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The Therapeutic Use of Peritoneal Macrophages in Infected Leukaemic Patients

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Abstract—*Ten patients with acute leukaemia and infection unresponsive to antibiotics received single or multiple transfusions of peritoneal cells, mainly macrophages, obtained from donors undergoing peritoneal dialysis for renal failure. In six patients the cultures became negative and the temperature returned to normal and remained there, in two, there was temporary return of the temperature to normal and two patients showed no response. No significant side-effects were encountered. No changes in leukaemic blast cell numbers were seen in any of the patients.*

Bleeding and infection are the major causes of death in patients with bone marrow suppression. While bleeding can be controlled by platelet transfusions, infection remains a major problem as the effectiveness of antibiotics is greatly reduced in patients with granulocytopenia [1]. Attempts to reduce the impact of neutropenia by granulocyte transfusions are complicated by the need for a large number of neutrophils. Cells are usually obtained from healthy donors or from patients with chronic myelogenous leukaemia by continuous flow centrifuge or filtration leucapheresis.

The possibility that the immunogens identified in the mixed lymphocyte reaction (LD antigens), which are responsible for initiating both graft-versus-host (GVH) and host-versus-graft reactions (HVG), might not be present on macrophages led to the suggestion that macrophages might survive longer than granulocytes when transfused into LD incompatible individuals [2]. Their action might be potentiated if they are directed against target cells such as bacteria or tumour cells which have been coated with andogenous antibodies for which macrophages have Fc receptors [3].

It was then identified that there are strong differences between macrophages of peritoneal origin and those of alveolar or splenic origin. Whereas peritoneal macrophages express weak or no LD antigens [4, 5] and are rich in hydrolytic enzymes, alveolar or splenic macrophages express strong antigens but are poor in hydrolytic enzyme contents [6].

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In a preliminary study [7], peritoneal cells (mainly macrophages) were used in small numbers to combat infections in leukaemic patients not responding to antibiotics. Such cells were also used to combat infections and to induce rapid wound healing when transfused into a patient with resistant wound infection who was no longer responding to antibiotics [8].

In further studies, human peritoneal cells were transfused intravenously into normal rabbits and into rabbits previously made septicemic by injecting them with *E. coli* intravenously, while the normal group showed no signs of GVH or HVG reactions, the septicemic group were freed from the infection [9].

In this paper we report the results obtained when transfusing a larger number of peritoneal cells to infected leukaemic patients than to those transfused earlier [7].

PATIENTS AND METHODS

Donors

Patients with chronic renal failure who required peritoneal dialysis were used as donors. All patients were having peritoneal dialysis for the first time and their condition was stable enough to allow electively timed dialysis. They were all free of infection and were HBsAg negative. Each patient was dialysed for 3 successive days at a rate of six litres per day using a standard technique. On the first day, only a few cells were collected. On the second day, the number of cells released was higher (5–15 times the first day) and the percentage of macrophages was about 80–90%.

On the third, the number of cells fell [11]. The peritoneal dialysate was collected under sterile conditions in polyvinyl chloride (PVC) blood collection bags containing 70 ml ACD. The bags were then centrifuged at 1000 rpm for 10 min at 4° C. Excess fluid was removed with the aid of a plasma extractor. The cells from collecting units were then pooled into one bag, washed twice with normal saline and finally resuspended in a small volume of isotonic saline. A sample was taken for counting and cell morphology, and the final suspension was adjusted to contain 10^7 cells/ml. The number of cells required was then infused intravenously without delay at a rate of 10 ml/min. Previously, tests were made to ensure that the macrophages were not agglutinated by the recipient serum.

Recipients

Ten informed consenting recipients received cells (for children their parents gave consent). All patients had acute leukaemias as proved by the presence of high percentages of blast cells in the peripheral blood and bone marrow smears. They were all febrile (sustained temperature 38.5–40.5° C) mostly due to bacterial infections and were on proper antibiotic treatment with no response. All patients (except one patient G.K.) received chemotherapy for leukaemia prior to or simultaneously with macrophage transfusions. Table 1 summarises the status of the recipients, and their haematological data prior to macrophage transfusions.

RESULTS

Table 2 summarises the response of the patients to the transfusions of peritoneal cells. The response fell into three categories:

1. Six patients had complete and sustained normalisation of temperatures and their cultures became negative (patients 2, 4, 5, 7, 8 and 9).
2. Two patients had temporary normalisation of temperature (patients 3 and 6).
3. Two patients had little or no response (patients 1 and 10).

All patients (categories 1 and 2) responded by a rapid fall in temperature within 4–7 hr. There was no significant or noticeable change in the WBC count, (total and differential) or in the percentage of blast cells in the peripheral blood smears 1–4 days after transfusion. In patients 1–4 counts were

Table 1. *The haematological data of the patients who received macrophage transfusions*

No.	Patient	Age/sex	Diagnosis	WBC count $\times 10^9$/l (% blast)	Hb (g/100ml)	Platelets ($\times 10^3$)	Bone marrow	Chemotherapy for leukaemia
1	G.K.	40/F	ALL-R	7.0 (87%)	7.1	2	Hypercellular ↑ ↑ Blasts	None given yet
2	H.M.	30/M	AMMOL-R	4.4 (22%)	11.6	28	Hypercellular ↑ ↑ Blasts	Dau + cyto
3	B.M.	20/M	AML-R	3.1 (24%)	7.5	5	Hypercellular ↑ ↑ Blasts	Dau + cyto
4	K.S.	25/F	AML-R	1.2 (8%)	6.8	7	Hypocellular ↑ Blasts	Dau + cyto
5	J.M.	16/M	AML-R	1.0 (62%)	4.8	10	Hypocellular ↑ Blasts	Dau + cyto
6	A.J.	5/M	ALL-R	3.0 (0%)	10	50	Hypercellular ↑ Blasts	VC + pred
7	F.H.	20/F	AMMOL-R	1.5 (10%)	3.7	57	Hypocellular ↑ Blasts	Dau + cyto
8	A.A.	6/M	ALL	10.2 (49%)	8.5	15	Hypercellular ↑ ↑ Blasts	VC + pred
9	T.M.	20/M	A. Prol.	6.1 (85%)	4.5	10	Hypercellular ↑ ↑ Blasts	Dau + cyto
10	A.A.+	11/M	ALL/R	6.6 (22%)	9.5	5	Hypercellular ↑ ↑ Blasts	VC + pred

Cellularity of bone marrow ↑ ↑ = 60–80% blasts in the marrow, ↑ = 30–50% blasts
Chemotherapy: Dau = daunorubicin, cyto = cytosine arabinoside, VC = vincristine, pred = prednisolone.
Diagnosis: All = acute lymphoblastic leukaemia, AML = acute myeloblastic leukaemia, APL = acute promyeloctyic leukaemia, AMOL = acute monocytic leukaemia, AMMOL = acute myelo-monocytic leukaemia, R = in relapse.

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Table 2. The response of the patients to macrophage transfusions

No.	Patient	Initial temp. (° C)	Infection	Infecting organism	Antibiotics	No. of transfusions & no. of peritoneal cells given ($\times 10^9$)	Temp down to (° C)	Onset of response(hr)	Duration of response (hr)	Culture after transfusions
1	G.K.	39–39.5	Broncho-pneumonia	Cultures Neg.	Amp	(1) 1	37.5	6	15	Neg.
2	H.M.	38–38.5	Orchitis (Epididymitis, Pseudomonas)	*Pseudomonas**	Tobromycine	(1) 1	37.5	5	>72	Neg.
3	B.M.	38–38.5	Septicaemia	*Salmonella**	Chloramphen.	(1) 1	36.6–37.4	5	15	Pos.
4	K.S.	40–40.5	U.T.I.	*E. coli**	K + G	(1) 2	35.5–37.5	7	>20	Neg.
						(2) 0.5	35.8–37.6	4	>72	
5	J.M.	38–38.7	Septicaemia	*E. coli*	K + G	(1) 1	No response	–	–	Neg.
						(2) 1	37.2–37.5	6	13	
6	A.J.	38–40	?	Cultures Neg.	Amp + G	(1) 0.8	37.0–37.4	4	8	Neg.
7	F.H.	38–39.5	Infected gums	B-hemolytic Strept.	Pen.	(1) 1	37.0	5	20	Neg.
						(2) 1	37.0	7	>72	
8	A.A.	38.5	Septicaemia?	*Streptococcus viridans**	K + G	(1) 1	37.0	4	24†	Neg.
9	T.M.	38.0	Skin boils	*Staph aureus* (from boils)	K + G	(1) 0.8	37.0	5	10	Neg.
						(2) 0.6	37.0	–	>72	
10	A.A.	38–40.5	Septicaemia	Cultures Neg.	Amp + G	(1) 1	37.5	8	22	Neg.
						(2) 1	No response			
						(3) 0.5	37.0	7	5	

Infecting organisms as cultured from various sites: local lesions, urine etc.

* Positive blood cultures for organisms mentioned.

Antibiotics: Amp = ampicillin, K = keflin (iv), G = gentamicin (iv), Pen = penicillin

† Temperature rose again and then fell gradually to normal by 48 hr. It remained normal thereafter until the patient's discharge.

obtained within 2 hr after transfusion, with no change. No side-effects were encountered except in two patients (K.S. and T.A.) who developed chills lasting for 1 hr. We noted complete resolution of the localised gum infections in patient F.H. shortly after the second dose of peritoneal cells and who remained afebrile until he was discharged, and complete resolution of skin boils in patient (T.M.) within 3 days after the second dose of peritoneal cells and remained afebrile until discharged.

DISCUSSION

The donors were in renal failure but were otherwise normal. They were chosen not to have previous dialysis which might have exhausted their macrophages. The sharp increase in the number of cells during the second day of dialysis is possibly due to the irritation resulting from the procedure which causes mobilisation of cells present in the stationary phase in the peritoneal cavity. The fluid was collected in PVC blood bags, because macrophages are known to stick to glass. The bags were carefully centrifuged at low speed to avoid clumping of the macrophages.

The 10 recipients chosen had acute leukaemia and were febrile, mostly due to bacterial infection. They were not responding to antibiotics. The failure in these patients to control infection is probably due to general suppression of the bone marrow and RES, partly due to the use of cytotoxic drugs [10]. The number of cells infused into the original three patients was kept relatively low in order to avoid significant damage in terms of GVH. However, when no side-effects were encountered in those patients [7], the dose was increased and multiple transfusions were attempted in this study. No side-effects occurred.

The infusion of peritoneal cells has the advantage in that they are rich (80–90%) in the high phagocytic tissue macrophage [11]. Though the infusion contains about 10% lymphocytes ($10–20 \times 10^7$), these numbers are unlikely to cause significant GVH reactions where the recipient has some persisting mixed lymphocyte reactivity. Thus we did not irradiate the cell suspension in order to destroy the radiosensitive lymphocytes as suggested earlier [2], but would always do so where the recipient's immunity is severely depressed. Moreover, lymphocytes taken from uraemic patients may be less able to produce GVH than those collected from normal individuals [12].

The rapid fall in temperature in the responding group (patients 2, 3, 4, 5, 6, 7, 8 and 9) 5–7 hr after administration of macrophages is consistent in all patients and might have been due to the effectiveness of the macrophages against the infecting macroorganisms. This is further supported by the negative cultures after transfusion. The macrophages would be expected to home on to antibody-coated target cells, as they have receptors for activated Fc. The microorgans are dealt with efficiently by the high hydrolytic enzymes contents of peritoneal macrophages [6]. The macrophages are able to kill target cells with an efficiency increased up to 200-fold in the presence of antibodies [3]. This will explain the effectiveness of small cell doses. The effect obtained with 10^9 macrophages is comparable to that obtained with 2×10^{11} neutrophils [13, 14]. The temporary effect in the second group of patients might have been due to the number of macrophages administered, being enough to cope with most but not all the microorganisms, so that the rest were able to multiply. The complete resolution of infection in patients (FH) and (TM) was obvious macroscopically and was confirmed by cultures. In other instances, the effect of macrophages on a patient with wound infected with pseudomonas that was resistant to antibiotics were seen to resolve macroscopically and was confirmed by negative cultures after transfusion with allogeneic macrophages [8]. Patients with septicaemia responded to macrophage transfusions as demonstrated by the negative culture.

Xenogeneic macrophages from humans were also seen to be effective to combat infections in rabbits made septicemic by intravenous injection of *E. coli* [9].

The lack of response in patients (A.A. and T.M.) was possibly due to the active leukaemic process with or without infections.

The reason why we found no change in the WBC count (total and differential) 2 hr after peritoneal cell transfusions in patients 1–4, and 1–4 days in the rest of the patients is because of the small dose of cells administered and that the macrophages might have homed into the site of infection shortly after the transfusion. In another study [15] peritoneal cells were transfused into a patient with aplastic anaemic who was neutropenic. The patient's white cell and platelet counts started to rise 2 weeks after transfusion. They increased to twice the pre-transfusion level, remained there for 2 weeks and then started to drop again. The rise in the white cell count was possibly due to the secretion by the macrophages of colony stimulating factor [16] which stimulates colony growth *in vitro*. These results justify a long-term follow-up of the patient's white cell count after macrophage transfusion to look for any long-term changes which might be induced similarly by macrophage transfusions.

In previous work [17] we demonstrated that antibody-coated tumour cells are destroyed by macrophages *in vivo* and *in vitro*. Macrophage has also been shown to destroy murine hepatoma cells

in vitro by collaborating with monoclonal antibodies [18]. The lack of effect on the leukaemic blast cells in the patients studied suggest absence of antibodies to such cells.

Monocytes collected from peripheral blood by filtration leucapheresis was therapeutically used [19] to combat fungal infections in cancer patients without much success. They also reported the incidence of febrile reactions in 70% of their patients following monocyte transfusions. Our results suggest that peritoneal macrophages are therapeutically more effective than blood monocytes. Side-effects were seen in 2/10 of our patients (K.S. and T.A.) who developed chills lasting for 1 hr. The discrepancy between our results and those of Djerassi *et al.* [19] could be due to the following reasons:

1. The peritoneal macrophages are prepared by centrifugation of the dialysis fluid at low speed (1000 rpm) with little damage to the cells. White cells prepared by filtration leucapheresis are known to be more damaged and cause more side effects than those prepared by continous flow centrifuge. This is related to the cell damage associated with adherence or removal of cells from nylon fibre [20].

2. Evidence was provided by Volkman [21] that resident macrophage populations are self-sustaining and virtually excluded blood monocytes as an antecedent of these cells.

We conclude from these and previous studies that macrophages have the following advantages over neutrophils in the treatment of infected leukaemic patients:

1. Macrophages have greater avidity for antibody-coated mircoorganisms and can home on to and kill target cells within 30–60 min [3].
2. Peritoneal macrophages have a high hydrolytic enzyme content [6].
3. They have a low immunogenicity as demonstrated by negative mixed lymphocytes cultures [2].
4. The effective dose is very small, and therefore less side-effects are encountered than when using large numbers of neutrophils.
5. The preparation of macrophages when available, is simple and does not need any complicated machinery. It does not traumatise the cells during preparation as may occur when granulocytes are prepared by filtration leukapheresis. There is, therefore, a better chance of administering the cells in good condition.
6. Macrophage transfusions might induce two phases of protection: (a) short phase after transfusion, by the macrophages, (b) long-term protection by stimulating the patients's own bone marrow to produce white cells and platelets [15].
7. Macrophages have longer half life (71 hr) [22], than neutrophils ($T1/2$–7 hr) and consequently should give longer effect.

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