CONCISE REPORT

Plasmapheresis With Return of Cryoglobulin-Depleted Autologous Plasma (Cryoglobulinpheresis) in Cryoglobulinemia

By Bruce C. McLeod and Richard J. Sassetti

Three patients with cryoglobulinemia have been treated with automated plasma exchange procedures in which the replacement fluid was autologous plasma, obtained at a previous plasmapheresis and incubated in the cold to precipitate abnormal protein. All three responded with a reduction in cryoglobulin level and an improvement in clinical manifestations of cryoglobulinemia. The cost of treatment was less than that of conventional plasmapheresis, even though the quality of the replacement fluid was superior. The term "cryoglobulinpheresis" is suggested for this treatment process.

Therapeutic plasmapheresis with blood-separation instruments has been recommended in a variety of immune diseases, such as Goodpasture’s syndrome,1 thrombotic thrombocytopenic purpura,2 systemic lupus erythematosus,3 myasthenia gravis,4 and cryoglobulinemia.5 One drawback of these extensive plasma exchange protocols has been the requirement for large quantities of costly protein-containing plasma replacement solutions.

We have encountered three patients with cryoglobulinemia in whom repeated plasma exchange was an important component of therapy. In treating these patients, we used a refinement of plasmapheresis that avoids several problems with replacement fluid. The method consists of a cold incubation of plasma removed at plasmapheresis, to precipitate abnormal proteins, followed by recovery of the supernatant plasma and its use as the replacement fluid in a subsequent plasma exchange. "Cryoglobulinpheresis" (CG-pheresis) is an accurate term for the overall treatment process.

MATERIALS AND METHODS

Cryoglobulin Studies

Cryoglobulins were isolated by standard techniques. Precipitate was washed 3 times in a volume of ice-cold buffer (0.01 M phosphate, 0.15 M NaCl, pH 7.4) equal to the original serum volume and dissolved in a known volume of buffer at 37°C. Protein was quantified by the Folin or biuret method.

Cryoglobulinpheresis

Plasmapheresis has been carried out with a Haemonetics Model 30 Blood Processor. Special precautions taken to minimize cooling included the following: (1) anticoagulant and plasma replacement solutions were warmed to 37°C before use and were infused through a 37°C warming coil; (2) patients were warmed externally with an electric blanket throughout the exchange procedures. In the first exchange on each patient, plasma was replaced with a 5% albumin solution. In subsequent exchanges, the replacement fluid was cryoglobulin-depleted autologous plasma plus enough saline to maintain positive fluid balance.

For production of cryoglobulin-depleted autologous plasma, plasma was collected in increments of 300–500 ml in transfer packs having a capacity of 600 ml. These were incubated in an ice-water bath in a refrigerator. The time allowed for cryoprecipitation was 72–120 hr in case 1, and 48 hr in cases 2 and 3. At the end of this time, the bags were centrifuged at 4°C and 2500 g for 15 min (1–2 hr in the early treatments of patient 3). The supernatant plasma was then separated with a standard plasma extractor. In case 1, the supernatants were pooled and cultured prior to freezing at −20°C. In cases 2 and 3, the cold incubations and intervals between treatments were shorter, and cultures were not done.

Cost Analysis

At the time of this writing, our institution purchases fresh-frozen plasma from suppliers for approximately $100.00/liter and 5% albumin solution for $200.00/liter. Preparation of 3 liters of cryoglobulin-depleted autologous plasma may entail the following expenses: 20 plastic “transfer packs,” $20; about 1 hr technician time, $10 (maximum); one blood culture, $15 (optional). Thus, the maximum cost for 3 liters is $45, or $15/liter.

CASE REPORTS

Case 1

Patient 1 is a 43-yr-old white male who was first seen in 12/75 because of progressive purpura and arthralgia of the lower extremities that had begun to interfere with his work. Past history revealed mild hypertension controlled with methyldopa. Physical examination revealed palpable purpuric lesions involving the legs and scrotum, with brownish discoloration of the intervening skin. Both ankles were warm, swollen, and painful upon motion.

A CBC and RPR were normal. Urinalysis revealed hematuria and a few granular casts. Cryoglobulin was present in the serum at a concentration of 90 mg/dl. Further analysis of washed cryoprecipitate by immunoelectrophoresis revealed both IgA and IgG. Rheumatoid factor was not demonstrable in the serum or in the cryoprecipitate. Liver function tests and coagulation factors were normal. IgG was 16.4 mg/ml, IgA was 4.0 mg/ml (normal <3.2 mg/ml), and IgM was 1.8 mg/ml. Hemolytic complement, C3 and C4, were normal. Immunofluorescent analysis of the serum was negative for autoantibodies. A 24-hr urine collection contained 130 mg protein; creatinine clearance was 111 ml/min. A bone marrow aspirate...
revealed a slight increase in lymphocytes (23%) and plasma cells (7%). A skin biopsy showed leukocytoclastic angiitis. Renal biopsy revealed normal findings on light and immunofluorescent microscopy. The patient was considered to have IgA-IgG essential mixed cryoglobulinemia.

The patient's purpura-arthralgia syndrome was severe enough to warrant treatment; however, since no life-threatening manifestations of cryoglobulinemia were evident, the hazards of steroid or immunosuppressive drug therapy were difficult to justify. We therefore undertook cryoglobulinpheresis (CG-pheresis) as the sole therapeutic maneuver. Throughout 1976, a 2-liter plasma exchange was performed on an outpatient basis every 10–14 days. The baseline cryoglobulin level fluctuated between 80 and 130 mg/dl. CG-pheresis consistently brought about a reduction of 50%–70% in the cryoglobulin level; the level then returned toward the baseline over a period of several days (data not shown). Despite the brief reduction in cryoglobulin level, biweekly therapy gave prolonged benefits. On this treatment schedule, the patient had relief of arthralgias; outbreaks of purpura became less frequent, seldom occurring more than twice in an interval between treatments; and the rash became confined to the feet and ankles.

In 12/76, after 1 yr of therapy, the patient felt well. A complete blood count, serum protein electrophoresis, coagulation profile, and complement profile were normal. Microscopic hematuria and occasional granular casts were still seen. Urinary protein excretion was 19 mg in a 24-hr collection, and the creatinine clearance was 140 ml/min.

In order to exclude a spontaneous remission in symptoms of cryoglobulinemia, CG-pheresis was stopped in 1/77. There was gradual deterioration. After 3 mo without treatment, extensive rash was continuously present, and disabling ankle pain had recurrd. CG-pheresis was resumed in 4/77 at a rate of 2 liters every 14 days. This brought prompt control of symptoms and the patient continued to do well on this therapy.

Case 2

Patient 2 was a 65-yr-old white male who complained of painful ulcers on both legs. In 1/77 he was found to have multiple myeloma with cryoglobulinemia. Melphalan and prednisone were given elsewhere for several months, but he did not return for further treatment. He did well until 12/77, when he noted acral and scrotal purpura. Serum protein electrophoresis of a specimen processed at 37°C revealed an "M" component in the γ region that was absent in serum that had been incubated in the cold (Fig. 1). Analysis of the cryoglobulin revealed a monoclonal IgG-κ. There were lytic lesions in the skull and an excess of immature plasma cells in the marrow. Treatment with melphalan and prednisone was resumed, and there was a decrease in his paraprotein level and improvement in his purpura. In 3/78, the γ-globulin level was 2.7 g/dl.

By 4/78, the γ-globulin level had increased to 3.3 g/dl. Leg cramps, purpura, and ulceration developed and the patient was referred to this institution for plasmapheresis. Physical examination revealed extensive purpura of the legs and scrotum, and large pretribial ulcers that exuded serous fluid. Outpatient CG-pheresis was begun at a rate of 3 liters/exchange, with the results shown in Table 1 and in Fig. 1. CG-pheresis consistently produced a drop of 50%–70% in his cryoglobulin level. The protein reaccumulated between procedures, perhaps due to reequilibration with the extracellular pool, but there was a trend to lower levels. During this period of treatment, the patient's leg cramps improved. The ulcers did not heal completely, but exudation ceased. Unfortunately, he became impatient because plasmapheresis did not produce an immediate cure and declined further treatment of any kind.

Four weeks later he was returned to the hospital by his family because of fever and obtundation. His upper and lower extremities were gangrenous below the elbows and knees, respectively, and proximal portions of the arms and legs were involved with purpura and large ulcers. There was gangrene of the lips, the ear lobes, and the tip of the nose. The rectal temperature was 40°C. Antibiotics were given and an emergency plasmapheresis was performed, but he died within hours after admission.

<table>
<thead>
<tr>
<th>Table 1. Results of CG-Pheresis Case 2</th>
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<tr>
<td>Treatment date</td>
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<tr>
<td>Prepheresis cryoglobulin level (g/dl)</td>
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<td>Postpheresis cryoglobulin level (g/dl)</td>
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Fig. 1. Cellulose acetate electrophoretic patterns of serum from patient 2. (A) Serum drawn just before CG-pheresis and kept at 37°C until electrophoresis. There is an "M" spike in the gamma region. (B) Serum drawn just after CG-pheresis and kept at 37°C until electrophoresis. The size of the "M" spike is substantially decreased. (C) Serum drawn before CG-pheresis and incubated for 36 hr at 4°C. The "M" spike is absent.
**Case 3**

Patient 3 is a 47-yr-old white male who was hospitalized with complaints of exertional calf pain and paresthesias in the hands and feet. He had been treated for hypertension for 2 yr but his blood pressure had been poorly controlled and was 200/130 on admission. Physical examination showed only mottling of the skin of the legs. A CBC and platelet count were normal. Urinalysis showed proteinuria, hematuria, and numerous red cell casts. The BUN was 17 mg/dl and the creatinine was 1.4 mg/dl. Twenty-four-hour urinary protein was 1 g and the creatinine clearance was 74 ml/min. IgG was 5.1 mg/ml, IgA was 1.3 mg/ml, and IgM was 11.9 mg/ml. A test for cryoglobulins was strongly positive and high titer rheumatoid factor was present. Electrophoresis of warm-processed serum revealed an “M” component in the γ region that was absent in serum incubated overnight at 4°C. The cryoprecipitate contained monoclonal IgM with antiglobulin activity, plus polyclonal IgG. Relative serum viscosity was 1.9 at 37°C. A bone marrow aspirate revealed 60% lymphocytes, but a radiographic bone survey showed no destructive lesions. The patient was thought to have Waldenström’s macroglobulinemia with cryoglobulinemia, glomerulonephritis, and refractory hypertension. Chlorambucil was begun and hydrochlorothiazide, propranolol, and prazosin were continued.

After 2 mo of this therapy, his complaints were unchanged and his blood pressure remained elevated. Electrophoresis revealed a γ "M" component of 3.6 g/dl. He was referred to this center for plasmapheresis, where his blood pressure was 180/120. Outpatient CG-apheresis was begun at a rate of 2.5 liters/exchange. Changes in the patient’s clinical course with twice weekly CG-apheresis are summarized in Fig. 2. The cryoglobulin level dropped 50%-70% after each plasma exchange. It increased in the intervals between treatments but showed an overall downward trend. In addition, the hematocrit rose and the blood pressure decreased, indicating a decrease in plasma volume. After two exchanges, his paresthesias disappeared and his blood pressure fell to normal for the first time in 6 mo. Over the next few weeks, his leg cramps diminished, proteinuria decreased, and his urinary sediment improved. He began to have side effects from his antihypertensive medicines, and his blood pressure remained normal when they were reduced. Coagulation parameters were studied before and after a 2.5-liter CG-apheresis with the results shown in Table 2.

After 2 mo, CG-apheresis was stopped in order to assess his response to chlorambucil. Within 2 wk, the cryoglobulin level rose above 3 g/dl. Paresthesias, hematuria, and proteinuria recurred. CG-apheresis was resumed, with relief of these manifestations of cryoglobulinemia, and chemotherapy was changed to cyclophosphamide and prednisone.

**DISCUSSION**

The optimal treatment of cryoglobulinemia depends on the clinical circumstances, since a cryoglobulin may be associated with a variety of underlying disorders and with a broad spectrum of clinical findings. Plasmapheresis can be uniquely effective in achieving rapid initial control of symptoms and has been used as an adjunct in long-term therapy when other measures do not give adequate control.56

Automated blood-separator machines have made plasmapheresis more convenient. However, problems with replacement fluid have intensified. Protein deple-
tion is inevitable if replacement is limited to crystal-
loid in vigorous plasma-exchange protocols. On the
other hand, commercial colloid solutions have draw-
backs as plasma substitutes. Plasma protein fraction
and albumin solutions fail to replenish many plasma
constituents, such as coagulation factors, complement
components, and immunoglobulins. Hypogamma-
globulinemia and transient coagulopathy are seen
after large exchanges of plasma for these fluids. Fresh-
frozen plasma replaces most normal proteins, but it
carries an appreciable risk of hepatitis and of trans-
fusion reactions when long-term infusion of large
volumes is undertaken. Furthermore, all of these prod-
cts are expensive, and the supply of each is limited.

In the technique of CG-apheresis, autologous plasma
is recycled to avoid some of the shortcomings of these
plasma substitutes and to achieve a natural extension
of the concept of plasmapheresis. Selective depletion
of cold-insoluble protein permits the normal fluid,
electrolyte, and macromolecular content of the
patient’s plasma to be returned to him, as well as the
normal cellular constituents of the blood that are
recycled in a conventional plasmapheresis. This
provides a safer, more physiologic, and cheaper
replacement for the plasma removed than any of the
commercial colloid preparations. It also seems a more
satisfactory “solution” for the problems of the patient
who suffers from a single species of abnormal protein,
just as plasmapheresis is an improvement upon phle-
botomy. Some proteins, such as labile coagulation
factors, may undergo functional deterioration during
liquid storage in the cryoprecipitation phase, but the
majority of plasma constituents are preserved.

Our experience supports the expectation that cryo-
protein-depleted autologous plasma is an adequate
substitute for native plasma removed at plasmaphere-
sis. None of our patients had experienced peripheral
dema or unusual infections during the period of
treatment. Coagulation parameters remain in the
normal range after CG-apheresis, and none of our
patients reported abnormal bleeding during therapy.
None of our patients has been exposed to hepatitis or
has had a transfusion reaction.

In each of our cases, the overt signs of illness were
due primarily to the cryoglobinulin, so that plasma
exchange was an appealing treatment modality. Each
patient improved with CG-apheresis and in each case,
the contribution of CG-apheresis to improvement was
documented by recurrence of symptoms when pheresis
was stopped. Patient 1 was debilitated by his purpura-
arthritis syndrome. He responded immediately to
CG-apheresis and was managed successfully with biweekly treatment for several years. Patients 2 and 3
had B-cell neoplasms, but were free of bone pain,
fractures, cytopenias, hypercalcemia, or any other
manifestations of tumor burden per se. After a period
of response to chemotherapy, a syndrome of purpura
and distal gangrene developed in patient 2; the
progression of this syndrome was temporarily arrested
by CG-apheresis. Cessation of pheresis contributed to a
fatal outcome before an alternative drug regimen
could be tried. Patient 3 suffered from leg cramps and
paresthesias. His renal function was threatened by
paraprotein deposition and by severe hypertension.
None of his clinical problems improved in the early
phases of chemotherapy, but all showed a prompt and
gratifying response to CG-apheresis.

CG-apheresis would seem to be an attractive format
for plasma exchange in many patients with cryoglobu-
linemia whose abnormal protein is completely precip-
itable. It is an uncomplicated technique that could be
accomplished in any blood bank facility that already
does plasmapheresis. The fact that it is less expensive
than routine plasmapheresis has made us more willing
to offer it to patients with cryoglobulinemia. Indeed,
given the low cost and the high likelihood of benefit, it is
probably one of the most cost-effective forms of
plasmapheresis now available. Except for case 2, no
case acceptance has been good, even over several
years of regular treatment.

Selective removal of abnormal proteins from plasma
may become practical in other diseases in which
conventional plasmapheresis is effective. Immunoab-
sorbent columns have been used to remove anti-DNA
antibodies in systemic lupus erythematosus and
blocking factors in disseminated cancer. These
elegant devices accomplish rapid removal of the
abnormal protein in a single stage and can return
plasma to the subject immediately. Our experience
suggests that processing stored plasma for return in a
subsequent plasma exchange can be equally effective.
This “two-stage” approach can take advantage of
slower separatory processes and may require less
complicated instrumentation.

ACKNOWLEDGMENT
We wish to thank Dr. Thomas Coogan, Jr. and Dr. Robert Stein,
who referred their patients for plasmapheresis.

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