Clin. exp. Immunol. (1975) **22,** 54–61.

CELL-MEDIATED IMMUNITY IN PATIENTS ON LONG-TERM HAEMODIALYSIS

CAROL A. KAUFFMAN, A. D. MANZLER AND J. P. PHAIR

Department of Internal Medicine, Infectious Disease Division, University of Cincinnati Medical Center and Cincinnati Veterans Administration Hospital, and Department of Internal Medicine Division of Nephrology, Christ Hospital, Cincinnati, Ohio, U.S.A.

(*Received* 5 *November* 1974)

SUMMARY

Several parameters of cell-mediated immunity were assessed in thirty patients with chronic renal failure treated with long-term haemodialysis. Lymphopenia was uncommon, and only two patients showed diminished numbers of thymus-derived peripheral blood lymphocytes. Skin test energy to three antigens was documented in only one patient. No serious infections with intracellular organisms were noted in any of the patients. Phytohaemagglutinin (PHA) induced DNA synthesis in eleven patients was lower than that shown by normal subjects. This poor responsiveness to PHA was not corrected by haemodialysis, and in only two patients was the response improved by substituting normal human plasma for uraemic plasma in the lymphocyte cultures. Thus with the exception of PHA response, other parameters of cell-mediated immunity were intact in this group of chronic stable haemodialysis patients.

INTRODUCTION

Uraemia has been termed 'nature's immunosuppressive device' and in previous studies has been associated with prolonged homograft survival (Dammin, Couch & Murray, 1957), skin test anergy (Wilson, Kirkpatrick & Talmage, 1965; Huber *et al.*, 1969; Selroos, Pasternack & Virolainen, 1973), marked lymphopenia (Wilson *et al.*, 1965; Jenssen, 1958), and abnormal *in vitro* lymphocyte responsiveness to mitogenic stimulation (Huber *et al.*, 1969; Nakhla & Goggin, 1973; Ming, Ming & Dammin, 1968; Elves, Israels & Collinge, 1966). In addition, uraemic serum has been found to contain factors which suppress the mitogenic response of normal lymphocytes (Newberry & Sanford, 1972; Silk, 1967). However, much of the data, especially that generated from *in vitro* lymphocyte transformation, is contradictory; recent work would suggest that lymphocyte function is not abnormal in chronic renal failure (Webel, Briggs & Light, 1974; Daniels *et al.*, 1970, 1971; Kasakura & Lowenstein, 1967). Some of these discordant results have arisen because heterogeneous groups of patients, with varying degrees of renal failure, have been studied. Earlier studies involved patients whose 'uraemic state' was not as efficiently treated as is possible today. It

Correspondence: Dr Carol A. Kauffman, Infectious Disease Division, University of Cincinnati Medical Center, Cincinnati, Ohio 45267, U.S.A.

was of interest to determine the extent of immunosuppression present in patients with well-compensated uraemia in comparison with previous studies involving persons with severe and sometimes terminal renal failure. The present report is an evaluation of several aspects of cellular immunity in a relatively homogeneous population of stable chronic haemodialysis patients.

MATERIALS AND METHODS

The study population consisted of thirty patients with chronic renal failure of various aetiologies who underwent thrice weekly haemodialysis. Most dialysis periods lasted for 6 hr utilizing an Ultra-Flow II haemodialyser with a cuprophane membrane. Ages of the patients ranged from 13 to 74 years with a mean of 48 years. There were twenty men and ten women. The mean duration of haemodialysis prior to the study was 10 months with a range from 1 to 39 months. Serum creatinine ranged from pre-dialysis values of 8·3 mg/100 ml to 19·5 mg/100 ml to post-dialysis values from 2·0 mg/100 ml to 9·3 mg/100 ml. No patient was acutely ill, and all were able to maintain their anticipated level of function at home, work or school. No patient had a clinically overt intercurrent viral or bacterial illness when studied. Charts were reviewed retrospectively for evidence of infectious complications due to intracellular organisms frequently associated with immunosuppression.

Peripheral blood total leucocyte counts and lymphocyte counts were performed by standard methods.

Thymus-derived (T) lymphocytes in blood were identified by their capacity to form spontaneous rosettes with sheep red blood cells. The technique of Jondal *et al.* was utilized (Jondal, Holm & Wigzell, 1972) except that lymphocytes were separated from other leucocytes on a cotton column (Rocklin & David, 1971). At least 200 lymphocytes were counted using phase microscopy, and those with three or more erythrocytes attached were scored as rosettes.

Intradermal skin tests were performed by one observer with 0·1 ml of the following antigens: trichophyton (Hollister Stier, Incorporated, Spokane, Washington) 1000 PNU/ml, histoplasmin (Parke-Davis, Incorporated, Detroit, Michigan) and streptokinase–streptodornase (SK–SD) (Lederle Laboratories, Pearl River, New York) 50 units of SK/ml and, when indicated, 400 units of SK/ml. Reactions showing greater than 5 mm of induration at 48 hr were recorded as positive.

PHA-induced DNA synthesis in cultured lymphocytes was assessed using a modification of the method of Fernald & Metzgar (1971) as previously described (Kauffman *et al.*, 1974). Briefly, heparinized blood was allowed to sediment, the leucocyte-rich plasma was collected, and the cells were washed three times and resuspended in Eagle's minimum essential medium with 20% heat-inactivated human plasma. Cultures were carried out in both autologous uraemic and pooled normal human group AB plasma. Aliquots of 0·2 ml of the cell suspension containing $2{\cdot}5 \times 10^5$ lymphocytes were cultured in 6×50 mm culture tubes. PHA-M (Difco, Incorporated, Detroit, Michigan) was reconstituted with 5 ml of buffered saline, pH 7·3, and was added to each culture in amounts varying from 0·03 ml of an undiluted solution to 0·01 ml of a 1:10 dilution; control cultures contained no mitogen. At the end of the 72-hr incubation period the cells were pulse-labelled with 1 μCi of tritiated thymidine, harvested by standard methods, and assayed for radioactivity in a beta liquid scintillation counter. Results were expressed as disintegrations per minute (d/min) per $2{\cdot}5 \times 10^5$ mononuclear cells. The response to PHA was considered low if DNA synthesis in the study population was less than that shown by any of the control subjects. Twelve normal control subjects, 28–40 years of age, were concomitantly studied.

Viability of lymphocytes after 72 hr in culture was determined using the trypan blue dye exclusion technique (Murphy, Wiens & Watson, 1958).

To assess the effect of uraemic plasma on normal lymphocytes, normal donor cells were cultured as described above except that a similar concentration of uraemic plasma was substituted for autologous plasma. Donor cells obtained from two or more normal individuals were cultured with the plasma from each uraemic patient.

RESULTS

Lymphocyte counts and T-cell numbers

Pre-dialysis leucocyte counts of twenty-seven patients ranged from 4000/mm^3 to 15,800/

mm^3 with a mean of $8330/mm^2$. Lymphocyte counts varied from $820/mm^3$ to $4500/mm^3$ (mean $\pm$ 1 s.d. = $2397/mm^3 \pm 1038/mm^3$). Four of the twenty-seven patients were mildly lymphopenic (lymphocyte count between $1000/mm^3$ and $1500/mm^3$); only one patient had a lymphocyte count less than $1000/mm^3$. Haemodialysis did not change the total leucocyte count nor the lymphocyte count significantly in the eight patients in whom these values were compared. The mean lymphocyte count was $2368/mm^3$ pre-dialysis *vs* $2286/mm^3$ post-dialysis ($P > 0{\cdot}5$ by Student's *t*-test).

The percentage of circulating lymphocytes which were T cells was $70{\cdot}6 \pm 9{\cdot}7\%$ (mean $\pm$ 1 s.d.) in twenty-eight haemodialysis patients studied pre-dialysis compared with $71{\cdot}7 \pm 6{\cdot}2\%$ in eighty normal controls ($P > 0{\cdot}1$ by Student's *t*-test). The absolute numbers of T lymphocytes ranged from $756/mm^3$ to $3195/mm^3$ in the haemodialysis patients (lower limit of normal was $889/mm^3$ in the control group). Of the total group of patients only two had diminished T-cell numbers (absolute counts and percentage of T cells were more than two standard deviations below the mean seen in the normals). Haemodialysis did not change the number of circulating T cells in seven patients studied, including the two with low numbers of T cells; the mean number of T cells pre-dialysis was 65·4% compared with 64·4% post-dialysis ($P > 0{\cdot}5$ by Student's *t*-test).

Delayed cutaneous hypersensitivity

Skin tests with three antigens were performed on eighteen patients. Fourteen of eighteen patients (78%) responded to at least one antigen; nine of these fourteen responded to two or

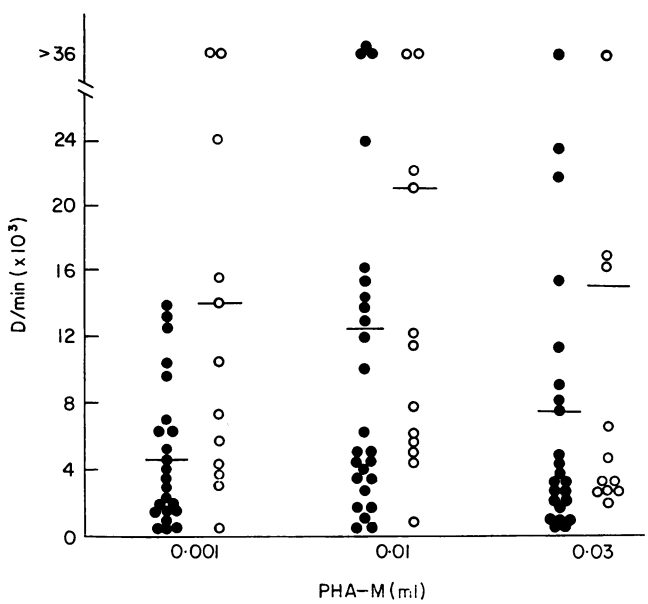

FIG. 1. Responses to varying doses of PHA shown by lymphocytes from haemodialysis patients and control subjects. Lymphocytes were cultured in autologous plasma. PHA-induced DNA synthesis is expressed as d/min/$2{\cdot}5 \times 10^5$ cells. Student's *t*-test revealed the following *P* values: 0·001 ml, $0{\cdot}01 < P < 0{\cdot}02$; 0·01 ml, $0{\cdot}05 < P < 0{\cdot}1$; 0·03 ml, $P > 0{\cdot}2$. (●) Haemodialysis patients. (○) Control subjects.

more antigens. Only four persons were unresponsive to all three antigens. However, repeated testing of three of these four persons with 40 units of streptokinase showed that two gave a positive reaction to this larger dose.

Response of lymphocytes to PHA

PHA-induced DNA synthesis was evaluated in twenty-five of the thirty patients in the study. Lymphocytes from these twenty-five persons cultured in autologous pre-dialysis plasma appeared to respond less well at all doses of PHA than did lymphocytes from controls cultured in autologous plasma (Fig. 1). However, these differences were statistically significant only for the 0·001-ml dose of PHA ($0{\cdot}01 < P < 0{\cdot}02$ by Student's *t*-test). The majority of persons in both patient and control groups showed the maximum response to the dosage of 0·01 ml of an undiluted solution of PHA. When only the maximum response to PHA was considered, eleven patients had a lower response than did concomitantly run control subjects. The difference in the maximum response to PHA between control and patient

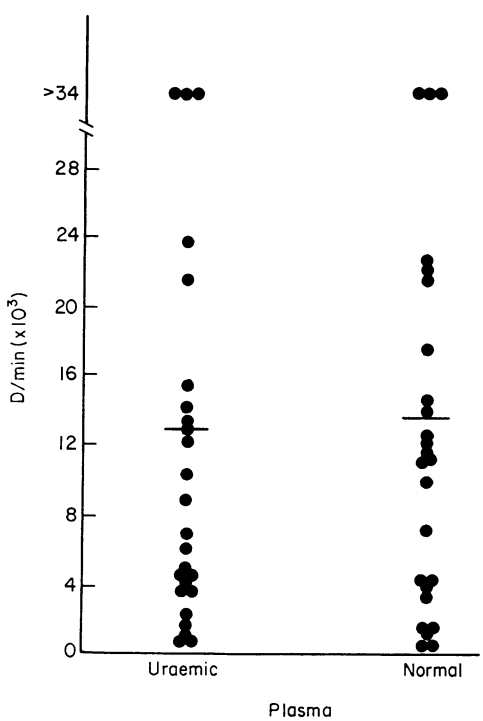

FIG. 2

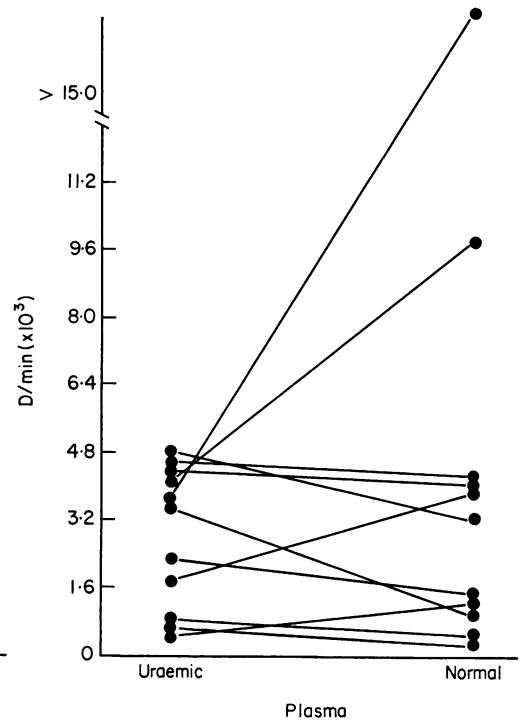

FIG. 3

FIG. 2. The effect of uraemic *vs* normal human plasma on the maximal PHA response of lymphocytes from haemodialysis patients. PHA-induced DNA synthesis is expressed as d/min/$2{\cdot}5 \times 10^5$ cells. The difference between the lymphocyte response in uraemic *vs* normal plasma is not statistically significant ($P > 0{\cdot}5$ by Student's *t*-test).

FIG. 3. Attempt to correct the low PHA response shown by some haemodialysis patients-by substitution of normal human plasma for autologous uraemic plasma. PHA-induced DNA synthesis is expressed as d/min/$2{\cdot}5 \times 10^5$ cells.

lymphocytes was not statistically significant. Substitution of pooled normal human plasma for autologous uraemic plasma did not enhance lymphocyte responsiveness to PHA (Fig. 2). Of the eleven persons with abnormally low maximum responses to PHA in autologous plasma, nine also demonstrated low responses in normal plasma (Fig. 3). Only two people showed an improvement in lymphocyte function when their cells were removed from autologous uraemic plasma. Studies were performed pre- and post-dialysis in seven patients, five of whom showed a low response to PHA pre-dialysis. This procedure did not significantly change the response to PHA (Fig. 4). Background DNA synthesis in cultures without mitogen were in the same range for controls (72–1022 d/min) and haemodialysis patients (80–1628 d/min). Lymphocyte viability after 72 hr in culture was $91{\cdot}5 \pm 8{\cdot}0\%$ (mean $\pm$ 1 s.d.) for haemodialysis patients and $89{\cdot}8 \pm 7{\cdot}1\%$ for control subjects ($P > 0{\cdot}5$ by Student's t-test).

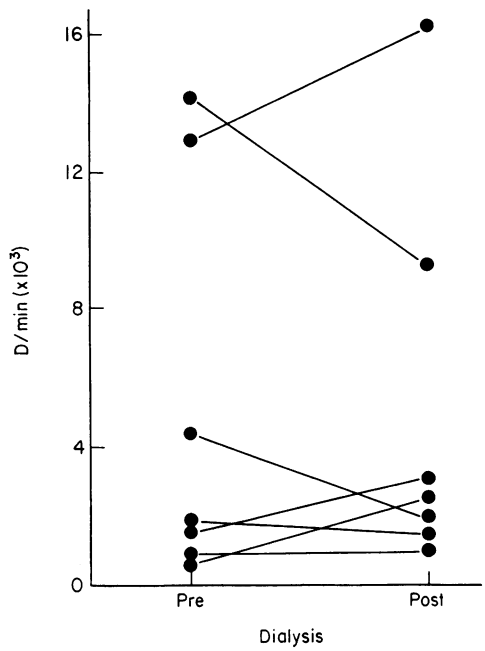

FIG. 4.

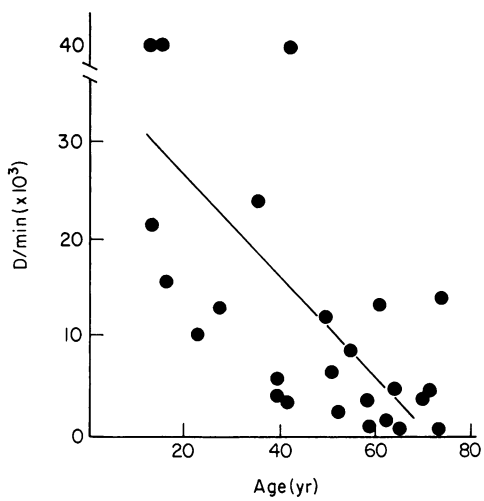

FIG. 5.

FIG. 4. The effect of haemodialysis on the PHA response of lymphocytes from haemodialysis patients. PHA-induced DNA synthesis is expressed as d/min/$2{\cdot}5 \times 10^5$ cells. The difference between pre-dialysis d/min and post-dialysis d/min is not statistically significant ($P > 0{\cdot}5$ by Student's t-test).

FIG. 5. The relationship of PHA response to age in haemodialysis patients. PHA-induced DNA synthesis is expressed as d/min/$2{\cdot}5 \times 10^5$ cells. The correlation coefficient (r) is $-0{\cdot}64$ with $P < 0{\cdot}01$. $y = -508x + 36908$.

Evaluation of correlation cofficients and chi-square analyses showed that the following were not related to lymphocyte responsiveness to PHA: serum creatinine or BUN; underlying renal disease; the total time spent on haemodialysis; the drugs the patients were receiving (thiazide diuretics, methyldopa, digitalis, allopurinol). However, there was a strong negative correlation with the age of the patient (Fig. 5). Sixty-four per cent of those

over 50 years had a poor response to PHA while 75% of those over 60 responded poorly. Nine of the eleven patients with a poor response to PHA were older than 50 years of age. Since it was important to assess the role of age alone compared with the combination of chronic renal disease and older age, the PHA response of a group of fifteen healthy non-hospitalized persons 60–80 years of age was evaluated. No decrease in response to PHA was found when lymphocytes from these elderly persons were cultured in normal plasma. The mean PHA response of this group was 35,611 d/min with a range of 4786–114,053 d/min; the mean response of lymphocytes from haemodialysis patients over 60 years of age when cultured in normal human plasma was 11,954 d/min with a range of 404–63,752 d/min.

When uraemic plasma was used in cultures of normal lymphocytes, it was found that plasma from six patients consistently suppressed the PHA response of lymphocytes from several normal donors. The other thirteen plasmas tested were not inhibitory to normal lymphocyte function. Five of the six patients with plasma inhibitory factors were among the eleven whose lymphocytes showed a low response to PHA; this included the two patients whose plasma was suppressive to their own lymphocytes. Haemodialysis did not remove this inhibitory activity from uraemic plasma.

Prevalence of opportunistic infections

No patient in the study group had infection with herpesviruses, parasites such as *Toxoplasma gondii* and *Pneumocytis carinii*, or intracellular bacteria such as *Mycobacterium tuberculosis*. No fungal infections were documented except two cases of candidal vaginitis which were easily treated with topical nystatin.

DISCUSSION

Previous studies of cellular immunity in persons with chronic renal failure have emphasized the findings of lymphopenia (Wilson *et al.*, 1965; Jenssen, 1958), diminished dermal hypersensitivity (Wilson *et al.*, 1965; Huber *et al.*, 1969; Selroos *et al.*, 1973), and abnormal lymphocyte responsiveness *in vitro* to various mitogens (Huber *et al.*, 1969; Nakhla & Goggin, 1973; Ming *et al.*, 1968; Elves *et al.*, 1966). Many of these studies involved patients who were terminally uraemic or who were dialysed less frequently than recommended today. Only one study investigated the role of chronic haemodialysis in correcting these defects in cellular immunity (Webel *et al.*, 1974). Our survey of several paramenters of cellular immunity in a group of stable haemodialysis patients with well-compensated uraemia indicated that overall these patients had little impairment of cellular immunity.

In contrast to previous studies which found moderately severe lymphopenia (lymphocyte counts between 500/mm^3 to 1000/mm^3) to be commonplace in persons with chronic renal failure (Wilson *et al.*, 1965; Jenssen, 1958), only 18% of our patients were lymphopenic, and in four of five the lymphopenia was mild (1100/mm^3 to 1400/mm^3).

Enumeration of T lymphocytes in the blood of persons with chronic renal failure has not been reported previously. Using a technique which probably measures the total population of circulating T lymphocytes, we found that only two of our patients had reduced numbers of T cells. These patients' lymphocytes did not respond to PHA *in vitro*; unfortunately skin tests were not performed on these two persons.

Previous studies of delayed dermal hypersensitivity revealed that 50–58% of persons with chronic renal failure were anergic to a battery of antigens (Wilson *et al.*, 1965; Huber *et al.*,

1969). We found that only four of eighteen (22%) haemodialysis patients were unresponsive to the three antigens used. Retesting of three of these four persons with a higher dose of SK–SD produced a positive response in two. Thus, true skin test unresponsiveness was very uncommon in this population.

Even though there was little evidence of lymphopenia or suppression of lymphocyte function *in vivo*, the maximal lymphocyte response to PHA *in vitro* was lower in eleven out of twenty-five patients than in the concomitantly tested control subjects. These results corroborated those of other workers who have shown a poor response to PHA in persons with chronic renal failure (Huber *et al.*, 1969; Nakhla & Goggin, 1973; Ming *et al.*, 1968; Elves *et al.*, 1966). Of these eleven persons, three were mildly lymphopenic. Since all cultures contained the same number of lymphocytes, lymphopenia was not the cause of the diminished mitogenic response. However, two patients had low numbers of circulating T cells; this depletion could account for their poor response to PHA, which is probably a T-cell mitogen in man (Lohrmann, Novikovs & Graw, 1974). The low PHA response was primarily cell-associated since substitution of normal human plasma for autologous uraemic plasma resulted in greater DNA synthesis in only two subjects. Skin tests performed in six of these eleven patients showed that only one person was unresponsive to the three antigens used, and unfortunately, he was not available for testing with a higher dose of SK–SD. Lymphocyte viability after 72 hr in culture was the same for those patients who were poorly responsive to PHA as those who responded well. The PHA response could not be related to clinical features such as type and duration of renal disease or drug therapy nor to frequently measured laboratory parameters of uraemia.

PHA responsiveness was related to age in that all but two subjects, demonstrating a low response were older than 50 years of age. The role of age alone in determining mitogenic responsiveness was assessed by testing a group of healthy controls 60–80 years of age. These persons responded as well to PHA as did the younger control group. Thus it appeared that only persons who were both elderly and had chronic renal disease demonstrated a lower than expected response to PHA.

Factors which inhibit normal donor lymphocyte transformation have been found in uraemic plasma by some investigators (Newberry & Sanford, 1972; Silk, 1967) but were absent in other studies (Webel *et al.*, 1974). Only six of our patients manifested plasma inhibitory factors, none of which were removed by dialysis. The reasons for the discordant results among different studies might be related to differences in patient populations. However, Newberry & Sanford (1972) did evaluate a relatively homogeneous group of chronic haemodialysis patients and found dialysable inhibitory factors in nine out of ten patients (Newberry & Sanford, 1972). Another explanation of the absence of similar dialysable factors in our patients and those of others (Webel *et al.*, 1974) could revolve around differences in the haemodialysis membranes utilized or the length of the haemodialysis period in different renal units.

Our patients had no history of infection with pathogens often associated with depressed cellular immunity. In fact, there is little evidence that infections with fungi, intracellular bacteria, parasites, and certain DNA viruses are prevalent among haemodialysis patients unless they have undergone renal transplantation with immunosuppression (Montgomerie, Kalmanson & Guze, 1968; Pien *et al.*, 1973; Strauch *et al.*, 1974). The most commonly seen infections in haemodialysis patients involve shunt colonization and bacteraemia with organisms such as *Staphylococcus aureus* and *Pseudomonas* species (Kaslow & Zellner, 1972).

Neutrophil phagocytosis and killing seem to be far more important than lymphocytes in host defence against such infections.

Considering the lack of infections by intracellular pathogens and the nearly intact delayed dermal hypersensitivity and T-cell numbers, it is unlikely that the low mitogenic response found in some persons on chronic haemodialysis implies a significant suppression of cellular immunity in this group of patients.

The expert technical assistance of Mrs Janet Chatterjee is gratefully acknowledged. This work was supported by USPHS Training grant number TI AI 87, Research grant number HDO7769, and the Morton Hamburger Memorial Fund.

REFERENCES

DAMMIN, G.J., COUCH, N.P. & MURRAY, J.E. (1957) Prolonged survival of skin homografts in uremic patients. *Ann. N.Y. Acad. Sci.* **64,** 967.

DANIELS, J.C., SAKAI, H., COBB, E.K., REMMERS, A.R., JR, SARLES, H.E., FISH, J.C., LEVIN, W.C. & RITZMANN, S.E. (1970) Altered nucleic acid synthesis patterns in lymphocytes from patients with chronic uremia. *Amer. J. med. Sci.* **259,** 214.

DANIELS, J.C., SAKAI, H., REMMERS, A.R., JR, FISH, J.C., COBB, E.K., LEVIN, W.C. & RITZMANN, S.E. (1971) *In vitro* reactivity of human lymphocytes in chronic uremia: analysis and interpretations. *Clin. exp. Immunol.* **8,** 213.

ELVES, M.W., ISRAELS, M.C.G. & COLLINGE, M. (1966) An assessment of the mixed leucocyte reaction in renal failure. *Lancet*, **i,** 682.

FERNALD, G.W. & METZGAR, R.S. (1971) *In vitro* studies of the response of hamster lymphocytes to phytohemagglutinin and antigenic stimulation. *J. Immunol.* **107,** 456.

HUBER, H., PASTNER, D., DITTRICH, P. & BRAUNSTEINER, H. (1969) *In vitro* reactivity of human lymphocytes in uremia—a comparison with the impairment of delayed hypersensitivity. *Clin. exp. Immunol.* **5,** 75.

JENSSEN, O. (1958) Observations on the leucocyte blood picture in acute uremia. *Brit. J. Haemat.* **4,** 422.

JONDAL, M., HOLM, G. & WIGZELL, H. (1972) Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming non-immune rosettes with sheep red blood cells. *J. exp. Med.* **136,** 207.

KASAKURA, S. & LOWENSTEIN, L. (1967) The effect of uremic blood on mixed leukocyte reactions and on cultures of leukocytes with phytohemagglutinin. *Transplantation*, **5,** 283.

KASLOW, R.A. & ZELLNER, S.R. (1972) Infection in patients on maintenance hemodialysis. *Lancet*, **ii,** 117.

KAUFFMAN, C.A., PHAIR, J.P., LINNEMANN, C.C., JR & SCHIFF, G.M. (1974) Cell-mediated immunity in humans during viral infection. I. Effect of rubella on dermal hypersensitivity, phytohemagglutinin response and T-lymphocyte numbers. *Infect. Immun.* **10,** 212.

LOHRMANN, H.P., NOVIKOVS, L. & GRAW, R.G., JR (1974) Cellular interactions in the proliferative response of human T and B lymphocytes to phytomitogens and allogeneic lymphocytes. *J. exp. Med.* **139,** 1553.

MING, P.L., MING, S.C. & DAMMIN, G.J. (1968) Effect of uraemia and azathioprine on leucocyte response to phytohemagglutinin. *Fed. Proc.* **27,** 432.

MONTGOMERIE, J.Z., KALMANSON, G.M. & GUZE, L.B. (1968) Renal failure and infection. *Medicine*, **47,** 1.

MURPHY, W.H., JR, WIENS, A.L. & WATSON, D.W. (1958) Impairment of innate resistance by triiodothyronine *Proc. Soc. exp. Biol. (N.Y.)*, **99,** 213.

NAKHLA, L.S. & GOGGIN, M.J. (1973) Lymphocyte transformation in chronic renal failure. *Immunology*, **24,** 229.

NEWBERRY, W.M. & SANFORD, J.P. (1972) Defective cellular immunity in renal failure: depression of reactivity of lymphocytes to phytohemagglutinin by renal failure serum. *J. clin. Invest.* **50,** 1262.

PIEN, F.D., SMITH, T.F., ANDERSON, C.F., WEBEL, M.L. & TASWELL, H.F. (1973) Herpesviruses in renal transplant patients. *Transplantation*, **16,** 489.

ROCKLIN, R.E. & DAVID, J.R. (1971) Method of production of MIF by human blood lymphocytes. *In Vitro Methods in Cell-Mediated Immunity* (ed. by B. R. Bloom and P. R. Glade), p. 281. Academic Press, New York.

SELROOS, O., PASTERNACK, A. & VIROLAINEN, M. (1973) Skin test sensitivity and antigen-induced lymphocyte transformation in uraemia. *Clin. exp. Immunol.* **14,** 365.

SILK, M.S. (1967) The effect of uremic plasma on lymphocyte transformation. *Invest. Urol.* **5,** 195.

STRAUCH, B., SIEGEL, N., ANDREWS, L. & MILLER, G. (1974) Oropharyngeal excretion of Epstein–Barr virus by renal transplant recipients and other patients treated with immunosuppressive drugs. *Lancet*, **i,** 234.

WEBEL, M.L., BRIGGS, W.A. & LIGHT, J.A. (1974) Lymphocyte transformation in hemodialyzed renal failure patients. *Clin. Res.* **22,** 52A.

WILSON, W.E.C., KIRKPATRICK, C.H. & TALMAGE, D.W. (1965) Suppression of immunologic responsiveness in uremia. *Ann. intern. med.* **62,** 1.