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THE INFLUENCE OF BLOOD TRANSFUSIONS ON LYMPHOCYTE REACTIVITY IN MAN

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Summary

The effect of planned blood transfusion (BT) on lymphocyte reactivity in previously non-transfused uraemic patients has been investigated. A sustained and non-specific decrease in MLR was observed in approximately 60 per cent of the cases. Other patients had only a transient decrease, normal or increased response. Lymphocyte suspensions whose proliferation was reduced after BT suppressed the response of autologous cells taken before BT. Neither pre-BT degree of immune responsiveness nor clinical status of the patients had any influence on this phenomenon.

Introduction

The mechanism of the beneficial effect of prior blood transfusion (BT) on subsequent cadaver kidney transplant survival [1] is still unknown. Among the numerous hypotheses that have been put forward one of the most probable should be the induction of non-specific suppression [2].

To investigate this we have analysed the influence of planned BT on in-vitro lymphocyte reactivity in previously non-transfused uraemic patients awaiting transplantation.

Patients and methods

Twenty-four patients entered the protocol, nine not yet on regular dialysis, one was treated with chronic ambulatory peritoneal dialysis, and only three had been on dialysis for more than six months. None had ever been previously transfused, and the only female had never been pregnant.

Each received three transfusions of packed blood from different donors, given one month apart. Patients and donors were typed for blood groups and HLA specificities, but only matched for ABO, Rhesus and Kell antigens.

Lymphocytes were obtained from each donor. They were collected sequentially from the patients prior to and after each transfusion, till 90 days after the third. They were cryopreserved in liquid nitrogen and all samples from a given patient were assayed in a single retrospective experiment to eliminate test-to-test variations. One-way mixed lymphocyte reactions (MLR) were performed with stimulating cells from each of the blood donors and from a control lymphocyte pool of 10 unrelated subjects. Proliferative responses to PHA, ConA, PPD and Streptokinase-Streptodornase (Varidase®) were measured in parallel. The geometric mean cpm of ^{3}H-Thymidine incorporation of triplicates were considered for analysis [3].

Results

Modification of proliferative responses after BT

BT produced a significant effect on MLR in the majority of cases. However, the fluctuation noted in the level of reactivity to mitogens or to soluble antigens did not appear to be clearly related to the administration of BT, except for PPD in patients with sustained MLR reduction.

With respect to MLR three patterns of responses, which were not related to HLA matching of the blood received, could be distinguished (Table I):

Pattern I: An early and marked decrease in the MLR to the specific donor and to other cells occurred 10 days after the first BT in nine cases (37.5%). This effect subsequently waned, but it was boosted after each additional BT. It still existed three months after the last BT in six cases (25%).

TABLE I. Examples of changes in MLR after BT. Results obtained in three representative patients

Response pattern	Stimulating cells*	Days after the first BT 0	10	30	40	60	70	90	150
I (SEL)	D1	35.3†	49‡	9	30	20	57	25	41
	D2	17.9	59	−13	49	62	68	57	69
	D3	17.7	39	66	33	36	88	77	89
	C	60.7	24	40	−12	43	21	50	37
II (MIL)	D1	24.4	5	40	12	21	60	57	−48
	D2	38.9	12	39	41	47	77	48	15
	D3	120.6	8	9	37	33	45	47	9
	C	128.6	−2	14	−6	32	66	25	−10
III (COM)	D1	19.8	−63	−34	36	−53	−75	−20	−8
	D2	23.6	−32	−26	60	19	−44	17	13
	D3	19.1	−5	−28	35	−39	−17	−15	13
	C	34.1	−31	−94	34	−24	−51	−71	−28
BT		1		2		3			

* D1, D2, D3 and C indicate cells from the first, second, third donor and from the stimulating pool respectively.

† Net cpm ($\times 10^{-3}$) = cpm of allogeneic cultures minus cpm of unstimulated cultures

‡ % reduction of response = (1 − net cpm post-BT/net cpm pre-BT) × 100

Pattern II: A similar prolonged and non-specific reduction in the MLR appeared later on, after the second or third BT in five other patients (21%).

Pattern III: A transient non-specific decrease in MLR occurred after either one of the BT in nine cases (37.5%). Lymphocyte reactivity from other samples was otherwise normal or even increased.

In 13 patients a specific reduction of reactivity to the donors, but not to the lymphocyte pool, could also be observed with some of the lymphocyte samples (case MIL in Table I). One patient, however, had only such a specific response to the third donor.

To ascertain whether the observed MLR reduction was actually related to active suppression, lymphocyte suspensions obtained after BT were mixed with autologous lymphocytes taken before BT in 'three-cell' cocultures. Only modulating cells whose own blastogenesis was decreased could suppress pre-BT reactivity (Table II). As for MLR decrease, this inhibition was noted with the stimulating cells of the blood donor as well as with other cells.

TABLE II. Assay for MLR suppression (patient AYM)

Day of sampling*		Response to the stimulating pool		
Responders†	Modulators	net cpm ($\times 10^{-3}$)	% Reduction‡	% Suppression
0		36.0	Control	
60		71.1	−97	
0	60	44.7		−24
90		17.6	51	
0	90	16.4		54

* Day 0 = before the first BT; Day 60 = 30 days after the second BT; Day 90 = 30 days after the third BT

† Either 1×10^5 responders or 5×10^4 responders + 5×10^4 non-irradiated modulators were cultured with 1×10^5 irradiated stimulating cells

‡ % reduction and % suppression were calculated as:

$$(1 - \text{net cpm test/net cpm control}) \times 100$$

Immunological parameters that might affect change in MLR

Uraemia is thought to be an important factor modifying immune responses [4]. It was therefore of interest to investigate whether patients displayed low pre-BT immune reactions, and whether the level of responsiveness correlated with further changes in MLR.

Prior to BT, lymphocyte reactivity did not differ from that of normal donors in MLR or in response to mitogens. However, many patients did not react to PPD (7/24 as compared to 1/40 controls; $p < 0.01$) or to either of the antigens used

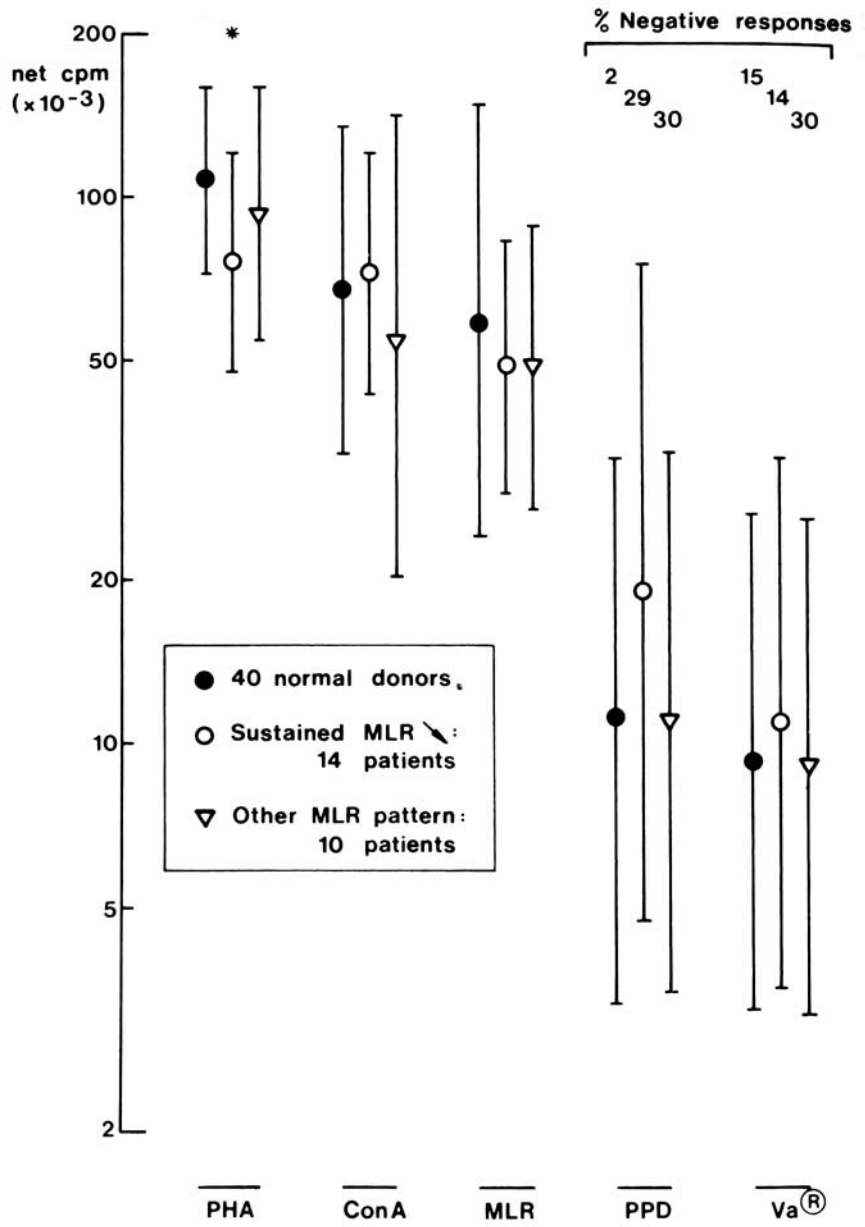

Figure 1. Pre-BT proliferative responses of the patients as compared to their donors (geometric mean ± SE). MLR was performed with the control lymphocyte pool as stimulating cells.
* Indicates a difference at the $p < 0.01$ level with the reactivity of normal donors.

(11/24 versus 7/40; $p < 0.02$), but the level of blastogenesis was otherwise comparable in responder patients and in the donors. Except for reactivity to PHA ($p < 0.01$), the degree of responsiveness that existed at that time did not correlate with MLR modifications that occurred later (Figure 1).

Other factors which might possibly effect immune responses were investigated. Neither the presence of HBs antigenaemia, nor that of actively acquired antibodies to this antigen or passive seroprophylaxis, had any influence on the BT effect. Furthermore, hepatitis B vaccination did not interfere with BT since among the six patients who were vaccinated in the course of the protocol, four displayed sustained MLR reduction (patterns I + II) and two showed another pattern ($p > 0.95$).

Clinical parameters in the different MLR patterns

In order to analyse whether the clinical status could be important for inducing the BT effect, patients were compared regarding dialysis treatment, renal function and degree of anaemia. Thus, the proportion of patients not yet on dialysis was higher in groups I and II (6/14 versus 3/10 other patients) but the difference was not significant. Pre-BT serum creatinine (950 ± 240 in group I + II versus 1235 ± 595) and its increase during the protocol (90 ± 235 and 125 ± 565) were in the same range for all patients. Similarly, haemoglobin (9.6 ± 1.8 versus 9.3 ± 2.8) and the increase after BT (0.35 ± 2.1 versus 1.35 ± 1.5) did not vary from one group to another.

Discussion

Altered immunologic competence due to induction of suppressor cells in transfused patients has been recently increasingly discussed [5–7]. In the prospective study reported here, BT were associated with a sustained and non-specific decrease in MLR in approximately 60 per cent of patients tested. Furthermore, when lymphocytes taken after BT were mixed with cells obtained before BT, inhibiting cells were found in all the samples with decreased allogeneic response but not in those with normal or increased response. Suppressor cells or cytotoxic lymphocytes generated after alloimmunisation could explain these results but, since these lymphocyte suspensions were not cytotoxic to the stimulating cells in direct ^{51}Cr release assays [3], inhibition could be attributed to suppressor cells.

It is possible that the BT effect might be enhanced by a previously deficient reactivity [8]. Indeed there was a certain degree of depression of cellular immunity in the patients but, except for PHA, pre-BT responses did not correlate with the MLR changes that occurred later. Moreover patients with circulating antibodies to HBs antigen, which reflects normal immunity, could also display depressed MLR.

Several possibilities have been suggested to explain lowered immunity in uraemia: toxic elements accumulated by the patients, nutritional deficiency [4], or even anaemia [9]. Therefore we investigated the influence of different clinical parameters on the BT effect, but none played any significant role.

It has been repeatedly demonstrated that the response to a given antigen can possibly be enhanced or conversely depressed when injected concurrently with another antigen than when injected alone [10]. Since many dialysis patients are now submitted to hepatitis B vaccination it was important to ascertain that this procedure does not interfere with BT administered to prepare them for kidney transplant.

In conclusion, the results presented here show that BT can induce in vivo the generation of suppressor cells which are non-specifically active toward allogeneic cells. It seems therefore that this is one of the mechanisms by which BT improves kidney transplant survival.

Acknowledgments

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Open Discussion

SUC (Chairman) Did you plan to use the same blood donor for the transfusion?

GLUCKMAN This has been suggested, but we did not do it with unrelated blood donors because of logistical reasons. Nevertheless we currently have a protocol using donor specific blood transfusions in our living related donor kidney transplant programme. We are studying the mixed lymphocyte reaction in these cases.

LENHARD (Heidelberg) Have you any idea on the type of the suppressor cells, is it a monocyte or is it a suppressor T-cell, which mediate MLR suppression after blood transfusions? Have you carried out experiments using purified cell preparations not just mononuclear cell preparations?

GLUCKMAN No, up to now we recognise two types of suppressor cells, one type is radio resistant, and persists after irradiation of the modulator cell in

the co-culture and one type is radio sensitive and disappears. The radio sensitive cells are non-specific, the radio resistant cells are, I think, specific for HLA antigens of the blood donor. We have looked at the variation of OKT4 and OKT8 before and after blood transfusion, but as in your paper we did not find any modification but we did not look at the monocyte count. We are planning to separate the cell to look at what is suppressed. I think that we will find a mixture of cells, probably monocytes like you have described, but also T-cells.