Clin. exp. Immunol. (1986) **65**, 336–344.

Blood transfusion suppresses cutaneous cell-mediated immunity

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(Accepted for publication 19 March 1986)

SUMMARY

Cutaneous cell-mediated immunity (CMI) evoked by dinitrochlorobenzene (DNCB) was evaluated in end-stage renal disease patients on regular haemodialysis and before renal transplantation. Twenty-seven per cent of the patients had suppression of cutaneous CMI as shown by a negative response upon DNCB challenge. We analysed seven factors known or postulated to have an influence on renal allograft rejection for their effects on cutaneous CMI. Age, sex, red blood cell groups, pathogenesis of the underlying kidney disease, and HLA-DRw6 status had no direct effect on the DNCB response. The number of blood transfusions and the duration of haemodialysis were related to a decrease of the DNCB response but were at the same time correlated. By multiple regression analysis it was shown that the number of blood transfusions had a major suppressive effect on the DNCB response, whereas the duration of haemodialysis had a minor suppressive effect if any. Thus the cutaneous CMI evoked by DNCB partly reflects a general CMI response involved in allograft rejection as well. At the same time the effect of blood transfusion on cutaneous CMI restricts the application of the DNCB test for prediction of future renal allograft rejection.

Keywords immunosuppression DNCB blood transfusion haemodialysis cell-mediated immunity

INTRODUCTION

The DNCB skin test evaluates cutaneous immune reactivity to a primary antigen *viz* dinitrochlorobenzene (DNCB) (Catalona *et al.*, 1972; Bleumink *et al.*, 1974). After sensitization with a standard dosage of DNCB a series of DNCB dosages are applied at the time of the challenge. Erythema, induration and skin lesions at the challenge test areas result in a total DNCB score, which reflects the degree of cutaneous cell mediated immunity (CMI) of the individual tested (Catalona *et al.*, 1972). The DNCB score measured before kidney transplantation could thus provide an estimate of the strength of the CMI response to be expected in allograft rejection. The DNCB score would then be a simple method to judge the necessary vigilance in selection of renal grafts by histocompatibility tests.

In different studies the value of the DNCB score for prediction of the vigour of renal allograft rejection was not consistent (Hamilton, Ledger & Diamandopoulos, 1976; Rolley *et al.*, 1977; Wedgewood *et al.*, 1981; Dick *et al.*, 1983; Harris *et al.*, 1983). Confounding factors in the analyses might explain the variation in the results. Different degrees of matching for the major histocompatibility determinants encoded by the HLA loci influence rejection of renal allografts. It has been proposed that HLA-DRw6 positive persons have a stronger CMI response than HLA-

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DRw6 negative persons (Hendriks *et al.*, 1983a). Histo-incompatibility could thus have an overruling effect on CMI differences. Age, sex and the red blood cell ABO determinants of the recipient have been associated with the celerity of graft rejection (Joysey *et al.*, 1973; Opelz & Terasaki, 1977). Immunosuppression induced by blood transfusion (Opelz, Mickey & Terasaki, 1973), may mask inherent differences in rejection tendency. An immunosuppressive effect of haemodialysis was substantiated by the demonstration of suppressive factors in the serum of haemodialysis patients (Goldblum & Reed, 1980). The underlying kidney disease may also have an effect. In some immunopathological kidney diseases disordered CMI has been postulated (Atkins *et al.*, 1982). Thus, when the DNCB score is analysed for its predictive value in allograft rejection, other influences should be ruled out or taken into account in the analyses. Furthermore, if the cutaneous CMI response reflects at least part of the immune response to allografts the cutaneous CMI should be subject to at least some of the factors of influence on allograft rejection.

The response of the DNCB challenge tests depends on the dosage of DNCB used during sensitization. However sensitization of healthy persons with more than 0·5 mg DNCB induces a positive response to a challenge with 12·5 μg or less DNCB in all those individuals (Friedmann *et al.*, 1983). In this study, which had been approved by the local ethical committee, sensitization was elicited using 2 mg DNCB.

The influence of seven factors, known or postulated to have an influence on renal allograft rejection, *viz* age, sex, ABO red blood cell groups, HLA-DR type, underlying kidney disease, number of blood transfusions and the duration of haemodialysis, on the DNCB score was evaluated before renal transplanation. The relative influence of those factors on cutaneous CMI, and the interrelations between them were analysed.

MATERIALS AND METHODS

Patients

All patients with end-stage renal failure were on regular haemodialysis, had no treatment with immunosuppressive drugs and had had no former kidney transplants. One hundred and forty patients from eleven regional dialysis centres gave their informed consent and were investigated.

Patients with glomerulonephritis with or without systemic lupus erythematosus were registered as having an immunopathological basis for their kidney disease. All other patients had none.

Patients were typed for HLA-A, -B, -C, and -DR antigens according to standard methods and the serological definition of HLA-DRw6 was based on several groups of oligospecific sera (Hendriks *et al.*, 1983b). Patients with at least one HLA-DRw6 positive allele were designated HLA-DRw6 positive. The duration of haemodialysis was defined as the time between the first date of haemodialysis and the date of the DNCB sensitization.

DNCB skin test

Patients were sensitized by the application of 2 mg DNCB, dried onto 2 cm^2 felt pads, to the skin of the upper arm for 24 h. The sensitization and the measurement of the DNCB response upon challenge were performed by the same investigator to avoid bias between observers. Sixteen patients showing no reactivity upon sensitization were excluded from the analysis, because the possibility that they had not been adequately exposed to DNCB sensitization could not be excluded. Challenge dosages of 30, 15, 7·5, 3·7, and 1·8 μg of DNCB on 1 cm diameter felt pads were applied to the skin of the forearm 2 weeks later. After 3 days each of the five test areas was assigned a score: 0 = no reaction or erythema only; 1 = erythema and induration confined to the patch area; 2 = erythema and induration extending beyond the patch area; 3 = 2 plus blistering. The total DNCB score was the sum of the responses to each of the five challenge tests (range 0–15).

Statistical analyses

Normalization of the distribution of the DNCB scores was reached by pooling the patients with negative (score 0), low (score 1–2), intermediate (score 3–5) and high (score 6–11) DNCB responses into four classes which encompassed 33, 46, 26, and 19 patients respectively (Table 1).

Table 1. DNCB responses in relation to duration of haemodialysis, number of blood transfusions and age of the patients

DNCB response class			
1	2	3	4
DNCB score			
0	1–2	3–5	>5
Number of patients			
33	46	26	19
Duration of haemodialysis*			
34·0±26·8	19·4±15·4	24·8±35·4	17·9±15·7
Blood transfusions†			
15·9±18·4	6·1± 8·7	5·4± 5·7	4·7± 5·9
Age‡			
40·9±11·1	38·5±11·0	36·4± 8·1	39·8± 7·9

* Mean and standard deviation of the duration of haemodialysis in months.

† Mean and standard deviation of the number of blood transfusions.

‡ Mean and standard deviation of the age in years.

The distribution of the number of blood transfusions was normalized into five classes with 0–1, 2, 3–5, 6–12, and 13–67 transfusions, which encompassed 26, 27, 24, 23 and 24 patients respectively.

The duration of haemodialysis was normalized into six classes with 0–5, 6–10, 11–16, 17–24, 25–46, and 47–136 months of haemodialysis representing 20, 22, 24, 19, 21, and 18 patients respectively.

Correlation coefficients were determined between the DNCB score or four DNCB response classes, the number of blood transfusions or five blood transfusion classes, the duration of haemodialysis or six haemodialysis classes and the age of the patients to validate the normalization applied (Table 2). Nine non-transfused patients were regarded as missing values in the analysis of the relation between transfusion free interval and other factors.

Table 2. Correlations between the DNCB response, blood transfusions, haemodialysis, age and transfusion free interval

	DNCB	DNCBResp	NBldtr	BldCl	DurDia	DiaCl	Intvl
DNCB		0·95					−0·02
NBldtr	−0·26	−0·31		0·72			−0·22
BldCl	−0·19	−0·22					
DurDia	−0·18	−0·18	0·42	0·47		0·84	0·03
DiaCl	−0·22	−0·22	0·44	0·51			
Age	−0·11	−0·08	−0·06	−0·05	0·03	0·10	

DNCBResp = DNCB response classified into four groups (see text); NBldtr = number of blood transfusions; BldCl = number of blood transfusions classified into five groups (see text); DurDia = duration of haemodialysis in months; DiaCl = duration of haemodialysis classified into six groups (see text); Intvl = time between last blood transfusion and DNCB sensitization.

Table 3. Multiple regression analyses of the effects of blood transfusion and haemodialysis on the DNCB response

Selected variable	Method	*P* value	Other variable	*P* value
NBldtr	Forward	0·001	DurDia	NS
NBldtr	Forward	0·001	DiaCl	NS
BldCl	Forward	0·015	DurDia	NS
BldCl	Forward	0·015	DiaCl	NS
DurDia	Enter	0·049	NBldtr	0·004
Diacl	Enter	0·016	NBldtr	0·008

For abbreviations see Table 2.
NS = not significant.

Table 4. Distribution of patients according to bloodgroup, sex, HLA-DRw6 status, and pathogenesis of kidney disease

	DNCB response class					
	1	2	3	4	Significance	
Bloodgroup						
Bloodgroup O	22	20	15	10	$\chi^2 = 4{\cdot}33$	NS
Bloodgroup non-O	11	26	11	9		
Sex						
Males	13	29	16	14	$\chi^2 = 7{\cdot}23$	NS
Females	20	17	10	5		
HLA-DRw6						
HLA-DRw6 negative	24	29	15	12	$\chi^2 = 1{\cdot}56$	NS
HLA-DRw6 positive	9	17	11	7		
Pathogenesis						
Non-immunological	27	24	19	14	$\chi^2 = 8{\cdot}74$	$P = 0{\cdot}03$
Immunological	6	22	7	5		

NS = not significant.

To analyse which of the variables or which combinations of the variables gave the best estimate of the corresponding DNCB value, multiple regression equations were performed using the forward and enter method in the SPSS$^{\times}$ regression program (SPSS$^{\times}$, 1983). By the 'forward method' the program selected the variable which had the largest effect, followed by an analysis of the contribution other variables still had on the DNCB response, given the effect of the first variable (Table 3). In the 'enter' method we choose a variable, analysed its effect and, given the effect of the variable chosen, analysed the contribution of the other variables.

The significance of the differences in the distribution of the ABO bloodgroups, pathogenesis of renal disease, HLA-DRw6 and sex among the patients in the four DNCB response classes was determined by Chi squares' (Table 4). The *P* values in Table 5 were derived from two tailed Student's *t*-test for two means.

Table 5. Number of transfusions (mean ± s.d.) in groups of patients divided according to HLA-DRw6, sex, bloodgroup and pathogenesis of the kidney disease.

	n	Transfusions	*P* value
HLA-DRw6			
Positive	44	5·6 ± 8·9	NS
Negative	80	9·9 ± 13·5	
Sex			
Males	72	5·4 ± 7·7	< 0·002
Females	52	12·4 ± 15·7	
Bloodgroup			
O	67	8·8 ± 12·2	NS
Non-O	57	7·8 ± 12·2	
Pathogenesis			
Non-imm.path.	84	9·1 ± 13·3	NS
Imm. path.	40	6·7 ± 9·5	
Imm. path.			
Resp cl 2	22	3·7 ± 2·8	< 0·04
Resp cl 1 + 3 + 4	18	10·3 ± 13·2	
Non-imm. path.			
Resp cl 2	24	8·3 ± 11·5	NS
Resp cl 1 + 3 + 4	60	9·5 ± 13·9	

Non-imm. path. = non-immunological pathogenesis of the underlying kidney disease; imm. path. = immunological pathogenesis of the underlying kidney disease; Resp cl 2 etc. = DNCB response class 2 etc; NS = not significant.

RESULTS

DNCB response in the patient population

Twenty-seven per cent of the patients did not respond to the DNCB challenge, which indicated suppression of the DNCB response when compared to a 100% positive response of healthy persons (Table 1, and Friedman *et al.*, 1983).

The distribution of the DNCB scores in the population of patients was gradual and not bimodal (Fig. 1). Consequently a division of the patients into four groups of DNCB responders (Table 2–5) became arbitrary but was validated by a high correlation coefficient ($r = 0{\cdot}95$)) between the original DNCB scores and the normalized DNCB response classes (Table 2).

DNCB response in relation to seven factors postulated to influence CMI

Seven factors known or postulated to be involved in allograft rejection were tested for their effect on cutaneous CMI evoked by DNCB.

The influence of blood transfusions, haemodialysis and age was surveyed by calculation of the mean value of each factor within the four DNCB response classes (Table 1). High DNCB scores were related to a shorter duration of haemodialysis and to a lower number of blood transfusions. The age of the patients had no effect on the DNCB response.

Three other factors, i.e. O versus non-O red blood cell groups, sex, and the HLA-DRw6 positive versus negative phenotype of the patients, also had no effect on the DNCB response (Table 4).

Patients with glomerulonephritis (Table 4) appeared to have a significant higher mean DNCB score than patients with non-immunological pathogeneses of their underlying kidney diseases ($\chi^2 = 8{\cdot}74$, $P = 0{\cdot}03$).

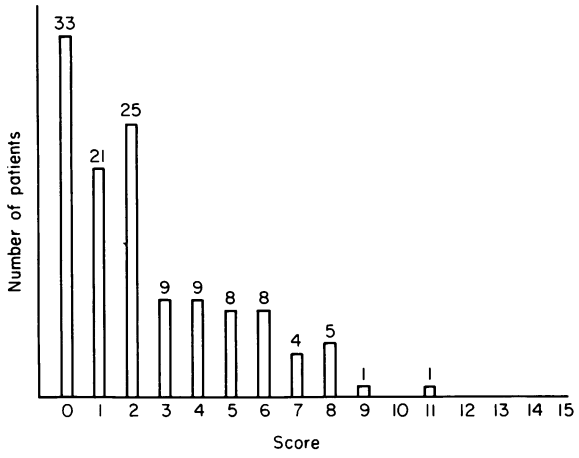

Fig. 1. Distribution of the DNCB scores in 124 haemodialysis patients. For DNCB scores, see Materials and Methods.

A correlation matrix between DNCB scores, DNCB response classes, number of blood transfusions, blood transfusion classes (BldCl), duration of haemodialysis, haemodialysis classes (DiaCl), age and transfusion free interval (Table 2) demonstrated high correlation coefficients between DNCB scores and DNCB response classes, between number of blood transfusions and BldCl and between the duration of dialysis and DiaCl which validated the normalizations applied.

Negative correlations between the DNCB responses and the number of blood transfusions ($r = 0{\cdot}19$–$0{\cdot}26$) and between the DNCB response and the duration of hemodialysis ($r = 0{\cdot}18$–$0{\cdot}22$) were found as was expected from the results in Table 1.

The age of the patients showed no significant correlation with any of the other factors analysed, which denied any substantial effect of age on the DNCB score (Table 2).

A high correlation between blood transfusions and duration of haemodialysis became evident ($r = 0{\cdot}42$–$0{\cdot}51$, Table 2).

Multiple regression analysis of the influence of blood transfusions and haemodialysis on the DNCB score

A consequence of the correlation between blood transfusions and haemodialysis was that when their separate effects on the DNCB responses were measured the influence of the correlated factor should be taken into account in the analysis. This was accomplished by multiple regression analysis using the forward method (Table 3).

The number of blood transfusions emerged as the variable with the largest influence. Given the effect of blood transfusion on the DNCB response ($P = 0{\cdot}001$–$0{\cdot}0015$) the duration of haemodialysis had no significant residual effect on the DNCB response. When the duration of haemodialysis was first entered in the program an effect of haemodialysis on the DNCB response that was just significant was demonstrated ($P = 0{\cdot}016$–$0{\cdot}049$), but blood transfusion still provided a major contribution ($P = 0{\cdot}004$–$0{\cdot}008$). However, a concomitant influence of blood transfusion in the haemodialysis effect could not be excluded in this type of analysis. We concluded that blood transfusions had a major effect on the DNCB response and that if haemodialysis had any influence at all, its effect was small.

The interval between blood transfusion and the DNCB skin test

The major suppressive effect of blood transfusion on the DNCB response raised the question how long this effect persisted. The interval between the last blood transfusion and the time of the DNCB skin test was analysed in relation to the DNCB response, number of blood transfusions and the

duration of haemodialysis. The range of the transfusion free interval was from 0·5 to 48 months with a mean of 6·4±9·1 months. There was a negative correlation ($r = -0{\cdot}22$) between the interval and the number of blood transfusions (Table 2). We concluded that the interval between the last blood transfusion and the time of application of the DNCB skin test had no significant influence on the DNCB response.

The influence of blood transfusion on the factors analysed

Because of the major suppressive effect of blood transfusion its influence on the other factors was evaluated to find out whether the results had been affected by differences in the number of blood transfusions (Table 5). Such an effect had already been excluded for the age of the patients because we could not discover any significant correlation between age and blood transfusions (Table 2).

The mean and standard deviation of the number of blood transfusions were calculated for the groups of patients analysed for the effects of the other factors (Table 5). The mean number of blood transfusions in the HLA-DRw6 positive group was lower than in the HLA-DRw6 negative group, but the difference was not significant (Table 5).

Although the mean DNCB scores for males and females were 2·86±2·52 and 2·00±2·43 respectively, we found no significant effect of sex on the DNCB response (Table 4). However, the mean number of blood transfusions in male patients was significantly lower ($P < 0{\cdot}002$) than in female patients (Table 5). Thus the higher DNCB score in males could be explained by a lower number of blood transfusions, which excluded a significant effect of sex on the cutaneous CMI.

The distribution of the number of blood transfusions in the patients with red blood cell group O versus A and/or B was almost equal, which sustained the earlier conclusion that the ABO type had no influence on the DNCB response (Tables 4 and 5).

A significant increase of the number of patients with an immunological pathogenesis of their underlying renal disease was found (Table 4). This was in particular based on the high number of patients with an immunological pathogenesis in the DNCB response class 2. When the mean number of blood transfusions was calculated for all the four DNCB response classes, the patients with an immunological pathogenesis in DNCB response class 2 had a significantly lower number of blood transfusions than the patients with an immunological pathogenesis in DNCB response classes 1, 3 and 4 (Table 5). We concluded that the suppressive effect of an immunological pathogenesis of the renal disease on the DNCB score was a spurious result dependent on a lower number of blood transfusions.

DISCUSSION

We postulated that inconsistency in the results of studies on the predictive value of the DNCB score for successive renal allograft rejection might depend on overruling influences of other factors involved in allograft rejection. Furthermore if the cutaneous CMI response upon DNCB reflects CMI involved in allograft rejection, at least some of the factors of influence on allograft rejection should be involved in cutaneous CMI as well and thus may confound the actual CMI response. Application of a sensitization dosage of 2 mg DNCB, which was amply sufficient to evoke a primary CMI response in 100% of healthy subjects, restricted the study to an investigation of relative immunosuppression in the effector phase of the cutaneous CMI response in haemodialysis patients. About 30% of the population of haemodialysis patients did not respond to the challenge test, which indicated suppression of cutaneous CMI during haemodialysis.

A gradual distribution of the DNCB scores in the population of haemodialysis patients was found (Fig. 1). Thus any division into high and low responders was arbitrary whatever cut off points were used. We made a division into four groups of patients with DNCB scores of 0, 1–2, 3–5 and 6–11 respectively which was validated by a high correlation with the original DNCB scores.

Of seven factors known or postulated to be involved in allograft rejection, *viz* ABO bloodgroups, age, sex, HLA-DRw6 effect, pathogenesis of the underlying kidney disease, number of blood transfusions and duration of dialysis, only the last two factors have been reported to be associated with immunosuppression. However, the mechanisms by which the other factors mediate

their influence on renal allograft rejection are so cryptic that we included those factors in the analysis. HLA-DRw6 negative kidneys transplanted into HLA-DRw6 positive recipients have a low graft survival rate whereas HLA-DR mismatched kidneys in HLA-DRw6 negative recipients have a better survival rate. This might be confined to a histo-incompatibility induced immune response but was also postulated to be dependent on an inherent high CMI response associated with HLA-DRw6. We did not find a high responder status associated with HLA-DRw6 in the cutaneous CMI test. The DNCB challenge test was adequate to test at least the efferent phase of the immune response. Thus we conclude that HLA-DRw6 is not associated with a high responder status in the efferent phase of cutaneous CMI. The same conclusion holds for the ABO red blood cell groups, age and sex.

Two factors, the number of blood transfusions and the duration of haemodialysis, were found to be interdependent. The necessity for blood transfusions increases with the duration of haemodialysis and is most likely to be associated with insufficient erythropoietin production. The correlation between the number of blood transfusions and the duration of haemodialysis implied that when a correlation between one of these factors and the DNCB score was found, the other factor should be analysed for its confounding effect.

The number of blood transfusions was correlated with suppression of cutaneous CMI. By multiple regression analysis we investigated whether, given the effect of blood transfusions, still other factors contribute to suppression of cutaneous CMI. The analysis in fact addressed the question whether such factors really contribute or entail spurious effects. The duration of haemodialysis was related with cutaneous CMI responses, but the effect of the duration of haemodialysis was dependent of the effect of blood transfusions. The duration of haemodialysis, given the effect of blood transfusions, had no direct suppressive effect on cutaneous CMI. Given the effect of the duration of haemodialysis, the number of blood transfusions still had a large suppressive effect on cutaneous CMI. This last analysis revealed an effect of haemodialysis that was just significant. However, the enter method applied in the statistical program does not exclude a concomitant effect of blood transfusions.

We conclude that the number of blood transfusions has a major suppressive effect on cutaneous CMI whereas a small effect of the duration of haemodialysis can not be proven or disproven.

An immunological pathogenesis of the underlying kidney disease appeared to be associated with an increase of the cutaneous CMI score. A relative increase of cutaneous CMI associated with an immunological pathogenesis of the kidney disease could in fact have been dependent on fewer blood transfusions in this group of patients. The effect associated with glomerulonephritis was indeed a spurious result introduced by the lower number of blood transfusions in patients with glomerulonephritis. This finding stressed the relevance of being aware of other influences on the factor analysed in relation to CMI responses.

Blood transfusions not only have a suppressive effect on cutaneous CMI responses but also an allograft rejection. Investigations on the mechanism of the blood transfusion effect in allograft rejection are hampered by the concurrent treatment with immunosuppressive drugs after transplantation. It is not known how many blood transfusions should be given to haemodialysis patients before transplantation because it is not clear whether the immunosuppressive effect increases with increasing numbers of transfusions. Our data suggest that it does.

Furthermore it is not known how long the immunosuppressive effect persists after blood transfusions. Consequently, we addressed the question whether the interval between the last blood transfusion and the application of the DNCB skin test had a noticeable effect on the DNCB score. No significant effect of the interval was found despite a range of 0·5 to 48 months (mean $6{\cdot}4 \pm 9{\cdot}1$ months). We conclude that blood transfusions have a lasting suppressive effect on cutaneous CMI responses.

The DNCB score has been advocated as a suitable test for predicting future renal allograft rejection. We argue that blood transfusion confounds the DNCB score and thus confounds the analysis of its predictive value.

To circumvent this confounding effect the DNCB skin test should be applied before any blood transfusion is given. In our experience this is hard to achieve because it was found that many patients had already received blood transfusions long before being subjected to regular haemodialysis.

We conclude that a main factor influencing renal allograft rejection, *viz* blood transfusion, also influences cutaneous CMI. This implies that the DNCB response at least reflects part of the CMI response involved in renal allograft rejection.

We would like to express our gratitude to all the haemodialysis patients from the dialysis centres in Alkmaar, Haarlem, Leiden, Leiderdorp, 's-Gravenhage, Rotterdam and Breda who gave informed consent to perform the DNCB skin test. The support of physicians and nursing staffs attending and the staff of the Eurotransplant Foundation is gratefully acknowledged. Valuable statistical advice was obtained from Drs G.C. Endel and E.A.van der Velde. This work was supported in part by grant C 82.332 of the Dutch Kidney Foundation.

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