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The effect of maintenance dialysis on lymphocyte function

I. HAEMODIALYSIS

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SUMMARY

The effect of haemodialysis on mitogen-stimulated lymphocyte DNA synthesis was determined in twenty-four uraemic patients. Lymphocytes pre-haemodialysis were significantly less responsive to phytohaemagglutinin (PHA) than were control lymphocytes and showed the same degree of impairment as uraemic lymphocytes. Intrinsic lymphocyte responsiveness improved immediately after each haemodialysis. Pre-haemodialysis plasma inhibited control lymphocyte responsiveness and this inhibition was even greater in post-haemodialysis plasma. This effect of haemodialysis on plasma lasted for 4–8 hr. Similar alterations of response were noted, despite the use of different dialysers and also when two other mitogens were substituted for PHA. This deleterious effect of haemodialysis on lymphocyte function is important for its possible immune consequences, and may indicate a deficiency in current haemodialysis technique.

INTRODUCTION

Patients with chronic renal failure have a defect in cellular immunity (Merrill, 1968; Huber *et al.*, 1969; Dammin, Couch & Murray, 1957; Boulton-Jones *et al.*, 1973; Hosking *et al.*, 1976). The lymphocyte response to PHA is a well accepted measure of cellular immunity and is reduced in uraemia (Nakhla & Goggin, 1973; Newberry & Sanford, 1971; Hosking *et al.*, 1976). However, the effect of haemodialysis on the depressed uraemic lymphocyte response is controversial. Newberry & Sanford (1971) found that haemodialysis improved the plasma capacity to support lymphocyte response, whilst Nelson & Penrose (1973) reported the opposite effect. To delineate the effect of haemodialysis, we studied the individual components of lymphocyte PHA responsiveness. The systems used were the intrinsic lymphocyte responsiveness, the isolated effect of uraemic plasma on normal lymphocytes and the combined effect of lymphocytes in autologous plasma Changes occurring before and after the initiation of maintenance haemodialysis and between individual haemodialyses were assessed with respect to each of these components.

This study was performed to determine the interrelated effect of haemodialysis on uraemic lymphocytes and plasma as assessed by PHA responsiveness.

MATERIALS AND METHODS

Haemodialysis patients. Twenty-seven patients (fifteen males and twelve females), whose ages ranged from 26 to 65 years (median 44 years), were studied. Twenty-four were on long-term hospital-centred maintenance haemodialysis for a period from 90 to 380 days with a median of 250 days on haemodialysis. Three patients were studied at end-stage chronic renal failure before maintenance haemodialysis was begun.

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All patients had chronic renal failure with creatinine clearances less than 2·0 ml/min/1·73 m^2. Glomerulonephritis was the underlying disease in fourteen, hypertension in six, polycystic disease in two and analgesic nephropathy in two, as well as one patient each with pyelonephritis, diabetic nephropathy and renal failure following haemolytic uraemic syndrome. Nine patients had received renal transplants and returned to chronic haemodialysis after graft nephrectomy because of rejection. The minimum time between total cessation of immunosuppressive drug therapy and the study was 6 months.

Uraemic patients. Nine uraemic patients were also studied. This group consisted of five women and four men whose ages ranged from 31 to 56 years, with a median of 43. All had a creatinine clearance of less than 5·0 ml/min/1·73 m^2. The aetiology of their renal failure was glomerulonephritis in six patients, hypertension in two patients and one had analgesic nephropathy. Although all patients were on standard medication at the time of the study, none of the drugs was known to influence immune function.

Haemodialysis technique. Haemodialysis was performed thrice weekly for 4 hr on each occasion. Blood flow rates were greater than 120 ml/min (mean 170 ml/min). Dialysate flow rates were maintained at 500 ml/min. No patient was transfused with blood during the study.

Two types of haemodialyser were used, hollow fibre (Dow Mk IV, Miami, USA) and parallel plate kidneys (Gambro, 1·5 m^2 or 1·0 m^2, Lund, Sweden) according to patient size and dialysis needs. Both new and formalin re-sterilized haemodialysers were used.

Culture technique. Each 1·0 ml culture contained $0{\cdot}25 \times 10^6$ mononuclear cells from a Ficoll-Isopaque density gradient separation. The cells were cultured in medium 199 (Commonwealth Serum Laboratories, Melbourne) containing 20% of either calf serum or patient plasma or control plasma from healthy volunteers, to which 0·6 μg purified PHA (Burroughs Welcome) was added. The culture was maintained at 37°C in 5% CO_2 for 3 days. The cells were pulsed with 2·0 μCi tritiated thymidine for 2 hr prior to termination of culture. A sampling manifold collected the cells on glass fibre filters, which were then washed with 2% acetic acid, 5% trichloracetic acid and 50/50 ether/alcohol. Each filter was placed in a vial containing 0·5 ml Soluene and 5·0 ml liquid scintillation fluid and counted in a Packard 2450 Tricarb liquid scintillation spectrometer. The means of counts from duplicate culture were expressed as counts per minute (ct/min).

The pre-haemodialysis lymphocytes were cultured after standing as whole blood at room temperature for up to 4 hr. Previous studies (Fitzgerald *et al.*, 1978) have shown no deleterious effects of such storage on lymphocyte function. This storage allowed pre- and post-dialysis lymphocytes to be tested in the same assay.

Culture combinations. Three different aspects of lymphocyte response to PHA were tested.

(i) *Intrinsic lymphocyte function.* The patient's lymphocytes were tested in calf serum.

(ii) *Plasma effect.* The effect of patient's plasma on responsiveness of control lymphocytes was studied.

(iii) *Lymphocytes in autologous plasma.* Lymphocytes were assessed by culture in their own plasma.

These three combinations of lymphocyte responsiveness were tested in eight patients with uraemia prior to initiation of haemodialysis, and compared with twelve patients on maintenance haemodialysis immediately prior to and following an individual haemodialysis. The effect of dialysis procedure on plasma was assessed in twenty-four patients before and after eighty-two individual haemodialyses.

Duration of haemodialysis effect. Two patients were tested at intervals over 20 hr after haemodialysis. One of the patients was retested on two further occasions.

The effects of different types and re-use of haemodialysers. To determine the influence of various types of haemodialyser on lymphocyte function, an analysis of pre- and post-haemodialysis plasma was performed. Hollow fibre formalin-sterilized haemodialysers (Dow Mk IV) (Cordis Corp. Miami, USA) were compared with new (ethylene oxide-sterilized) and re-used (formalinsterilised) parallel plate haemodialysers (Gambro, Lund, Sweden).

Other mitogens. The culture technique using pokeweed mitogen (PWM; Gibco) and insoluble concanavalin (ICon A; Pharmacia) was similar to that used for PHA, except that the cultures were continued for 6 days and utilized thymidine incorporation over the last 6 hr of culture. The concentration of PWM used was 40 μg/ml and that of ICon A 20 μg/ml. The system investigated was the culture of control cells in medium containing 20% patient plasma together with the appropriate control culture. The effect of haemodialysis on lymphocyte stimulation by these mitogens was assessed in five patients.

Creatinine levels. Serum creatinine and blood urea were measured prior to and immediately following each haemodialysis by a Technicon Autoanalyser.

Analysis of results. All results are expressed as the median. Comparison between groups was performed by the Wilcoxon rank sum test (Mann & Whitney, 1947) or the Wilcoxon signed ranks test (Wilcoxon, 1945).

RESULTS

Intrinsic lymphocyte function

Fig. 1 outlines the PHA responsiveness of lymphocytes from uraemic patients and from patients pre- and post-haemodialysis. The PHA responsiveness of lymphocytes from uraemic patients (34×10^3 ct/min) was significantly less ($P < 0{\cdot}05$) than control lymphocytes (75×10^3 ct/min). Intrinsic lymphocyte

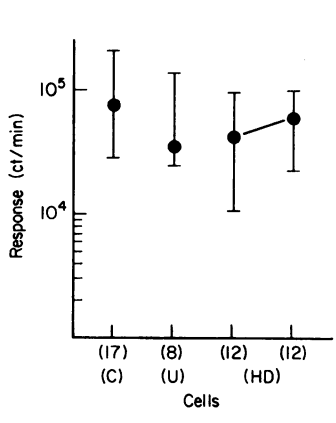

FIG. 1.

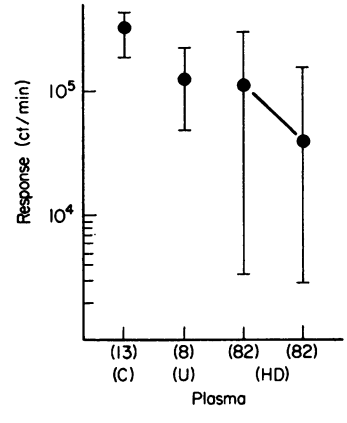

FIG. 2.

FIG. 1. Intrinsic lymphocyte function in calf serum. The PHA response of control lymphocytes (C), lymphocytes from uraemic undialysed (U) and paired pre- and post-maintenance haemodialysis (HD) patients assayed in calf serum, are compared. Uraemic and pre-haemodialysis lymphocytes were significantly less than controls ($P< 0{\cdot}05$). Post-haemodialysis lymphocyte responsiveness returned to control levels. Figures in parentheses show number of patients studies; (●) median values with ranges.

FIG. 2. Plasma effect on control lymphocytes. The effect of plasma from normal controls (C), compared with plasma from uraemic undialysed patients (U) and paired pre- and post-haemodialysis (HD) plasmas, on the responsiveness of control lymphocytes stimulated with PHA is illustrated. Control lymphocytes assayed in uraemic and pre-haemodialysis plasma were significantly less responsive than when tested in control plasma ($P< 0{\cdot}002$). Plasma after haemodialysis showed a further significant deterioration in control lymphocyte responsiveness when compared with pre-dialysis plasma ($P< 0{\cdot}001$). Figures in parentheses show number of patient plasmas studied; (●) median values with ranges.

function pre-haemodialysis (43×10^3 ct/min) was significantly less ($P<0{\cdot}05$) than control lymphocytes, but not significantly different from uraemic lymphocytes. Immediately following haemodialysis, lymphocyte responsiveness improved (61×10^3 ct/min: $P<0{\cdot}05$) and was not significantly different from controls.

Plasma effect on control lymphocytes

Fig. 2 shows that control lymphocytes stimulated by PHA in uraemic plasma had a reduced response (130×10^3) when compared with the response in control plasma (237×10^3 ct/min: $P<0{\cdot}002$). The lymphocyte response using haemodialysis plasma (117×10^3 ct/min) was not significantly different from the response in uraemic plasma. Immediately following haemodialysis, patient plasma showed a marked deterioration in its capacity to support PHA responsiveness of control lymphocytes (40×10^3 ct/min: $P<0{\cdot}01$).

Lymphocyte function in autologous plasma

Fig. 3 details the results of stimulation of uraemic lymphocytes in autologous plasma. The median response of 112×10^3 ct/min was significantly less ($P<0{\cdot}001$) than that of control lymphocytes in autologous plasma (250×10^3 ct/min). The response of pre-haemodialysis lymphocytes stimulated in their own plasma (72×10^3 ct/min) was not significantly different from uraemic lymphocytes in autologous uraemic plasma. However, immediately following maintenance haemodialysis, lymphocytes in autologous plasma deteriorated markedly with a median of 38×10^3 ct/min. These were significantly less responsive than pre-dialysis lymphocytes stimulated in autologous plasma ($P<0{\cdot}05$) and uraemic lymphocytes in uraemic plasma ($P<0{\cdot}05$).

Duration of dialysis effect

The duration of plasma inhibition of PHA responsiveness of control lymphocytes following haemodialysis is detailed in Fig. 4. It can be seen that the four samples of plasma taken up to 4 hr after

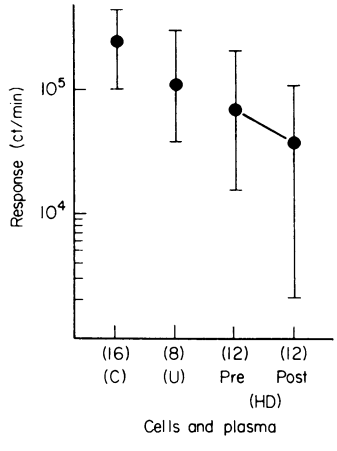

FIG. 3.

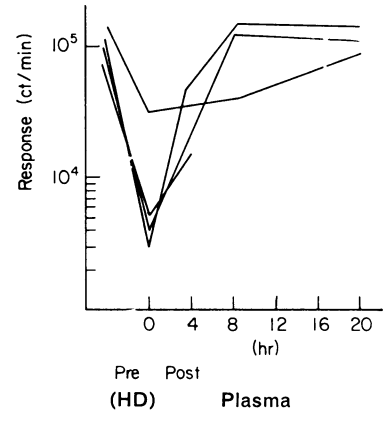

FIG. 4.

FIG. 3. Lymphocyte function in autologous plasma. The PHA stimulated lymphocyte response of control lymphocytes in autologous plasma (C) is compared with lymphocytes from uraemic undialysed patients (U) and paired pre- and post-haemodialysis patients' lymphocytes assayed in autologous plasma. Both uraemic and pre-dialysis lymphocytes were less responsive in autologous plasma than in the control ($P< 0{\cdot}001$). Immediately after haemodialysis a significant further deterioration in responsiveness occurred ($P< 0{\cdot}05$). Figures in parentheses show number of patient plasmas studied; (●) median values with ranges.

FIG. 4. Duration of dialysis effect. The duration of inhibition of PHA response of post-haemodialysis plasma on control lymphocytes has been followed after four dialyses. In all patients, plasma support of lymphocyte responsiveness 4 hr after completion of a haemodialysis was less than pre-haemodialysis. By 20 hr post-dialysis the plasma effect had reverted to pre-haemodialysis levels.

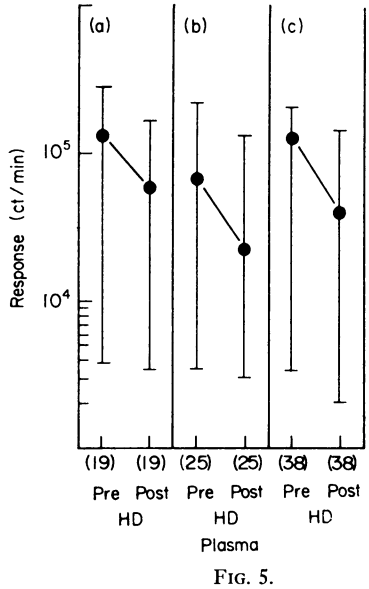

FIG. 5.

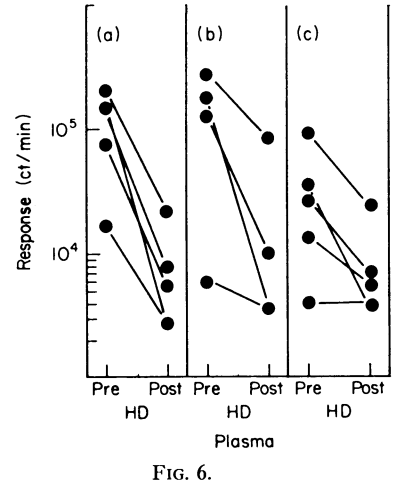

FIG. 6.

FIG. 5. Plasma effect with different dialysers. The effect of different haemodialysers on patients plasma effectiveness in supporting control lymphocyte responsiveness pre- and post-haemodialysis (HD) is shown. There was no significant difference in pre-haemodialysis plasma effect using formalin-sterilised hollow fibre dialysers (a), parallel plate (b), dialysers new (ethylene oxide sterilization) or re-used after formalin sterilization (c). Each type of haemodialyser gave the same degree of plasma effect after haemodialysis.

FIG. 6. Comparison of mitogens. Pre- and post-dialysis plasma from four patients have been compared using control lymphocytes stimulated with PHA (a), PWM (b) and ICon A (c). All lymphocytes showed an inhibited response in post-haemodialysis plasma.

haemodialysis still showed impaired ability to facilitate lymphocyte responsiveness compared with the pre-dialysis plasma. All had returned to pre-haemodialysis levels by 20 hr post-dialysis.

Effect of different types of haemodialyser

The significant post-haemodialysis deterioration in the capacity of plasma to facilitate PHA control lymphocyte responsiveness was seen with both types of haemodialyser and to the same degree (Fig. 5). Similarly, there was no significant difference between any of the pre-haemodialysis plasmas for the three haemodialysers studied, nor was the responsiveness significantly different following dialysis with each type of haemodialyser. Furthermore, the post-haemodialysis deterioration occurred whether the haemodialyser was new or re-used.

Pokeweed mitogen and insoluble concavalin A responsiveness (Fig. 6)

The same pattern of DNA synthesis was seen for PWM and ICon A as when PHA was used as the mitogen; that is, control lymphocytes grown in post-haemodialysis plasma responded less well to PWM and ICon A than when cultured in pre-haemodialysis plasma.

Creatinine and urea levels

Median creatinine levels were 0·97 (range 0·57–1·74) mmol/l before and 0·56 (range 0·23–1·17) mmol/l after maintenance haemodialysis. Urea was 28·8 (range 9·98–2·0) mmol/l before, and 14·3 (range 4·4–38·4) mmol/l after haemodialysis.

DISCUSSION

Many aspects of immune function have been found to be abnormal in uraemia. Impaired delayed hypersensitivity, as evidenced by prolonged skin graft survival (Dammin *et al.*, 1957; Smiddy, Burwell & Parsons, 1961), lymphopenia with decrease in both T- and B-cell subpopulations (Reddy, Goh & Cestero, 1975), decreased monocyte phagocytic ability (Ringoir *et al.*, 1975; Urbanitz & Sieberth, 1975; Montgomerie, Kalmanson & Guze, 1947), thymosin deficiency (Harris & Sengar, 1975) and impaired leucocyte migration capacity (Rosso di Secondo, Scalamogna & Sirchia, 1974) have all been reported. We have also shown that haemodialysis patients have a lymphopenia and a qualitative defect in lymphocyte function, but opsonic and neutrophil metabolic function are normal (Hosking *et al.*, 1976).

Overall lymphocyte function in chronic renal failure as assessed by DNA synthesis in response to PHA has been studied, but with quite variable results (Nakhla & Goggin, 1973; Newberry & Sanford, 1971; Nelson & Penrose, 1973; Elves, Israels & Collinge, 1966; Kasakura & Lowenstein, 1967; Byron, Mallick & Taylor, 1976; Daniels *et al.*, 1971). Many of the discrepancies relate to the particular culture protocol used. Cell plasma combinations have included uraemic lymphocytes assayed in control plasma, control cells assayed in uraemic plasma or uraemic cells assayed in uraemic plasma. We studied and compared all these combinations in uraemia, and also delineated the specific effects of haemodialysis on each component of PHA responsiveness, both before and after dialysis.

Differing results for intrinsic lymphocyte PHA responsiveness assessed by stimulating lymphocytes in control plasma have been reported both in uraemia and on haemodialysis. Uraemic lymphocytes have been found to be deficient in function (Nakhla & Goggin, 1973; Elves *et al.*, 1966), normal (Kaskura & Lowenstein, 1967) and increased (Daniels *et al.*, 1971; Byron *et al.*, 1976).

The lymphocyte response from patients on maintenance haemodialysis has been reported as normal (Sengar, Rashid & Harris, 1975) or deficient (Webel, Briggs & Light, 1974; Kauffman, Manzler & Phair, 1975). Our results demonstrate that the PHA responsiveness of lymphocytes pre-haemodialysis was significantly less than control lymphocytes and of the same degree of impairment as uraemic lymphocytes. However, immediately following haemodialysis, lymphocyte responsiveness improved to normal.

Uraemic plasma has been usually reported as less capable than normal of supporting lymphocyte PHA responsiveness of control lymphocytes (Newberry & Sanford, 1971; Hurst *et al.*, 1975; Nelson &

Penrose, 1973; Silk, 1967), although Kasakura & Lowenstein (1967) found it to be normal. Our results confirm that the plasma from uraemic patients inhibited the PHA response of control lymphocytes. Again, the results on maintenance dialysis studies vary. Plasma from patients on maintenance haemodilysis was found either to be less able than control plasma to facilitate lymphocyte responsiveness (Nelson & Penrose, 1973; Hurst *et al.*, 1975) or the same as control (Webel *et al.*, 1974). Our results indicate that pre-dialysis plasma inhibits normal lymphocyte responsiveness. Post-haemodialysis plasma was found to cause even further deterioration in the lymphocyte responsiveness. This could mean that haemodialysis either removes nutrients or molecules responsible for lymphocyte responsiveness, or conversely adds toxins which inhibit lymphocyte responsiveness. Nelson & Penrose (1973) found that *in vitro* dialysis further impaired pre-haemodialysis plasma. This suggested that haemodialysis, while removing uraemic inhibitors, also removed molecules necessary for lymphocyte responsiveness, and that this was the predominant effect. However, Newberry & Sanford (1971) reported that post-haemodialysis plasma, although less than control plasma, was better than pre-haemodialysis plasma in facilitating PHA responsiveness of control lymphocytes. They felt that a dialysable inhibitor was present in uraemic plasma. Elves *et al.* (1966) found that maintenance haemodialysis and peritoneal dialysis restored the uraemic plasma-inhibited lymphocyte responsiveness to normal. Sengar *et al.* (1975) found most haemodialysis patients' plasma normal in facilitating the control lymphocyte responsiveness, utilizing a mixed lymphocyte culture.

The disparity between various reports may be explained by the cyclical change of lymphocyte responsiveness from the start of one haemodialysis to the commencement of the next. Our studies have demonstrated that there is a plasma effect (which lasts for from 4 to 20 hr post-haemodialysis) induced by the haemodialysis procedure itself. In contrast, a haemodialysis improves intrinsic lymphocyte function.

A cycle of post-haemodialysis lymphocyte normality followed by deterioration in lymphocyte responsiveness up to the next haemodialysis is apparent. This is probably explained by the accumulation of uraemic toxins. The ability of plasma to inhibit control lymphocyte responsiveness also followed a cyclical pattern between haemodialyses. This was in the opposite direction to the lymphocyte pattern, with significant inhibition developing after each haemodialysis.

There have been few reports of the changing pattern of lymphocyte responsiveness with haemodialysis. Newberry & Sanford (1971) have reported an improvement in the plasma support of PHA lymphocyte responsiveness over each haemodialysis. Their findings seem at odds with the current report. However, mean pre-haemodialysis creatinine in the patient population was 1·8 mmol/l falling to 0·8 mmol/l post-haemodialysis. On haemodialysis, patients had much lower levels of creatinine, which may reflect more adequate haemodialysis and the two groups may not, therefore, be strictly comparable.

Lymphocyte response in autologous plasma reflected the predominant effect of plasma, with a significant fall in lymphocyte responsiveness after haemodialysis. The likely explanations to account for the overall effect on the plasma are the removal of essential molecules by the dialysis process and/or the addition of toxins from the dialysis apparatus, as previously mentioned. The same change in plasma effect was found when PWM or ICon A were substituted for PHA, i.e., a significant fall in responsiveness after haemodialysis. This makes it unlikely that any change in the post-haemodialysis plasma is acting through PHA binding. Other experiments with PWM and ICon A dose–responses suggest that this phenomenon is not due to plasma-binding of mitogen, as no increased response occurred with increasing mitogen beyond the usual optimal doses.

Analysis of each component of PHA lymphocyte responsiveness (cells and plasma) has shown that the decrease in lymphocyte responsiveness after haemodialysis is entirely due to the effect of haemodialysis on plasma. Lymphocytes themselves actually improve in responsiveness after haemodialysis when tested in calf serum. This deleterious change in plasma brought about by the haemodialysis procedure may indicate an inadequacy in the modern haemodialysis technique.

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