Output of peritoneal cells during peritoneal dialysis

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SUMMARY Peritoneal dialysis provides a good source for the collection of macrophages. Six patients with chronic renal failure undergoing peritoneal dialysis for the first time were studied, and maximum cell egress, mostly macrophages, occurred at 24-48 hours and diminished after 48 hours.

Collection of monocytes from peripheral blood is a cumbersome procedure and the yield is low. Human macrophages, together with other white cells, were obtained in large quantities from peritoneal dialysis fluid of patients undergoing dialysis for renal failure and were used therapeutically to combat infections in patients with uncontrolled leukaemia, who were no longer responding to antibiotics. The transfusion of peritoneal cells had no obvious side effects (Fakhri *et al.*, 1976a), and earlier *in vitro* experiments (Fakhri *et al.*, 1976b) suggested that macrophages lack the immunogens that generate the mixed lymphocyte reaction (lymphocyte-determined LD antigens). Furthermore, macrophages ($T\frac{1}{2}$ = 71 hours; Whitelaw, 1972) can enjoy much longer life than neutrophils ($T\frac{1}{2}$ = 6 hours) and might thereby be more beneficial. The number of lymphocytes and other white cells present with macrophages in the dose of peritoneal cells given was initially too small to cause any side effects, but such cells should be first irradiated with 1500 rads to prevent any subsequent risk of graft-versus-host disease in immunologically depressed recipients.

The number of peritoneal cells and the percentage of macrophages in them varied with time after dialysis. In this paper we present a study of the peritoneal cells removed by dialysis. Such information may be a useful guideline for those intending to use the macrophages therapeutically, or as a substitute for white cell transfusions, especially in centres where leucopheresis units are not available.

Material and methods

Patients with chronic renal failure, who required

peritoneal dialysis as part of their medical management, were chosen for this study. Those with acute superadded problems, for example, altered level of consciousness, infection, or other complications necessitating prolonged intensive dialysis, were excluded. The data reported in this study were collected from six patients whose condition was stable enough to allow electively timed dialysis and predialysis investigations.

The patients were dialysed on three successive days at a rate of six litres per day using standard techniques (dialysis solution A—see Appendix—was used in all six patients). The dialysate was collected in polyvinyl chloride (PVC) blood collection bags containing acid citrate dextrose (ACD). Each day's collection was then pooled into a plastic container and the total volume was measured. A representative sample of 30 ml was centrifuged in a plastic tube at 200 g for 15 minutes at 4°C. The pellet was then resuspended in 1 ml of saline and counted in a haemocytometer. The total daily output of the cells was calculated, and smears were prepared, stained with Leishman stain, and shown to be morphologically normal. Duplicate differential cell counts were performed, each counting 200 cells. Samples of the leucocytes recovered were studied by a stimulated nitro-blue tetrazolium test and by a candida-killing test (Lehrer and Cline, 1969) and found to have normal phagocytic function. A blood sample was taken from each patient for white cell count before, during, and after conclusion of dialysis. No significant change in the blood picture was noted. In a few patients dialysis and collection of cells continued on the fourth day.

Results

The Table shows a summary of the results for the

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Table Cell output during three days of peritoneal dialysis

Patient	First day				Second day				Third day			
	Total no. of cells $\times 10^6$	N	L	M	Total no. of cells $\times 10^6$	N	L	M	Total no. of cells $\times 10^6$	N	L	M
JT	125	12.5	75	37.5	1500	30	285	1185	225	54	26	145
RT	50	14	12	24	400	44	140	216	600	120	120	360
TR	60	6	36	18	400	140	40	220	150	9	36	105
FH	75	9	45	21	1050	63	231	756	125	4	30	91
LM	50	4	12	34	1500	35	200	1165	500	80	40	380
SH	450	50	266	134	2000	80	30	1620	900	135	117	648

first three days of dialysis. On the first day of dialysis the total number of cells was low and the macrophages were generally below 50%. On the second day, the total number of cells increased sharply (5-15 times on the first day) with increased numbers of macrophages. On the third day of dialysis the total number of cells dropped sharply, but the proportion of macrophages remained as high as on the second day, or was even higher. The drop in the cell output was not shown if the patients were not dialysed on the second day. Most of the cells appeared in the first 2-3 litres of the dialysis fluid, and the sixth litre of fluid contained few cells. On the fourth day of dialysis cell egress was very small (less than 50 million), and we thought it was unnecessary to continue the studies beyond the third day even though the dialysis was continued in some patients.

Discussion

The patients selected for this study were all young or middle-aged and had no problems other than chronic renal failure. It was thought they would give a high yield of cells. They were known to be negative for serum hepatitis B antigen and antibody, but if used for transfusion it would be wise also to know their status with regard to cytomegalovirus, for example, IgM antibody titre. We observed that patients who had secondary infections were shown to give a lower yield of cells, possibly due to the diversion of white cells into other sites, perhaps sites of infection. A considerable number of uraemic patients were shown to have a secondary infection (mainly of the chest and urinary tract), which could be the result of depressed leucocyte functions due to uraemia, and they were not considered for this study. Also the patients chosen were those who did not have a very high blood urea (below 50 mmol/l (300 mg/100 ml)) so that their condition did not necessitate continuous dialysis over a long period of time. They were dialysed for short periods over many days, thus allowing time for the cells to repopulate the peritoneum.

During the first day the total output of cells was low, presumably representing the cells available in the mobile phase in the peritoneum. On the second day the number of cells was increased considerably and the percentage of macrophages increased too. These cells are presumably from the stationary phase in the peritoneum as well as from the circulating blood due to peritoneal irritation.

On the third day the number of cells fell, possibly due to the exhaustion of the cells from such sources, and on the fourth day they fell even further. The exhaustion of such potentially effective macrophages could make the patient more prone to develop infection and peritonitis, so we would not encourage collection beyond two days and would encourage the return of the patient's own cells to their own peritoneal cavity when dialysis has to continue for more than four days.

The output from the second day of peritoneal dialysis would be the best choice for therapeutic purposes because of the high yield of cells and the high percentage of macrophages. The macrophages are long-lived ($T_{1/2} = 71$ hours; Whitelaw, 1972). Their effectiveness is enhanced some 200 times in the presence of antibodies (Fakhri *et al.*, 1973), and their demonstrated ability to combat infections (Fakhri *et al.*, 1976a) might suggest that they were circulating and functioning normally in the unrelated recipients.

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Appendix

FORMULA OF SOLUTION A FOR PERITONEAL DIALYSIS

Na	=	140 mEq/l (140 mmol/l)
K	=	4 mEq/l (4 mmol/l)
Ca	=	4.5 mEq/l (1.1 mmol/l)
Mg	=	1.5 mEq/l (0.6 mmol/l)
Lactate	=	45 mEq/l (5.0 mmol/l)
Chloride	=	105 mEq/l (mmol/l)
Glucose	=	1.5%

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