Membrane plasmapheresis with cryofiltration in rheumatoid arthritis[1]

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A technique for the on-line removal of macromolecules has been developed that involves the separation of plasma in an extracorporeal circuit by a membrane filter, its filtration in the cold through a second membrane for removal of macromolecules, and reinfusion of the treated plasma into the patient. This method was first applied in the chronic treatment of a patient with unremitting rheumatoid arthritis, and subsequently used in 5 other patients with rheumatoid arthritis. Significant improvements in clinical symptoms and biochemical factors have been noted. This technique allows the removal of macromolecules without requiring plasma replacement products when used twice weekly or at longer intervals of time.

Index terms: Arthritis, rheumatoid • Plasmapheresis, technique
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The presence of increased concentrations of macromolecular solutes in immunological disease states is well known. It is for this reason that clinicians have been prompted to apply plasma exchange in the treatment of immunological diseases. Plasma exchange has been carried out primarily by centrifugal methods. Recently, membrane separation techniques have become available.[1] A major disadvantage of plasma exchange is the requirement of plasma products for volume replacement. At present, over four million liters of plasma are collected annually in the United States.[2] If plasma exchange were shown to be beneficial in the various diseases under investigation, the supply of plasma would not be adequate. A second disadvantage of plasma exchange is that complications associated with plasma or plasma product infusion are not uncommon.

In an effort to overcome these disadvantages, the technique

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of on-line plasma treatment by cold membrane filtration was developed (cryofiltration). Preliminary reports of this technique have been published.[3-7] It is the purpose of this paper to outline the rationale for the application of this technique, give a more detailed description of the system, and summarize its clinical effects in the first patients treated.

Materials and methods

Clinical Circuit Design: The circuit design for the clinical application of the double membrane scheme with plasma cooling for the removal of macromolecules has been described.[3] In the extracorporeal circuit, plasma is separated on-line from whole blood in the plasma separator, a membrane hollow fiber cellulose acetate filter (Plasmaflo Hi-05, Asahi Medical Co., Tokyo, Japan) with 0.50 m^2 surface area. The plasma is then cooled before being filtered by the macromolecule filter (the Asahi Plasmaflo module, a membrane hollow fiber cellulose acetate filter of 0.65 m^2 surface area).

In the initial studies both the plasma cooling circuit and the macromolecule filter were cooled in an ice-water bath. The filtered plasma is united with the mainstream blood flow, and then warmed to physiological temperature before return to the patient. Priming volume of the plasma circuit is about 300 ml and that of the total system about 450 ml. Plasma flows are maximally 30 ml/min at a blood flow of 100 ml/min. Blood access consisted of veno-venous circulation, subclavian catheterization, or arteriovenous fistula, with blood flows of 50–100 ml/min. Anticoagulation consists of an initial bolus injection of 5000–7000 units of heparin followed by continuous heparin injection (2000–4000 units/hr) by an infusion pump to keep the activated clotting time at 200–300 seconds.

Patient selection: Patients were selected who demonstrated seropositive rheumatoid arthritis as indicated by elevated levels of rheumatoid factor, C1q-binding immune complexes, Westergren sedimentation rate and cryoglobulins and who were failing (or had failed) to respond to at least one major drug therapy regimen. Other criteria for inclusion in the study were evaluable joint involvement (not Class IV) with synovitis and radiographically demonstrated erosion (except in Case 1).

Case reports

Case 1. A 62-year-old white woman had had severe seropositive unremitting rheumatoid arthritis (RA) since age 15. Steroids, gold, penicillamine, cyclophosphamide and anti-inflammatory drugs had failed to control the symptoms over years of treatment. She had undergone multiple surgical procedures for deforming, disabling rheumatoid arthritis including bilateral knee replacement, left hip replacement and bilateral hand (distal interphalangeal) joint replacements. Some relief was obtained by means of conventional plasma exchange (three exchanges at 2 liters/exchange) with cell centrifugation performed eight months prior to treatment by membrane plasmapheresis with cryofiltration, but symptoms recurred a few days later. Serum immune complexes and cryoproteins measured 2296 U/ml and 3.0 mg/ml, respectively before the first treatment. At the time of the initiation of this trial, the patient was substantially wheelchair-bound, grip strength was 0, morning stiffness lasted the entire day, active synovitis was present in most of the palpable joints, and on a 0–10 subjective pain index, the patient was consistently reporting 10.

Case 2. A 61-year-old white man had a 6–7 year history of rheumatoid arthritis characterized by widely spaced acute attacks of tenosynovitis. By early 1981 he was suffering even greater restriction of activity. Given a course of penicillamine, the patient experienced a reaction including an acute polymyositis that gradually diminished after discontinuance of the drug and therapy with prednisone. Throughout this period, mild paresthesias of the toes continued and gradually led to anesthesia of each sural nerve. Increased sensory neuropathy occurred with sudden development of foot-drop bilaterally. The patient was hospitalized and a course of ten cryofiltration treatments instituted.

Case 3. A 59-year-old white woman had had severe rheumatoid arthritis since age 42. Over the years, aspirin, nonsteroidal anti-inflammatory drugs, steroids, nitrogen mustard and methotrexate had been used with no remarkable benefit. The patient's history also included multiple joint replacements in the hands and feet, and discectomy (she had received methylprednisone intravenously a few months before this treatment). With a less than favorable response to this therapy, it was decided to begin cryofiltration treatments and a left radiocephalic fistula was constructed.

Case 4. A 55-year-old white woman with seropositive active rheumatoid arthritis began to have ankle pain 28 months prior to initiation of treatment, and had to stop working. Since then she has had active synovitis of both wrists, all metacarpophalangeal joints, proximal interphalangeal joints 2-4 bilaterally, and all metatarsophalangeal joints. Nonsteroidal anti-inflammatory agents had been used with no effect.

Case 5. A 49-year-old white woman had been well until five years ago when she experienced the sudden onset of swelling and erythema of the left ankle. This subsequently progressed and involved all major joints. Eleven months after onset, the right hip was replaced and 6 months later, the left hip. Treatment has included all major therapies: gold, steroid, indomethacin (Indocin) and methotrexate with only moderate benefit. She was being maintained on 100 mg of azathioprine (Immuran) and 5 mg prednisone at the time of her first treatment.

Case 6. A 58-year-old obese white woman was given the diagnosis of rheumatoid arthritis in 1979. She was initially treated with doses of prednisone for pain in both hands, knees,

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shoulders and tarsometatarsal joints. Since then she has had intermittent flares. Inability to work and progressive deformity led her to become a candidate for cryofiltration. Beginning in January 1982, she has been undergoing cryofiltration treatments concurrent with 15 mg/day prednisone and D-penicillamine.

Table 1 summarizes the patient population and *Table 2* presents laboratory results from these 6 patients.

Biochemical and Hematological Analyses: Immune complexes were detected by C1q binding and precipitation with polyethylene glycol (PEG), as described by Zubler et al.[8] Results are expressed in binding equivalents of aggregated IgG and expressed in units/ml (10 units = ~1 μg/ml). Rheumatoid factor was determined by nephelometric assay. For cryoglobulin isolation and characterization, blood was drawn and allowed to clot at 37°C for 90 minutes. The serum was then stored at 4°C for 5 days and observed for the presence of cryoprecipitate. The cryoprecipitate was resuspended and washed in iced phosphate-buffered saline (PBS) three times, and the volume reduced to 1 ml. All calculations were adjusted for the amount of volume reduction. On the washed and concentrated cryoprecipitate, optical density measurement at 280 nm, or total protein determination by the Folin Lowry method was performed. Serum immunoglobulin levels were obtained by nephelometric assay and serum multiple analyses (SMA) and hematologic parameters were analyzed via standard automated procedures. The serum C3 was obtained by nephelometry assay, C4 by radial immunodiffusion, and CH_{50} by the Kabat-Mayer method.

Clinical Assessments: Clinical evaluations included assessments of the Ritchie index (objective articular index of pain and swelling assessed by the clinician), grip strength, 50-ft walk time, duration of morning stiffness, and subjective pain index (on a scale of 0–10, 10 being the highest pain scored by the patient).

Results

Clinical: In the first clinical application the patient was treated 3 times in the first week and maintained on a twice-weekly schedule for 10 weeks (21 treatments). Treatment was stopped in the 12th week due to surgery for replacement of the right elbow. In the second week after stopping treatment, pain levels increased and the patient was treated

Table 2. Biochemical and immunochemical changes in plasmapheresis patients

Case/ age/sex	Treatment number	Albumin (g/dl)	Fibrinogen (mg/dl)	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)	RF (RLS)	IC (U/ml)
1 62F	1	3.7	...	738	612	265	252	2296
	10	3.1	410	474	366	154	237	441
	20	3.6	...	505	444	145	208	142
	30	3.4	500	628	493	139	222	376
2 61M	1	3.3	362	769	204	110	213	104
	10	3.2	310	381	93	60	39	53
3 59F	1	3.8	390	894	309	362	144	85
	10	3.8	430	859	253	284	117	73
4 55F	1	4.4	285	648	259	231	175	135
	8	3.8	...	491	182	168	125	68
5 49F	1	3.5	465	965	163	265	180	242
	10	2.9	303	576	107	146	77	56
6 58F	1	3.5	525	729	101	136	46	58
	10	3.2	286	805	123	115	79	47

RF = rheumatoid factor; IC = immune complex.

Table 1. Changes in clinical factors in 6 rheumatoid arthritis patients

Case/age/sex	Treatment number	Ritchie index	Morning stiffness	50 ft walk times	Grip strength (L/R) (mm Hg)	Pain index	Westergren sedimentation rate (mm/hr)	Hgb (g/dl)	RBC ($\times 10^{12}$/L)	Platelet ($\times 10^{9}$/L)
1 62F	1	...	24 hr	∞	0/0	10	75–100	11.7	4.58	699
	10	...	...	...	...	5	...	9.2	3.52	626
	20	...	...	...	...	4	30–35	9.5	3.68	841
	30	...	30 min	...	...	2–3	...	9.1	3.62	840
2 61M	1	37	2 hr	∞	45/65	...	27	13.1	4.77	327
	10	4	0	∞	110/95	...	3	11.9	4.18	305
3 59F	1	22	1 3/4 hr	17	64/80	...	29	11.0	4.59	241
	10	8	0	11	88/98	...	16	9.5	4.24	322
4 55F	1	38	6 hr	20	58/28	...	26	12.8	4.14	321
	8	20	2 1/2 hr	13	195/185	...	...	11.2	3.78	357
5 49F	1	48	1 1/2 hr	33	44/34	10	83	10.9	3.68	935
	10	9	0	23	44/38	6–7	15	11.5	3.92	526
6 58F	1	34	4 hr	58	82/78	10	60	10.8	3.57	223
	10	7	1 hr	23	132/120	4	34	9.0	3.17	315

Hgb = hemoglobin (g/dl); RBC = red blood cell count.

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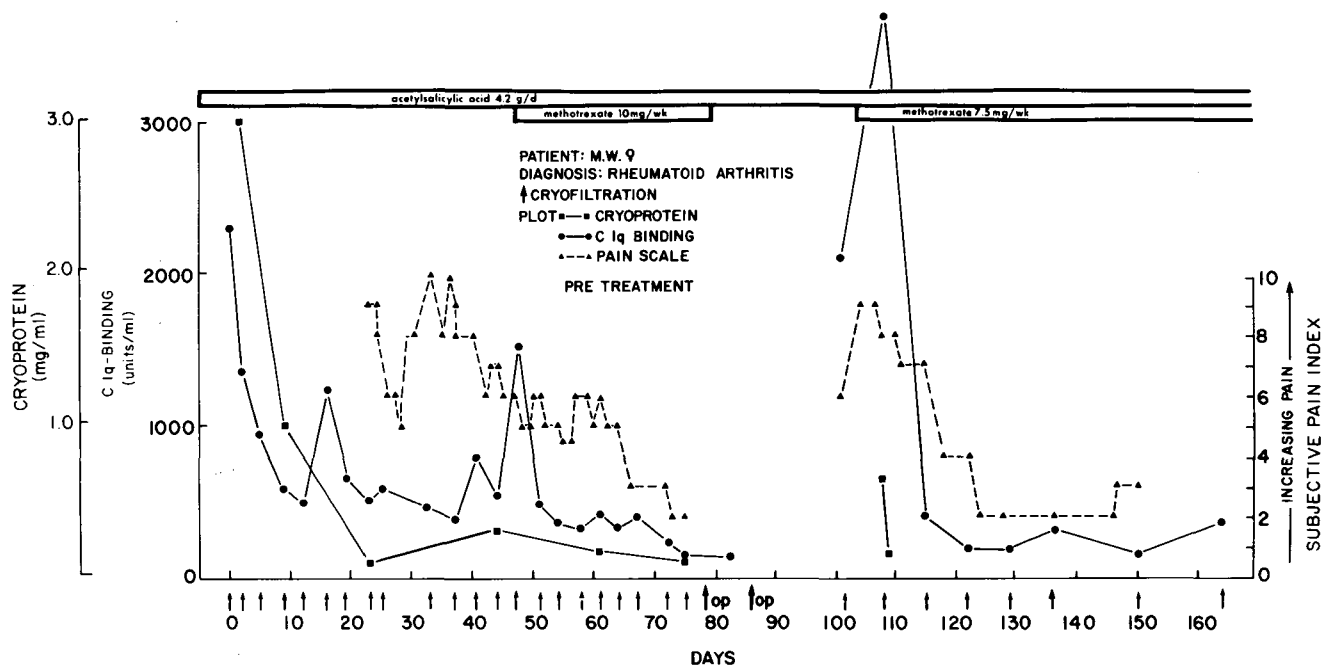
once a week for six weeks, then maintained with monthly treatments. Analgesics were taken as required. Methotrexate, 10 mg single dose per week, was begun on the seventh week of treatment (14th treatment) and given up to the 12th week, just prior to surgery. It was again started three weeks after surgery (25th treatment) at a single dose per week of 7.5 mg. Changes in serum cryoprotein and immune complex levels and also the change in subjective pain index in this patient are shown in the *Figure*. *Table 2* shows biochemical and immunological changes after 10 treatments.

After 10 cryofiltration treatments, circulating immune complex levels were reduced to 19% of the initial value and cryoprotein concentrations to 10% with use of this technique alone. Rheumatoid factor remained elevated and showed only a slight decrease (94% of initial value after 10 cryofiltration treatments) despite significant removal by the second filter as noted by sieving and extraction studies on this filter. In an attempt to further reduce the circulating immune complex level, administration of methotrexate was begun on the seventh week of treatment (14th treatment). This drug therapy and plasma treatment produced a further reduction in immune complex level to 150 U/ml. The combined regimen of cryofiltration plus methotrexate maintained the level of circulating immune complexes in the range of 10% to 15% of the initial values and the concentration of cryoprotein below 5% of the initial value. Serum immunoglobulins were decreased to 64% (IgG), 60% (IgA), and 58% (IgM) of initial values after 10 treatments, and remained in a stable normal range after that. Stopping both the methotrexate and cryofiltration treatments for surgery elevated C1q binding immune complexes, immunoglobulins and cryoprotein within three weeks. Following only two treatments, all chemistry readings returned to the presurgery levels (*Figure 2*).

Clinical measures of disease activity improved significantly. Morning stiffness decreased to less than half an hour, and the subjective pain index decreased from 10 to 2 or 3. Pain medication, such as oxycodone hydrochloride (Percodan) or Percocet, taken in daily doses of 4–6 tablets per day before the start of the procedures, was reduced gradually to an average of 1–4 tablets wk. Westergren erythrocyte sedimentation rate, which ranged from 75 to 110 mm/hr before the start of therapy decreased to 30–35 mm/hr. Although cryofiltration alone could improve the serological and clinical results, the administration of low dose methotrexate in conjunction with the treatment produced further lowering of immune complex levels and a diminished pain index. As this patient's condition had previously failed to improve with cytotoxic medication alone,



Changes in pretreatment C1q binding, immune complex level, cryoprotein and subjective pain index over a 5-month period in a patient (Case 1) with rheumatoid arthritis treated by cryofiltration.

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a synergistic effect between the filtration procedure and the drug must be considered.

Encouraged by this result, we applied the procedure to 5 other patients (*Table 1*) with rheumatoid arthritis or vasculitis. These patients were usually treated twice a week for five weeks for a total of 10 treatments, except in Case 4. In this patient, the treatments were discontinued after the eighth perfusion because of staphylococcus sepsis from the subclavian catheter site. All patients reported diminishing morning stiffness and in 3 of 5 patients, morning stiffness was completely gone after 10 treatments (*Table 1*). A dramatic improvement in the Ritchie index was noted in all cases and the subjective pain index also decreased substantially. Improvement in grip strength was noted in all patients except one (Case 5) who had severely deformed hands. Biochemical and immunochemical factors were closely followed (*Table 2*); circulating immune complex levels (IC) were reduced significantly in all patients. In patient 1, it was reduced to 19% after 10 treatments and to 6% after combined use with methotrexate. In the other 5 patients, IC levels were not so high initially but were reduced substantially after treatments (mean IC level was 52% of the initial value after 10 treatments), or fell within the normal range. Rheumatoid factor (RF), a form of immune complex in patients with rheumatoid arthritis, decreased in patients 2 and 5, but not in patients 1 and 6. After 10 perfusions, IgG, IgA, and IgM were reduced to 76%, 74%, and 67% of initial values, respectively.

Hematologic studies did not indicate any significant drops in platelet and red blood cell counts as a result of therapy. In patient 5, however, two units of packed red cells were infused after the eighth treatment. Hemoglobin levels decreased in most patients over the course of the first 10 treatments because of the large volumes of blood samples taken (600–700 ml) over the course of the first 10 treatments. In patient 1 who was treated for longer periods, the hemoglobin level after 30 treatments (six months after beginning therapy) was comparable to that after 10 treatments. Blood loss during this procedure resembled that in hemodialysis.

Electrolytes, liver function tests, BUN, and creatinine did not change significantly, even in patient 1 who has now undergone more than 70 treatments, thus indicating that the described methodology would be safe for chronic use.

Filtration Results: The macromolecule filter (cryofilter) was developed for use with blood and therefore was not optimized for this methodology. Plasma separation rates were based on the limitations imposed by this filter. In sieving studies of plasma solutes conducted throughout the course of these treatments, sieving for the plasma separator averaged 0.97, 0.97, and 0.91 for IgG, IgA, and IgM, respectively; 0.91 for rheumatoid factor and 0.96 for albumin. Sieving in the cryofilter averaged 0.70, 0.63, and 0.45 for IgG, IgA, and IgM, respectively; and 0.70 for albumin. Generally this low sieving of albumin occasionally required that albumin be infused, but for treatment schedules of twice per week or more, no albumin was required. A large variation in the sieving coefficients of the cryofilter occurred.[9] This variation is thought to be related to the plasma residence time in the cooling circuit, time of sampling after starting individual treatment, concurrent drug and anticoagulation therapy, and the macromolecule concentration in the blood. When the filter was placed at ambient temperature, the inlet pressure of the filter did not increase and sieving of immunoglobulins, rheumatoid factor and immune complexes exceeded 0.80, indicating the effect and importance of cooling. Although residence time of the plasma in the cooling circuit is short (approximately 10 minutes at 30 ml/min plasma flow), precipitation in the cooling circuit was evident in early series of patients with high cryoprotein concentrations.

A second limitation of the present cryofilter lies in the rate and total volume of plasma treated. This filter becomes plugged as the plasma is filtered, hence the flow rate is reduced from the optimum 30 ml/min. When the inlet pressure to the macromolecule filter reaches 300 mm Hg, plasma filtration is discontinued. In clinical practice, usually two filters are used consecutively and the treatment is terminated when the second filter is plugged. With two filters, 3-5 liters of plasma are treated in approximately three hours. In one treatment (Case 2), three filters were used to process 5.6 liters.

Following some treatments, the macromolecule filters were processed to collect the deposited materials. Analyses showed that various proteins including immune complexes, immunoglobulins, C3, C4, rheumatoid factor, albumin, and fibrinogen are contained in this material.

Discussion

Plasma exchange, by its nonspecific removal of plasma factors, is useful in reducing the levels of macromolecules like immune complexes or cryoglobulins. This technique, however, has limitations in chronic applications due to the losses of essential

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plasma substitutes[1]. An additional disadvantage is limited removal related to the volume of exchange. Processing more than two liters is costly because of the added plasma product required and efficiency is only marginally improved by the dilution of the plasma pool. Further, with increased volume of exchange, losses of plasma solutes are increased.

A preferred system of treatment would be to remove only the specific plasma factor(s) that may be associated with the disease state, e.g., by specific sorbents.[10] In many of the immunological disease states, multiple biochemical abnormalities exist, and because of the different chemical natures of these plasma solutes, multiple sorbent systems may be required. Usually, the cause of the disease is unknown, making identification of the pathologic substance even more difficult.

Plasma exchange, by its nonspecific removal of macromolecules, has been shown to be effective in various clinical applications. Developments to make this form of solute removal more clinically useful without the deterrents of the present methodologies would be desirable. In most immunologically related disease states, the abnormal plasma factors are of approximately 100,000 molecular weight or more, hence the application of membrane filtration is suggested.[3]

The scheme of operation is to separate the plasma containing the macromolecule(s) of interest by a membrane process and then pass this plasma through a second membrane of lower porosity, which would reject the macromolecules and allow passage of albumin and lower molecular weight solutes back to the patient. The use of a membrane filtration step to separate the plasma from the blood is preferred over a centrifugal process. Blood cell losses, particularly platelets, are significantly less with the membrane process. The advantage of this double filtration scheme is that there are only minimal or no requirements for plasma products, which also eliminates or reduces the hazards associated with their infusion. In addition, treatment times can be extended to process larger plasma volumes on a regular basis, since the removal of blood cells and low molecular weight plasma solutes is minimized.

Membranes that could pass albumin yet retain all molecules of a size greater than 100,000 could theoretically be selected. In practice, however, most membranes available or producible for medical use do not have such uniformity of pore size.

In in vitro investigations with available membranes of varying nominal pore size and human plasma collected by centrifugal plasmapheresis containing elevated levels of C1q binding immune complexes, complete sieving of low molecular weight solutes was achieved with varying rejection rates of high molecular weight solutes such as albumin and immune complexes.[11] With membranes of nominal pore size of 0.1μ through which greater than 95% albumin sieving was achieved, generally less than 30% rejection of immune complexes was possible. Although efficient rejection of macromolecules and passage of albumin depend to a large degree on the properties of the membrane, the available surface area and the concentration of the macromolecule are also important.

An important consideration in the selection of the membrane type is the requirement of the clinical situation. If the plasma solute to be removed has a size much greater than albumin's, the pore size of the membrane required may be closer to the size of the plasma solute than to that of albumin. Thus, a degree of selectivity in solute removal may be possible. The high incidence of cryoglobulins associated with immunological diseases, and the presence of pathologically related solutes in the cryoprecipitates suggest temperature as another variable in the filtration process.[12-14] Theoretically, temperature can provide an even more specific means of removing the macromolecules, thereby creating a more selective mode of removal compared to plasma exchange and the double filtration scheme alone.

Recently, a method of plasmapheresis with return of cryoglobulin-depleted autologous plasma in cryoglobulinemia was described.[15] However, recovery of the plasma requires complicated laboratory methods and care to prevent possible contamination. In evaluation of human plasma collected by centrifugal plasmapheresis containing elevated concentrations of cryoprecipitable material, the on-line cooling of the plasma and its filtration through the Asahi cellulose acetate hollow fiber filter allowed over 90% sieving of albumin with a cryoprotein reduction to less than 10% in a single pass.[3] In cryofiltration, the separated plasma is returned to the patient at once, hence the treatment time can be extended to process larger plasma volumes and the potential for contamination is reduced. Procedurally, this methodology is comparable to other extracorporeal treatments such as hemodialysis or centrifugal plasmapheresis.

In the first clinical application, a patient suffering from unremitting rheumatoid arthritis in whom administration of steroids and cytotoxic drugs had failed to control the disease has now undergone more than 70 treatments. Both immunologic and clinical signs of disease were greatly diminished;

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circulating immune complex levels and cryoprotein concentrations were reduced by this treatment alone. A further decrease was gained by the additional administration of cytotoxic drugs. No significant changes of pretreatment rheumatoid factor levels were noted in this patient despite a 30% rejection by the second filter. Similar clinical results were reported with use of plasma exchange or lymphoplasmapheresis[16] in patients suffering from rheumatoid arthritis in whom improvement was achieved without a decrease of rheumatoid factor levels. This phenomenon was also evident in 3 other patients in whom the RF level, despite substantial removel by the cryofilter, has been shown to be unchanged after treatment. Interestingly, RF did decrease in 2 other patients (Cases 2 and 5) after therapy. The explanation for these different responses of RF cannot be given at this time. The different production rate of RF in each patient might be one explanation for this phenomenon. Different forms of bound RF (IgG-RF or IgM-RF) might be another. In over two years of study, the first patient has shown no signs of adverse physiological reactions. The clinical improvement correlated with the decrease of immune complex levels and cryoglobulin concentration.

It is most likely that the rapid, sustained decrease in circulating immune complexes as measured by C1q binding and cryoprotein and the accompanying, though slower improvement in clinical factors of disease activity resulted from therapy. In this patient, who had severe seropositive rheumatoid arthritis for nearly 50 years and who gained no relief from all conventional and several experimental modes of therapy, the likelihood that spontaneous remission would have occurred at this time is negligible. In the 5 additional patients treated, reductions of C1q binding immune complex levels were achieved in each case with clinical improvement.

Based upon the sieving properties of the filters and the plasma volume of the patient, the percentage reduction in immune complexes was higher than the expected calculations.[4] This suggests changes in the body's clearing and production rates for these solutes. Studies have shown that plasma exchange causes reversal of splenic blockage of reticuloendothelial function and that there is an inverse correlation between splenic function and the level of C1q binding material.[17]

The on-line precipitation in the cooling circuit observed in the early treatments in part related to the high concentration of cryoglobulins in the plasma. Although on-line precipitation is not a requirement for macromolecule removal with this technique, precipitation has been reported to occur immediately[12] and may not in certain cases require long cooling periods.

A further point of discussion with regard to cooling is its effect on sieving. With the Plasmaflo device, previous studies have shown that complete albumin sieving occurs when particle-free solutions are filtered at 37°C.[18] Therefore, rejection of albumin by the cryofilter may be related to the presence of the higher molecular weight substances, possibly generated in the circuit. In studies of the filtered proteins collected by resuspension and reprecipitation, albumin is present in the precipitate. It is therefore most probable that in the cooling and filtration process, albumin is removed from the solution nonspecifically by entrapment among the macromolecules. Protein-heparin complexes may also be formed and affect sieving.

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