecipitation and did not contain rheumatoid factor activity. Precise characterization of the cryoglobulin could not be carried out, however, and

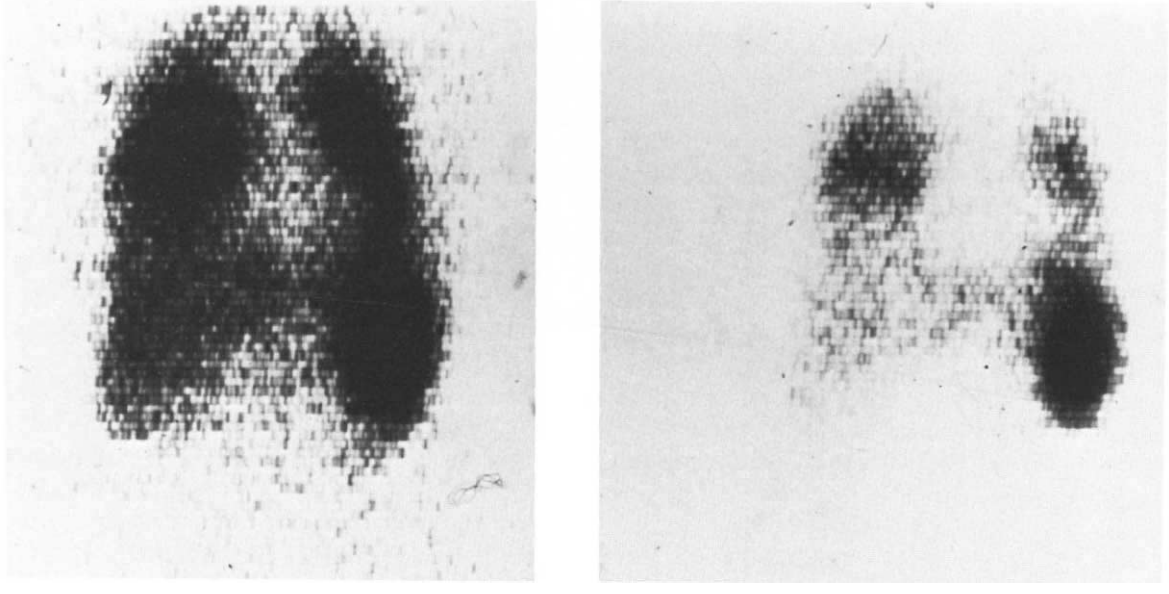

Fig. 1. *Distribution of ^{99}Tc labelled heat-damaged red blood cells in a patient with mixed cryoglobulinemia (patient 2, Table 1) before (left) and after (right) 4 plasma exchanges performed over 4 days.* The splenic uptake was 23% before exchange and 47% after: normal splenic uptake is from 50 to 60% of heat-damaged cells 1 hour after injection.

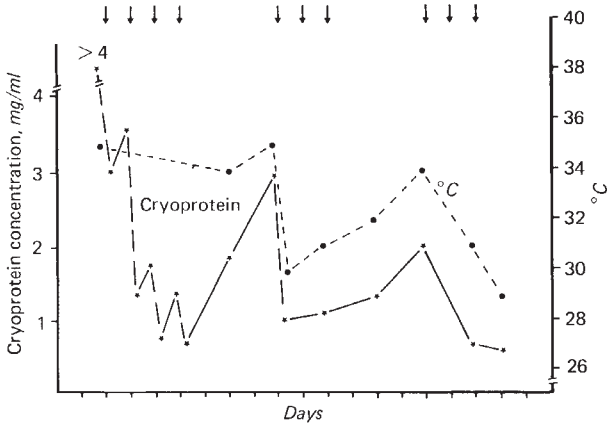

Fig. 2. *Cryoprotein concentration and temperature of precipitation of serial blood samples from a patient with mixed cryoglobulinemia (patient 2, Table 1) during 10 plasma exchanges (denoted by* arrows).

IgA and IgG were present either as contaminants of a monoclonal IgM cryoprecipitate or as trace components of a "mixed" cryoglobulin.

There is some difficulty in attempting to understand the combination of this patient's serologic findings and the renal involvement. The occurrence of glomerulonephritis in association with a cryoglobulin composed solely of a monoclonal immunoglobulin is extremely rare. There is no doubt, of course, that the complement system was activated, as shown clearly by the low levels of complement components, particularly the greatly depressed levels of C1, C2, and C4, and the lesser depression of C3. Such findings are especially characteristic of mixed cryoglobulinemia. Complement activation, however, by a monoclonal cryoglobulin alone has been described [23]. Presumably, activation occurs because aggregation of IgM at low temperature can be followed by fixation of Clq and subsequent activation of the rest of the complement system at 37° C. It is likely that the greater the concentration of the monoclonal protein, the easier the formation of the aggregate and the subsequent activation of the complement system. The type 2 cryglobulin would, however, be much more likely to be involved, and it is unfortunate that a renal biopsy was not performed: Immunofluorescent examination might have allowed comparison of the components of the cryoglobulin isolated from the serum with that deposited in the glomerulus. Histologic characterization also would have confirmed the diagnosis and allowed assessment of the degree of renal injury due possibly to the preceding hypertension. In view of findings such as the elevated serum creatinine concentration, the proteinuria, the red blood cells in the urine sediment, the presence of cryoglobulins, and the activation of the complement system, it seems likely that the renal lesion was glomerulonephritis and not another cause such as myeloma kidney or amyloidosis.

The persistence of C4 deficiency after restoration of normal levels of C3 in this patient is noteworthy because primary deficiency of complement components can be associated with the development of immune complex disease [24]. One explanation for renal involvement in these complement deficiency states is that removal of immune complexes by solubilization requires a functionally intact complement pathway [25]. As evidence in support of this hypothesis, studies now in progress in our laboratory by Drs. Pussell, Bartolotti, Peters, and myself show that many patients with immune complex disease have a defect in their ability to solubilize immune complexes in vitro and that this defect can be corrected by plasma exchange. Future investigation may shed more light on the nature of the C4 deficiency in this patient.

The patient responded to a therapeutic regimen that included two 4-liter plasma exchanges, cyclophosphamide, and prednisone by prompt loss of symptoms, sustained improvement in renal function, and virtual disappearance of the cryoglobulins. Four months later the monoclonal spike was still absent from the serum and at that time IgM levels were normal. Such control of immunoglobulin synthesis is remarkable. In the recent studies from our laboratory that I referred to before, however, we have evidence that plasma exchange plays a role in this control process. We have examined the effect of plasma exchange, cyclophosphamide, and prednisone on antiglomerular basement membrane (anti-GBM) antibody synthesis in patients with anti-GBM disease; in a group of patients matched for the severity of their disease, initial antibody titer, and duration of immunosuppression, we have shown that the half-life survival of circulating antibody was considerably shorter in those patients who received the most plasma exchanges.

Finally, I would like to return to the effect of protein concentration on cryoprecipitability. In the patient I described before, we showed that lowering the concentration of protein had a profound effect on the temperature at which precipitation occurred. It seems possible that the varying concentrations of protein found along the glomerulus and tubule may predispose toward cryoprotein precipitation. As in the patient discussed today, lowering the cryoprotein concentration abruptly by plasma exchange may lead to cessation of such precipitation. This hypothesis argues for the early use of plasma exchange in the management of patients with cryo-

globulinemia and evidence of progressive tissue damage, particularly those with progressive renal insufficiency.

Questions and Answers

DR. J. T. HARRINGTON: Dr. Agnello, would you care to comment on the quantitation and characterization of the cryoprecipitate in this patient?

DR. VINCENT AGNELLO (*Chief, Rheumatology and Clinical Immunology, NEMCH*): Unfortunately, the solubility properties of the cryoprecipitate were such that a precise quantitation and characterization was not possible; however, from the initial observations of the cryoprecipitate before washing, it was clear that large amounts of the cryoprecipitate were present. An IgM kappa monoclonal protein that did not have rheumatoid factor activity was present in the serum and cryoprecipitate. Little more can be said about the composition of the cryoprecipitate. The complement studies may, however, provide some clues as to the serological events that occurred in this patient. The changes in complement component profile during the acute and later phases suggest that during the acute phase there was activation of C3. It is conceivable that IgM kappa protein when present in very high concentrations may activate complement and produce the same sort of damage as found with immune complexes. Verroust et al have presented evidence in support of this mechanism [26]. Studies have not been done on the IgM kappa protein in this patient to determine its complement fixing activity, but such studies might resolve some of these questions.

Another possibility is that the monoclonal protein reacted with an antigen to form immune complexes during the acute phase; with the subsequent disappearance of the monoclonal protein, the immune complex formation ended. This hypothesis would explain the transient nature of what probably was a glomerulonephritic process.

DR. J. J. COHEN: I am not sure I understand. Do you mean there was an IgM monoclonal protein in the cryoprecipitate that was capable of combining with an antigen in the circulation?

DR. V. AGNELLO: Yes. I am raising that as an alternative hypothesis. The IgM kappa protein may be an antibody to an uncharacterized antigen and may form immune complexes that precipitate in the cold. This, in fact, has been demonstrated by Day et al in a patient whose course was very similar to that of this patient [27].

DR. C. M. LOCKWOOD: Other data have suggested a role for an antigen in that it has been shown that lipoprotein determinants can stimulate binding of Cl to monoclonal IgM and so activate the complement system [23].

DR. V. AGNELLO: I would not apply that hypothesis here. I think the glomerulonephritis is related to the underlying B-cell leukemia. The malignant B-cell is probably producing the monoclonal protein, as can be demonstrated in many such patients. In some instances, as in Day's patient, immune complexes involving the monoclonal antibody can be demonstrated. In that patient, the immune complexes consisted of an IgM kappa antibody directed to a lymphocyte antigen. Incidentally, the complement profile was very similar to that in the patient presented here.

DR. J. P. KASSIRER: That is an exciting and interesting integration of the pathophysiology in this patient. I take it that you could have been more confident of this hypothesis if you had been able to identify a monoclonal protein and complement components in the glomeruli during the period in which the glomerular disease was active.

DR. C. M. LOCKWOOD: Yes, it is a great drawback not to have the results of a renal biopsy, so we can only speculate.

DR. V. AGNELLO: But even without the biopsy information, I do not think there is evidence for especially nephrotoxic types of immune complexes in this patient; rather a transient high concentration of the IgM kappa antibody, as suggested, may have been involved in the renal process. The marked depression of complement in this patient is, however, a little misleading. The first and fourth components of complement were still undetectable following the disappearance of the monoclonal protein. It is likely that this patient had a lymphoproliferative disease with marked depression of Cl and C4 and probably Cl esterase inhibitor as well, similar to the patients reported by several centers [27–31]. This patient did not have angioedema but about one-half of such patients do because of the activation of the complement system.

After the monoclonal protein disappeared from the serum, evidence of the basic leukemic process was still apparent, but there was no ongoing process in the kidney. These results can probably be attributed principally to the treatment of the underlying condition. I think, however, you can make a case for some very beneficial effects of plasmapheresis: There were very high concentrations of cryoprecipitate, there was activation of C3, and a mild to moderate disturbance of renal function.

DR. C. M. LOCKWOOD: Yes, I agree with those comments. Concentration of cryoglobulin is very important in that there is reason to believe that lowering the concentration may avert the cryopre-

cipitation. It isn't necessary to get rid of the cryoglobulin entirely, but perhaps by lowering its concentration and thus changing the temperature at which cryoprecipitation occurs, it might be possible to protect target organs.

DR. DAVID J. SALANT (*University Hospital, Boston*): *First,* in the patients whom you have studied with circulating immune complexes, what evidence is there of immune complex disease? Did they have positive immunofluorescent findings for IgG and complement? *Second,* one can understand that the removal of circulating immune complexes, which are choking the RES, by plasmapheresis allows the heat-damaged red blood cells to be taken up by the system. I am not sure that I understand the discrepancy between your test of RES function improving with steroid therapy and the experimental data of Haakenstad, Case, and Mannik, which showed that steroids inhibited the RES clearance of circulating immune complexes [32].

DR. C. M. LOCKWOOD: In answer to your first question, we have more data available about the immune complex status of our patients with RPGN. Of the sera that we examined, we found that 10 out of 18 patients had Clq binding material initially, and 4 patients, 2 of whom weren't in that 10, had cryoglobulins. We had 12 biopsies available for immunofluorescent studies; 9 of those 12 showed granular deposits and 3 did not. One of those patients did not have granular deposits but had circulating immune complexes by our assay.

In answer to your second question, we do not know the effect of steroids on splenic function as judged by our tests in normal subjects; nor do we have sufficient data to analyze the effects of steroids in the short term—hours or days—in patients with immune complex disease and impaired splenic function. In some patients who have putative immune complex disease relatively long-term studies over weeks to months have shown that splenic function reverts to normal. Final analysis of the action of steroids on splenic function and immune complex clearance will probably require study in the experimental animal.

DR. D. SALANT: Have you looked for the factor or factors that impair RES function? Have you done sucrose gradient studies on the size of complexes?

DR. C. M. LOCKWOOD: We have taken the stored plasma of some of our patients in an effort to isolate Clq binding material on the sucrose gradient, but so far we have not been particularly successful.

DR. V. AGNELLO: The technical advances that have been made in the performance of plasmapheresis render small plasma exchange a maneuver of little risk. As in the patient under discussion, it wasn't absolutely clear that the immune complexes were playing a major role in the pathogenesis of glomerulonephritis, but the risk of waiting 1 to 2 weeks for cyclophosphamide and corticosteroids to have an effect was relatively great compared with the small risk of plasmapheresis. Have you found any major complications in performing multiple plasma exchanges?

DR. C. M. LOCKWOOD: Infection is the major potential complication. We are currently analyzing the incidence of infection in all of our patients who have received immunosuppressive agents—that is, those who have been given high-dose steroids and cytotoxic drugs—and included with these are patients who have had plasmapheresis. It is my feeling that plasma exchange does not carry any increased risk. A further factor to be considered is the role that splenic blockade may have in enhancing susceptibility to infection.

DR. J. J. COHEN: You have discussed the risks of the procedure and in focusing on the possible benefits you indicated that there are suggestions that cyclophosphamide or immunosuppressive agents either alone or coupled with steroids may be effective in some diseases. Whether the plasma exchange procedure adds anything to the effectiveness of the treatment is at issue. Until a truly controlled study to consider potential benefits is conducted, I'm afraid this will be a worrisome issue. Is there a plan to launch such a study?

DR. C. M. LOCKWOOD: We are currently conducting a randomized trial of patients who have severe glomerulonephritis and who fall into the putative immune complex category. One group receives prednisone and cyclophosphamide, and the other group receives prednisone, cyclophosphamide, and plasma exchange.

DR. J. J. COHEN: Whether or not there is any benefit from plasma exchange, the trial you are conducting should provide a wonderful opportunity to perform kinetic studies of the reappearance of the materials removed by the plasma exchange.

DR. C. M. LOCKWOOD: At the moment we have little data on changes of levels of immune complexes but we do have some information on the antibody-mediated diseases such as anti-GBM disease. We have studied how the anti-GBM antibody returns compared with the normal IgG. Our data show that there is very vigorous synthesis at least in some patients. We believe that the rapidity of synthesis may be important, in that there may be a

much more dynamic equilibrium between antibody fixed to the glomerular basement membrane and antibody in the circulation.

DR. J. P. KASSIRER: Dr. Berkman, can you tell us the cost of a single 4-liter plasmapheresis in this country?

DR. EUGENE BERKMAN (*Chief, Blood Bank, NEMCH*): The Blue Cross, Blue Shield billing would be approximately $1,800. The direct cost of albumin and disposable out-of-pocket expense to the hospital would be close to $800. The albumin is the major expense at $80/vial. One procedure usually requires 8 vials.

DR. J. T. HARRINGTON: Given the cost and differing views about when plasmapheresis is indicated in immune complex disease, do you have any "absolute" criteria for plasmapheresis? For instance, do you use it in all patients with anti-GBM disease?

DR. M. LOCKWOOD: Yes, we do, provided there is some residual renal function. We feel that both the recent data from our own and other's experience indicate the disease may have a very different outcome from the usually expected poor prognosis. We perform plasmapheresis on a daily basis until a satisfactory change in the serum creatinine concentration is evident. In our experience, the renal function in patients with a serum creatinine concentration below the level of 8 mg/100 ml has improved in every instance except one. By contrast, we have never been able to restore stable renal function in patients presenting with anuria or prolonged oliguria. In these patients, one indication for plasma exchange would be lung hemorrhage and our experience here is that this complication was controlled in 25 of 26 patients in whom it was a feature.

References

1. LERNER AB, BARNUM CP, WATSON CJ: Studies of cryoglobulins. The spontaneous precipitation of protein from serum at 5° C in various disease states. *Am J Med Sci* 214:410–415, 1947
2. GREY HM, KOHLER PF: Cryoimmunoglobulins. *Sem Haematol* 10:87–112, 1973
3. MELTZER M, FRANKLIN EC, ELIAS K, MCCLUSKEY RT, COOPER N: Cryoglobulinemia. A study of 29 patients. *Am J Med* 40:828–856, 1966
4. CREAM JJ: Cryoglobulins in vasculitis. *Clin Exp Immunol* 10:117–126, 1972
5. PUTNAM FW, MIYAKE A: Proteins in multiple myeloma. VI. Cryoglobulins. *Arch Biochem Biophys* 65:39–49, 1956
6. BROUET J-C, CLAUVEL, J-P, DANOU F, KLEIN M, SELIGMAN M: Biological and clinical significance of cryoglobulins. *Am J Med* 57:775–788, 1974
7. GREY HM, KOHLER PF, TERRY WD, FRANKLIN EC: Human monoclonal γG-cryoglobulins with anti-γ-globulin activity. *J Clin Invest* 47:1875–1884, 1968
8. MACKENZIE MR, GOLDBERG LS, BARNETT EV, FUDENBERG HH: Serological heterogeneity of the IgM components of mixed (monoclonal IgM-polyclonal IgG) cryoglobulins. *Clin Exp Immunol* 3:931–941, 1968
9. FRANKLIN EC, FRANGIONE B: Common structural and antigenic properties of human γM anti-γ globulins. *J Immunol* 107:1527–1534, 1971
10. LO SPALLUTO J, DORWARD B, MILLER W, JR, ZIFF M: Cryoglobulinemia based on interaction between a gamma macroglobulin and 7S gamma globulin. *Am J Med* 32:142–152, 1962
11. RIETHMULLER G, MELTZER M, FRANKLIN E, MIESCHER PA: Serum complement levels in patients with mixed (IgM-IgG) cryoglobulinemia. *Clin Exp Immunol* 1:337–339, 1966
12. CREAM JJ: Immunofluorescent studies of the skin in cryoglobulinemic vasculitis. *Brit J Derm* 841:48–53, 1971
13. MCINTOSH RM, GRISWOLD WR, CHERNACK WB, WILLIAMS G, STRAUSS J, KAUFMAN DB, KOSS MN, MCINTOSH JR, COHEN R, WEIL R: Cryoglobulins III. *Quart J Med* 174:285–307, 1975
14. HANAUER LB, CHRISTIAN CL: Studies of cryoproteins in systemic lupus erythematosus. *J Clin Invest* 46:400, 1967
15. MCINTOSH RM, KOSS MN, GOCKE DJ: The nature and incidence of cryoproteins in hepatitis B antigen positive patients. *Quart J Med* 177:23–28, 1976
16. BENGTSSON U, LARSSON O, LINDSTEDT G, SVALANDER C: Monoclonal IgG cryoglobulinemia with secondary development of glomerulonephritis and nephrotic syndrome. *Quart J Med* 175:491–503, 1975
17. PONTICELLI C, IMBASCIATI E, TARANTINO A, PIETROGRANDE M: Acute anuric glomerulonephritis in monoclonal cryoglobulinaemia. *Brit Med J* i:948, 1977
18. BARNETT EV, BLUESTONE R, CRACCHIOLO A, GOLDBERG LS, KANTOR GL, MCINTOSH RM: Cryoglobulinemia and disease. *Ann Intern Med* 73:95–107, 1970
19. GOLDBERG LS, BARNETT EV: Essential cryoglobulinaemia. Immunologic studies before and after penicillamine. *Arch Intern Med* 125:145, 1970
20. PUSSELL B, LOCKWOOD CM, SCOTT DM, PINCHING AJ, PETERS DK: Value of immune complex assays in diagnosis and management. *Lancet* ii:359–364, 1978
21. LOCKWOOD CM, PUSSELL B, WILSON CB, PETERS DK: Plasma exchange in nephritis. *Arch Nephrol* 8:383–418, 1978
22. LOCKWOOD CM, WORLLEDGE S, NICHOLAS A, COTTON C, PETERS DK: Reversal of impaired splenic function in patients with nephritis or vasculitis (or both) by plasma exchange. *New Engl J Med* 300:524–530, 1979
23. LINSCOTT WD, KANE JP: The complement system in cryoglobulinemia. *Clin Exp Immunol* 21:510–519, 1975
24. AGNELLO V: Complement deficiency states. *Medicine* (Balt.) 57:1–23, 1978
25. MILLER GW, NUSSENZWEIG V: A new complement function: solubilisation of antigen-antibody aggregates. *Proc Nat Acad Sci* 72:418–422, 1975
26. VERROUST P, MERY J, MOREL-MAROGER L, CLAUVEL J, RICHET G: Glomerular lesions in monoclonal gammopathies and mixed essential cryoglobulinemia IgG-IgM, in *Advances in Nephrology,* Vol. 1, edited by HAMBURGER J, CROSNIER J, MAXWELL MH, Chicago, Yearbook Medical Publishers, Inc. 1971, p. 161
27. DAY NK, WINFIELD JB, GEE T, WINCHESTER R, TESHIMA

H, KUNKEL HG: Evidence for immune complexes involving anti-lymphocyte antibodies associated with hypocomplementaemia in chronic lymphocytic leukemia (CLL). *Clin Exper Immunol* 26:189-195, 1976

28. CALDWELL JR, RUDDY S, SCHUR PH, AUSTEN KF: Acquired Cl Inhibitor deficiency in lymphosarcoma. *Clin Immunol Immunopathol* 1:39, 1972
29. OBERLING F, HAUPTMANN G, LAND GM, BERGERAT JP, MAYER G, BATZENSCHLAGER A, HAMMANN B, GILLETT B: Deficits acquis de l'inhibiteur de la Cl esterase au cours de syndrome lymphoides. *Nouve Presse Med* 4:2705, 1975
30. ROSENFELD SI, STAPLES PJ, LEDDY JP: Angioedema and hypocomplementemia: unusual features of lymphoma. *J All Clin Immun* 55:104, 1975
31. SCHREIBER AD, ZWEIMAN B, ATKINS P, GOLDWEIN F, PIETRA G, ATKINSON B, ABDOU NI: Acquired angioedema with lymphoproliferative disorder: association of Cl Inhibitor deficiency with cellular abnormalities. *Blood* 48:567, 1976
32. HAAKENSTAD AO, CASE JB, MANNIK M: Effect of cortisone on the disappearance kinetics and tissue localization of soluble immune complexes. *J Immunol* 114:1153-1160, 1975

The editors should like to expand the scope of these exercises by encouraging active participation of the journal's readership in *Nephrology Forum*. Questions or comments pertaining to this month's discussion may be submitted to Nephrology Forum, Box 212, New England Medical Center Hospital, 171 Harrison Avenue, Boston, Massachusetts 02111. To be eligible for publication correspondence must be received by November 30, 1979.