Leucapheresis in severe rheumatoid arthritis

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SUMMARY Two patients with severe seropositive rheumatoid arthritis previously unresponsive to conventional therapy have been treated with leucapheresis. This technique involves continuous cell separation daily to remove primarily lymphocytes. Clinical improvement was recorded with the use of standard rheumatological measures of inflammation. It is concluded that leucapheresis may help in the management of severely active rheumatoid arthritis when conventional therapy has been unsuccessful.

Both humoral and cell mediated immune mechanisms have been implicated in the pathogenesis of rheumatoid synovitis (Yu and Peter, 1974). Therapeutic response to immunosuppressive drugs such as cyclophosphamide (Cooperating Clinics Committee, 1970) and azathioprine (Urowitz, *et al.*, 1973) and to immunostimulants such as levamisole (Veys and Mielants, 1977) may support this hypothesis. Thoracic duct drainage has also been successful in the treatment of 12 patients with severe rheumatoid arthritis (Pearson *et al.*, 1975). We have treated 2 patients with rheumatoid arthritis unresponsive to conventional therapy with external lymphocyte depletion using a continuous cell separator (leucapheresis).

Patients and methods

Case 1 was a 63-year-old Chinese woman with severe, seropositive rheumatoid arthritis of five years' duration. She had been repeatedly admitted to hospital and treated with salicylates, prednisone, gold, chloroquine, azathioprine, and penicillamine. Despite this therapy her disease followed a relentless course, with continuous activity and progressive deformity.

Case 2 was a 41-year-old man with seropositive, nodular rheumatoid arthritis. He had had continuously active disease despite treatment in hospital and intra-articular steroid and gold therapy.

Both patients were admitted to hospital for a 2-week control period during which medications

were stabilised. Case 1 was receiving 3.6 g salicylate and 7.5 mg prednisone daily, while case 2 was receiving 4.8 g salicylate daily. These medications were then kept constant throughout the study.

LEUCAPHERESIS PROCEDURE

After obtaining the patients' informed consent leucapheresis was performed with the IBM blood cell separator, model 2990 (International Business Machines, Inc., Endicott, New York) as described previously (Curtis *et al.*, 1972). From a Teflon cannula in an antecubital vein blood entered the centrifuge. Continuous flow of blood through the centrifuge was maintained at a rate of 50-75 ml per minute. The centrifuge was calibrated to remove leucocytes, primarily lymphocytes (900 rpm). The entire volume of blood depleted of leucocytes was returned to the patient through a cannula inserted in the opposite arm.

In case 1, 19 daily treatments were performed over 6 1/2 weeks. In case 2, 18 daily treatments were performed over 3 1/2 weeks. Each day's treatment was carried out over 3-4 hours. Clinical and laboratory assessments were performed before, on 3 occasions during, and 3 to 7 days after leucapheresis.

CLINICAL ASSESSMENT

The clinical response was assessed by measurement of articular index (Lansbury, 1958), total active joint count, count of synovial effusions, grip strength (Savage, 1966), and 50 foot walking time.

LABORATORY METHODS

Routine tests. Routine laboratory tests included haemoglobin measurement, leucocyte count, and differential and platelet count, erythrocyte

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sedimentation rate, (ESR) (Westergren), serum protein electrophoresis, urine analysis, SGOT, alkaline phosphatase, electrolytes, and serum, calcium, and phosphorus.

Serological methods. Tests included latex fixation test for rheumatoid factor (Singer and Plotz, 1956), lupus erythematosus cell preparation (Hargraves, 1954), ANA by an immunofluorescent test using rat liver or mouse kidney (Friou and Quismorio, 1975), DNA binding by the Farr technique (Carr *et al.*, 1975), and complement studies (total haemolytic complement (Mayer, 1961), and C3 using Immunoplates (Kent Laboratories)). Immunoglobulin levels were also measured by means of immunodiffusion plates (Hyland Laboratories, IgG and IgM; Kallestad Laboratories, IgA).

Immunological methods. Cell mediated immunity was assessed *in vivo* by skin testing (candida, PPD, SK/SD, and trichophyton). T cells were identified by their capacity to form E rosettes (Jondal *et al.*, 1972). B cells were identified by the presence of complement receptors (EAC rosettes) (Ross *et al.*, 1973) and immunoglobulins on their surface (Clements *et al.*, 1974).

The proliferative capacity of lymphocytes was assessed by mitogen stimulation using phytohaemagglutinin (PHA), concanavalin (Con A), and pokeweed mitogen (PWM) in the following way.

Venous blood was collected with phenol-free heparin, and the mononuclear cells were separated by Ficoll-Hypaque density centrifugation. Triplicate 200 μ l cultures containing 5×10^4 cells were set up in flat-bottomed Linbro microtitre wells with 25% pooled human AB serum in RPMI medium (Grand Island Biological Co.). Mitogens (50 μ l per culture) were added over a wide dose range in the following dilutions: PHA (1:10, 1:15, 1:150) (Wellcome Reagents Ltd., England), Con A (1:1.25, 1:2.5, 1:25) (Calbiochem, USA), PWM (1:2.5, 1:25, 1:250) (Gibco-Biocult, USA). Tritiated thymidine (0.2 μ Ci per culture, specific activity 18.4 Ci per mmol) was added 4 hours prior to harvest. Thymidine uptake was determined by liquid scintillation counting and the results expressed as the mean counts per minute (cpm) from the replicate cultures. The data shown (Table 2) represent the maximum cpm obtained over the dose ranges indicated for each mitogen.

Humoral immunity in case 1 was assessed by the response to antigen challenge with salmonella.

Results

Separation of lymphocytes on a daily basis resulted

in an objective improvement during therapy in both patients (Fig. 1).

Case 1 showed improvement within 2 weeks, with maintenance of this response throughout leucapheresis therapy. Walking time (74 s to 36 s), active joint count (43 to 31), and articular index (123 to 80) all improved by the end of therapy, and this improvement persisted afterwards. Morning stiffness (2–3 hours) and grip strength (average 80) were unaltered, and extra-articular features of rheumatoid disease were not present. The patient reported a marked improvement in the degree of pain and in general wellbeing early in the course of therapy.

Case 2 showed similar improvement. Active joint count (32 to 12), articular index (130 to 72), and synovial effusion count (4 to 1) decreased by the end of therapy. Slight improvement was also documented in walking time (8 s to 6 s), grip strength (250 to 320), and in morning stiffness (45 min to 15 min). A rheumatoid nodule decreased significantly in size (from 20×14 mm to 5×4 mm) by completion of therapy.

Laboratory tests (Table 1) showed a fall in haemoglobin in both patients during leucapheresis, and in case 1 this necessitated transfusion on 3 occasions. Other routine tests were not altered. The ESR fell in case 1 but rose slightly in case 2.

Rheumatoid factor showed a decrease in case 1 (1:1280 to 1:320) but no change in case 2. Immunoglobulin levels showed a slight rise in case 1 and a slight fall in case 2 with a return to pre-leucapheresis levels at the end of therapy. Complement levels remained stable and tests for ANA, LE cells, and DNA binding were persistently negative in both patients.

Immunological studies (Table 2) showed a decrease in total lymphocyte count at the end of leucapheresis. Although the total number of T and B cells fell, the relative proportions were not altered.

Table 1 Laboratory studies

	Case 1			Case 2		
	Before	During	After	Before	During	After
Haemoglobin (g/100 ml)	12.7	11.0	9.6	15.1	13.5	12.6
WBC	6100	6700	8800	7200	7400	6400
Platelets	358 000	419 000	382 000	350 000	400 000	350 000
ESR	95	107	75	5	18	15
Rheumatoid factor	1:1280	1:640	1:320	1:320	1:160	1:320
IgG (mg/100 ml)	1200	1875	1200	715	585	610
IgA (mg/100 ml)	420	600	420	160	135	176
IgM (mg/100 ml)	212	415	150	50.5	50.5	44

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An initial low responsiveness to concanavalin A increased after treatment. In both cases PHA responsiveness remained unchanged during therapy. A fall from initially normal levels was seen in both patients with pokeweed mitogen.

Skin testing with a variety of antigens demonstrated preservation of response after leucapheresis. In addition, case 1 was able to mount a humoral response when challenged with salmonella antigen before and after therapy.

Daily leucapheresis in case 1 of a mean volume of 7.1 litres of blood resulted in removal of approximately 1.3×10^9 lymphocytes daily, and in case 2 leucapheresis of a mean volume of 7.3 litres of blood yielded a mean of 2.03×10^9 lymphocytes daily.

Lymphocytes removed from the centrifuge were examined at intervals. In case 1, 53–76% of lymphocytes removed were T cells and 21–35% were B cells approximating their percentage in the circulation. Similarly, case 2 66–83% were T cells and 11–33% were B cells. The lymphocyte yield was characteristically higher at the beginning of a week, with a trend toward a lower yield as treatment continued throughout the week.

It is interesting that in case 1 during a holiday period when no leucapheresis was carried out the joint count increased and the 50 ft (15 m) walking time plateaued (see Fig. 1). Reinstitution of leucapheresis therapy seemed to recapture the previous response. In case 1 the observed improvement was

Table 2 Immunological studies

	Case 1			Case 2		
	Before	During	After	Before	During	After
Lymphocyte number	1131	1118	581	1533	1044	798
T cells						
E rosettes						
Number	814	894	465	1058	752	485
%	72	80	80	69	72	61
B cells						
EAC rosettes						
Number	362	280	145	307	209	152
%	32	25	25	20	20	19
Surface Ig						
Number	192	290	151	138	84	72
%	17	26	26	9	8	9
Mitogens						
Con A	4400	7944	7210	11 126	14 833	31 959
PHA	13 890	15 916	11 797	34 586	45 099	36 059
PWM	21 137	10 196	15 958	40 890	33 886	28 915

maintained for approximately 3 months. Over the next 4 months the degree of disease activity gradually returned to its previous severe level. A second course of 24 leucapheresis treatments over a 6-week period met with similar success. The patient was then started on cyclophosphamide (100 mg daily) to maintain this level of improvement.

Case 2 was put on azathioprine (150 mg daily) at the completion of leucapheresis and has maintained an excellent response for 4 months and returned to full time employment.

Side effects from leucapheresis included minor intermittent dizziness and perioral tingling. The decrease in haemoglobin seen in both patients required transfusions in case 1 on 3 occasions. Other previously described complications (Oon and Hobbs, 1975) were not seen in our patients.

Discussion

Some patients with severe rheumatoid arthritis fail to respond to all conventional therapy including cytotoxic drugs. Thoracic duct drainage resulted in improvement in 12 such patients, suggesting that external removal of lymphocytes can result in a remission of symptoms (Pearson *et al.*, 1975). However, thoracic duct drainage is a major surgical procedure fraught with many potential complications.

External leucocyte depletion has been accomplished by leucapheresis in a small number of disease states. Large numbers of eosinophils have been removed in a patient with a hypereosinophilic syndrome (Ellman *et al.*, 1974). In the Sézary Syndrome, a T cell malignancy involving both the circulation and the skin, leucapheresis resulted in a decrease in circulating lymphocytes and in a depletion of lymphocytes from tissue infiltrates (Edelson *et al.*, 1974).

We have used this technique in the management of 2 patients with severe rheumatoid arthritis, successfully removing a large number of lymphocytes daily ($0.5-3.9 \times 10^9$). In the first case a modest clinical improvement and in the second case a marked improvement was documented. This was associated with a decrease in total lymphocyte count, though proportions of T and B cells remained normal. Furthermore, a subnormal response of lymphocytes to concanavalin A improved in both patients during leucapheresis therapy. In 1 patient a rheumatoid nodule was significantly reduced in size during therapy. A trend to a decrease in rheumatoid factor was seen in 1 patient. Other tests of cell mediated immunity and humoral immunity were not altered.

Both patients have been started on cytotoxic agents and have maintained their level of clinical improvement.

In consequence of the clinical improvement attained in these 2 patients a larger therapeutic trial in patients unresponsive to conventional therapy seems warranted.

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