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Effects of Large Doses of Dialysable Leukocyte Extract[1] (40939)

AMANULLAH KHAN, CYNTHIA BENJAMIN, AL ANTONETTI, AND J. M. HILL

Department of Immunotherapy, Wadley Institutes of Molecular Medicine, Dallas, Texas 75235

Abstract. The present study was designed to explore the effects of large doses of dialysable leukocyte extract (DLE) in two patients with advanced malignant melanoma. The doses ranged from 600 units/M^2 to 1200 units/M^2, given iv. The single dose of 1200 units/M^2 amounted to 2000 units. This represented an 80-fold increase over the previously reported highest single dose. Case 1 received 5100 units of DLE in three doses. Case 2 received 14,900 units iv in nine doses over a 6-week period. Slight, but temporary, tumor reduction was seen in patient 2. The high doses enhanced E-rosette formation and lymphocyte transformation of these patients. These doses also inhibited delayed hypersensitivity skin reactions, especially if the skin tests were given 4 hr after the injection of DLE. Inhibition of delayed hypersensitivity was observed even when E-rosettes and lymphocyte transformation were enhanced. The inhibitory effect was reversible. Blood chemistry, electrolytes, and peripheral blood count were unaffected except for some rise in LDH and increase in the absolute number of T cells.

The minimum dose of transfer factor (TF) capable of affecting delayed hypersensitivity is approximately 10^8 lymphocyte equivalents (1). This amount of TF was designated as a unit (2) (This unit was also approved for future use at the Third International Symposium on Transfer Factor, Dallas, Texas, October 12–14, 1978.) The term dialysable leukocyte extract (DLE) will be used in this paper instead of TF since our preparation represents a pool and its specificity cannot be ascertained. Previous clinical studies have been conducted at 5- to 10-unit dose levels if a unit is defined as 10^8 lymphocyte equivalents (4–16). South and Caleb (17) have given unconcentrated leukocyte dialysate iv. The availability of leukocytes in large volumes (7, 18) has now made it possible to investigate the effects of relatively large doses of DLE. Transfer factor may be of benefit in malignant melanoma (19, 20). The present study was undertaken to see if it would be advantageous to give large doses of DLE in advanced malignant melanoma and also to investigate the effects of such a treatment on the immune responses.

Materials and methods. Dialysable leukocyte extract was prepared from pooled buffy coats obtained from Wadley Central Blood Bank, according to the method of Lawrence and Al-Askari (21) as used in our laboratory (7, 18). The DLE (10 units/ml) was administered iv over a 15- to 30-min period. It was given to two patients with advanced malignant melanoma who were resistant to chemotherapy. Informed consent was obtained prior to therapy. Case 1 was a 29-year-old white male and case 2, a 49-year-old white female. Patient 1 received an initial dose of 800 units/M^2 iv and two subsequent doses of 1000 units/M^2 given iv on Days 8 and 14. The total amount of DLE given in three doses was 5100 units. Patient 2 received 600 units/M^2 iv on Day 1 and 1200 units/M^2 on Day 4. Her seven subsequent doses were 1000 units/M^2. The amount of DLE given in nine doses added up to 14,900 units over a 6-week period.

Skin tests. The method of performing and grading delayed hypersensitivity skin reactions has been described previously (7). The skin tests were done with the following antigens in a 0.1-ml volume of the appropriate diluent: mumps skin test antigen (Eli Lilly & Company), coccidioidin (Cutter Laboratories), purified protein derivative (PPD), 5 tuberculin units/0.1 ml (Parke, Davis & Co.), and varidase (streptokinase, 100 units/ml; streptodornase, 25 units/ml (SK-SD); Lederle Laboratories). The skin

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reactions were measured at 24 and 48 hr and only the maxima of the two are given in the tables.

E-rosettes. The percentage of E-rosette-forming T cells was determined (22) and the absolute number in the peripheral blood calculated.

Lymphocyte transformation. The transformation of lymphocytes in response to phytohemagglutinin (PHA) and pokeweed mitogen (PWM) was determined by a microculture method (23, 24).

Results. There was no antitumor effect observed in patient 1. However, an 18% reduction in the diameter of the abdominal mass was observed in patient 2 at 3 weeks. There was no further decrease in size during DLE treatment.

The effects on blood chemistry (SMA-12) are listed in Table I. The only notable change represented an increase in the serum lactic dehydrogenase (LDH) level. DLE did not contain LDH. Serum electrolytes, urinalyses, and peripheral blood count were also unchanged except for an increase in the absolute number of T cells in the peripheral blood.

The effects on the immune responses of the patients are listed in Tables II and III. Table II shows the diminished lymphocyte transformation and the low number of T cells before the initiation of treatment. There was gradual improvement in the lymphocyte transformation in response to PHA and the highest level was achieved following the third dose of DLE (normal, 45 ± 37). It declined again 14 days after the last dose of DLE. The response to PWM declined or remained unaffected at 4 hr, but improved at 24 hr. This response also declined to below the initial level at 14 days after the last treatment. The percentage and the absolute number of T cells in the peripheral blood fluctuated, but showed an overall improvement (normal levels: % T cells, 64 ± 6; absolute T cells, 2724 ± 1037/mm^3). The patient had positive skin reactions to PPD and SK-SD. The delayed reaction to PPD fluctuated while the reaction of SK-SD disappeared following the large doses of DLE.

Table III shows the effects of nine doses of DLE in patient 2. Again, the response to PHA improved gradually and stayed nor-

TABLE I. RESULTS OF BLOOD CHEMISTRY BEFORE AND AFTER DIALYSABLE LEUKOCYTE EXTRACT

	Case 1		Case 2	
	Before	After 1 week	Before	After 8th dose
Total protein (g/dl)	6.3	5.9	6.6	6.2
Albumin (g/dl)	3.4	3.1	4.2	3.7
Ca^{2+} (mg/dl)	8.7	9.3	9.5	9
Cholesteral (mg/dl)	150	135	195	185
Glucose (mg/dl)	110	135	108	95
BUN (mg/dl)	9	16	7	7
Uric acid (mg/dl)	6	5.4	3.4	3.3
Creatinine (mg/dl)	1	1	0.7	0.8
Total bilirubin (mg/dl)	0.4	0.5	0.4	0.5
Alkaline phosphatase (mU/ml)	135	185	115	110
LDH (mU/ml)	185	450	650	760
SGOT (mU/ml)	18	34	17	17

TABLE II. EFFECT OF HIGH DOSE DIALYSABLE LEUKOCYTE EXTRACT ON IMMUNE RESPONSES (CASE 1)

Time	Blastogenic index[a]		Percentage T cells	Absolute T cells/mm³	Delayed skin reactions	
	PHA	PWM			PHA	PWM
Before 1st dose	8.3 ± 0.9	12 ± 4	34	139	4+	3+
4 hr after	9 ± 3.5	1.5 ± 0.4	66	538		
24 hr after	9.3 ± 2.2	17.7 ± 5	25	382		
Before 2nd dose	13 ± 2.2	3.7 ± 0.7	65	889	2+	—
4 hr after	2 ± 0.4	1.7 ± 0.4	68	304		
24 hr after	14 ± 5	42 ± 3.5	68	557		
Before 3rd dose	14 ± 2.8	17 ± 3.5	49	367	4+	
4 hr after	31.5 ± 0.7	16 ± 2.8	50	267		
24 hr after	34 ± 4	33 ± 1.5	60	604		
14 days after	6.5 ± 1.2	1.5 ± 0.1	42	400		

Notes. 1st dose, 800 units/M² iv; 2nd dose, 1000 units/M² iv on Day 8; 3rd dose, 1000 units/M² iv on Day 15; PPD, purified protein derivative; SK-SD, streptokinase-streptodornase.

[a] Lymphocyte transformation tests were done in triplicate. Mean ± SD of the three indexes are given in the table.

mal after the third injection. The response to PWM was normal and it showed further improvement. The percentage of T cells was normal, but the absolute number of T cells was low in this patient, which improved gradually to normal levels. The patient had positive delayed hypersensitivity reactions to four recall antigens. The second set of skin tests was performed 4 hr after the third dose of DLE. Three of the reactions disappeared and the reaction to SK-SD was diminished. A subsequent set of skin tests, done immediately after the fifth dose of DLE, showed positive delayed hypersensitivity reactions to three antigens. The delayed reaction to coccidioidin was still negative. The follow-up immune studies are not reported since the patient received other modalities of treatment immediately after the last dose of DLE.

The injection of DLE was locally painful. This problem was minimized if DLE was injected through the tubing while the normal saline was running iv. Patient 1 had chills immediately following the first DLE injection and fever up to 37.8°. The subsequent infusions of DLE were tolerated without any problem. Patient 2 tolerated DLE well, but complained of burning at the site of iv infusion if DLE was given fast. The blood pressure remained within normal limits in both patients during DLE treatment.

Discussion. Transfer factor has been given in the past in doses up to 5×10^9 leukocyte equivalents, subcutaneously (approximately 2.5×10^9 lymphocytes) (16). The present report shows that DLE can be given safely in doses up to 1200 units/M² iv (single iv dose of 2000 units). This dose was 80 times higher than the previously reported high single dose. Certain parameters of T-cell function improved, as shown in Tables II and III. However, some of the delayed hypersensitivity skin reactions were inhibited by these doses. The maximum inhibition of skin tests occurred when the tests were done 4 hr after an injection (Table III). The existence of a component of DLE inhibitory for skin reactions has been described by Gottlieb *et al.* (25). The inhibition seen in our study could be related to this factor. The skin tests were also done immediately after the fifth dose of DLE in the same patient. The inhibition of skin tests was less on this occasion. The prior dose of DLE (fourth dose) was given 7 days earlier. These observations suggest that the effect of inhibitory factor becomes manifest a few hours after the DLE injection and is reversible.

It is interesting that while the delayed skin reactions were inhibited, the other parameters of cellular immunity, such as response to PHA and the number of E-rosette-forming cells, were not diminished. The question of specificity could not be addressed in this study since DLE was pre-

TABLE III. EFFECT OF HIGH DOSE DIALYSABLE LEUKOCYTE EXTRACT ON IMMUNE RESPONSES (CASE 2)

Time	Blastogenic index		Percentage T cells	Absolute T cells/mm^3	Delayed skin reactions			
	PHA	PWM			PPD	SK-SD	Mumps	Cocci
Before 1st dose	7.2 ± 6.2	40 ± 8	78	263	2+	4+	4+	2+
24 hr after	27 ± 4	62 ± 1.2	76	309				
Before 3rd dose	5.4 ± 0.3	24 ± 10	70	504				
4 hr after					—	3+	—	—
24 hr after	17.6 ± 2.8	15 ± 2.5	80	790				
Before 5th dose	12 ± 2.5	20 ± 5.8	65	369				
Immediately after					2+	4+	4+	—
24 hr after	71 ± 13	120 ± 21	74					
Before 9th dose	—	—	70	938				
24 hr after	63 ± 14	51 ± 3.6	53	1067				

Notes. 1st dose, 600 units/M^2 iv; 2nd dose, 1200 units/M^2 iv on Day 4; doses 3–9, 1000 units/M^2 iv 3 to 7 days apart; PPD, purified protein derivative; SK-SD, Streptokinase-streptodornase; cocci, coccidioidin.

[a] Lymphocyte transformation tests were done in triplicate. Mean ± SD of the three indexes are given in the table.

pared by pooling buffy coats in order to obtain large quantities. In addition to other immunologic properties, DLE has been shown to enhance E-rosettes and augment lymphocyte transformation (2, 21, 25–29). The DLE preparation used in this study was immunologically active since it possessed E-rosette-enhancing and lymphocyte transformation-enhancing properties. The full significance of the inhibitors needs to be explored. An important point that emerged from this study was that although the inhibitor was active in suppressing cutaneous reactions, it did not affect lymphocyte transformation and the number of E-rosette-forming cells. This also emphasized, once again, the desirability of fractionating TF to unravel various activities. It is unlikely that the results of this study were affected by the prior chemotherapy. Patient 1 received chemotherapy 4 months before DLE and patient 2 had chemotherapy 4 weeks prior to the initiation of DLE treatment.

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