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Immune potential in human uraemia

1. Relationship of glomerular filtration rate to depression of immune potential

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SYNOPSIS Aspects of immune potential in uraemic subjects, categorized by glomerular filtration rate, were intercompared and compared with results obtained from a group of normal volunteers. Evidence is presented to show that depression of both cellular and humoral immune potential occurs with progressive reduction of glomerular filtration rate. Lymphocyte transformation testing to the non-specific mitogen PHA revealed a significant elevation of blastogenic response in uraemia after 96 hours of incubation.

Infection is a serious problem in patients with acute and chronic renal failure (Merrill, 1968). It is probable that unselected protein restriction without attention to providing adequate calories and high biological value protein used to contribute to this susceptibility. With the selected low-protein diets used today in the treatment of renal disease, however, it is difficult to believe that malnutrition is the only cause of reduced immune potential.

Dammin *et al* (1957) showed prolonged survival of skin homografts in uraemic patients, normal electrophoretic patterns of antibody proteins, and normal γ-globulin levels. Smiddy *et al* (1960, 1961) supported the finding of prolonged survival of skin homografts in experimentally uraemic rabbits with a delay in the increase in weight of the draining regional lymph nodes as compared with healthy controls.

Lymphocyte population abnormalities are documented in uraemia by Daniels *et al* (1970, 1971a, 1971b) and Sakai *et al* (1970). These authors propose that the changes occurring in lymphocytopenic uraemic patients reflect the preferential decrease of long-lived small lymphocytes and a relative increase of short-lived lymphocytes with fast nucleic acid turnover rates.

The antibody-raising ability of uraemic subjects has been an area of some controversy. Stoloff *et al* (1958) reported a normal diphtheria antitoxin synthesizing ability in uraemia, while Wilson *et al* (1965) showed convincingly low agglutinin titres to *Salmonella typhi* somatic and flagellar antigens after immunization. More recently, Boulton-Jones *et al* (1974) found that antibody titres after immunization with keyhole limpet-haemocyanin were significantly lower in uraemic patients than in normal controls.

This study was undertaken to obtain information regarding the depression of immune potential in uraemia by comparing correlates of immune reactivity between subject groups with various degrees of renal function.

Material and methods

The immune potential of four subject groups has been investigated. One group had normal renal function while the patient groups were classified according to their glomerular filtration rate (GFR) as in table I. The type of renal disease was noted. One-third of the patients had chronic glomerulonephritis and the remainder suffered from a variety of renal diseases including polycystic disease, chronic pyelonephritis, hypertensive nephropathy, gout, and postpartum cortical necrosis.

The following test scheme was employed.

DAY ONE

Intradermal skin testing was performed using

Tuberculin PPD 10 units in 0·1 ml

Candida albicans 0·05 ml of 1% extract

Trichophyton 0·05 ml of 1% extract

Streptokinase (10 u), streptodornase (2·5 u) in 0·05 ml.

These tests were observed after 48 hours and the diameter of induration was recorded.

A serum sample was used to determine:

(1) IgG, IgM, and IgA concentrations by the technique of Mancini *et al* (1965);

(2) salmonella agglutinin titres by the standard method but with a modified dilution series.

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Female	Male	Classification	Group	Age (years)	Mean GFR (ml/min creatinine clearance)
10	4	GFR <5 ml/min	<5	34·6 ± 12·8	2·6 ± 0·8
3	2	GFR 5—10 ml/min	5—10	41·6 ± 16·6	7·3 ± 0·9
4	2	GFR 10—25 ml/min	>10	39·3 ± 12·6	18·6 ± 2·9
4	7	Normal controls	C	36·2 ± 10·7	—

Table I *The subject groups investigated*

Suspensions of somatic and flagellar antigens of *Salmonella typhi*, paratyphi A, and paratyphi B were employed;

(3) tetanus antitoxin titre by a modification of the technique described by Fulthorpe (1957).

A lyophilized standard antiserum was always titrated simultaneously with the test sera to ensure reproducibility of results.

DAY FIVE

The subject was immunized with 0·1 ml TABT vaccine (BP) intradermally.

Contact sensitization was induced by the application of 2 mg 2,4,dinitrochlorobenzene (DNCB—Analar, BDH) contained in 0·1 ml of 20% acetone in olive oil. The area of application was protected with an occlusive dressing for 24 hours, after which time the subject was instructed to wash the site.

A lymphocyte suspension was prepared by a method modified from Coulson and Chalmers (1967). The blastogenic response to 5 μl PHA (Wellcome Reagent Grade), tetanus toxoid at 0·01, 0·1, and 1·0 Lf/ml, a standardized *Candida albicans* antigen (1/10, 1/100, and 1/1000), and tuberculin PPD (250, 400, and 500 units per ml) were investigated. The cultures were pulsed with 2 μ Ci ^{3}H-thymidine for 3 hours before harvesting at 72, 96, and 120 hours. Blastogenic indices were expressed as:

(No. of mM ^{3}H thymidine ($\times 10^{10}$) absorbed per 10^6 lymphocytes in the stimulated culture) − (same value in unstimulated culture).

DAY SEVENTEEN

A serum sample was used to determine titres of Salmonella agglutinins and tetanus antitoxin.

Two patch tests of DNCB (0·05% and 0·1% w/v) were placed on the skin of the lower forearm and covered for 24 hours with occlusive dressings. Forty-eight hour-induration diameters were recorded.

DAY NINETEEN

Lymphocyte transformation testing was performed using as mitogenic stimulants 5 μl PHA and tetanus toxoid (0·01, 0·1, and 1·0 Lf/ml). Harvesting was performed as before.

Results

The results are presented in tables II and III and figures 1 and 2. The pooled variance *t* test was used to assess the statistical significance of inter-group variations. Log_{10} titres were compared. The 5% level was taken as the lower limit of significance.

Serum levels of IgG, IgM, and IgA did not differ significantly from the control group in any of the groups of patients with renal failure.

Significant differences in antibody to Salmonella somatic and flagellar antigens were not demonstrated in the pre-immunization sera, suggesting equality of previous antigenic experience in the four groups. Both the <5 and 5—10 groups had significantly lower titres of tetanus antitoxin than either >10 or the controls ($P < 0·05$). There was no significant difference between >10 and the controls.

Results of immunization-induced antibody production are presented in table II. Intradermal immunization with TABT vaccine showed *Salmonella paratyphi* A and B somatic and flagellar antigens to produce consistently low-titre sera in both normal and uraemic subjects. Statistical analysis of these results showed no significant difference between titres achieved by the four groups with respect to *Salm. paratyphi* B antigens. Significantly lower titres to *Salm. paratyphi* A somatic antigens were found in >10 than in the controls ($P < 0·01$) and to *Salm. paratyphi* A flagellar antigens in <5 than in the controls ($P < 0·02$).

Responses to *Salm. typhi* antigens were greater than to the other Salmonellae in all groups. Mean $\log_{10}$ antibody titres to *Salm. typhi* somatic antigens show a progressive increase from groups <5 to 5—10 to >10 to controls, the two groups with lowest GFR having significantly lower titres than the >10 group ($P < 0·05$) and this group in turn being significantly lower than the controls ($P < 0·01$). The response to *Salm. typhi* flagellar antigens also shows significant differences, both <5 and 5—10 producing lower titres than either >10 or the controls ($P < 0·002$). The responses to tetanus toxoid show the same pattern, the two groups with lowest GRF having significantly lower titres than either group >10 or the controls ($P < 0·05$).

Antigen	*Group*			
	<5	5—10	>10	*Controls*
Tetanus toxoid	1·72 (1·29)	1·32 (1·30)	2·56 (1·66)	2·99 (1·62)
Salm. typhi 'O'	1·23 (1·07)	1·44 (0·62)	2·04 (0·99)	2·64 (0·70)
Salm. typhi 'H'	2·13 (0·90)	2·18 (0·77)	3·26 (0·49)	3·16 (0·54)
Salm. paratyphi A 'O'	0·75 (0·57)	0·68 (0·37)	0·45 (0·16)	0·98 (0·41)
Salm. paratyphi A 'H'	0·37 (0·17)	0·68 (0·85)	0·88 (0·77)	0·99 (0·87)
Salm. paratyphi B 'O'	1·23 (0·70)	0·88 (0·44)	0·84 (0·41)	0·85 (0·31)
Salm. paratyphi B 'H'	1·07 (0·97)	0·74 (0·62)	1·35 (1·18)	1·83 (1·24)

Table II *Means and standard deviations (in parentheses) of log_{10} titres 17 days after TABT immunization in the four groups studied.*

Time (hours)	*Group*	*Blastogenic index*	
		Mean	*SE*
72	<5	247	73
	5—10	220	63
	>10	205	—
	Controls	187	29
96	<5	248	37
	5—10	268	125
	>10	72	16
	Controls	154	33
120	<5	176	28
	5—10	238	96
	>10	57	24
	Controls	128	35

Table III *The blastogenic indices of the four groups in response to phytohaemagglutinin*

The sum of the 48-hour skin induration diameters to the four antigens PPD, *C. albicans*, trichophyton, and streptokinase-streptodormase are compared in figure 1. A trend of diminishing response with decreasing renal function is shown with significant differences between <5 and >10 and <5 and controls.

Although extensive lymphocyte transformation testing was performed using the common antigens, tuberculin PPD and *C. albicans* extract, no significant differences were apparent between any of the four subject groups. The time of the peak blastogenic response changed with antigen concentration (eg, tuberculin PPD at 250 u/ml: peak at 96 hours; tuberculin PPD 400 and 500 u/ml: peak at 120 hours), but analysis of group data showed lymphocytes from all subject groups to respond similarly. Group-group variations in the ^{3}H-thymidine uptake of unstimulated lymphocyte cultures were insignificant and showed no relationship to glomerular filtration rate.

The timing of peak blastogenic response to PHA was different in the groups studied, the peak occurring later in groups <5 and 5—10 than in the controls (table III). The peak response achieved in the four groups did not differ significantly. When com-

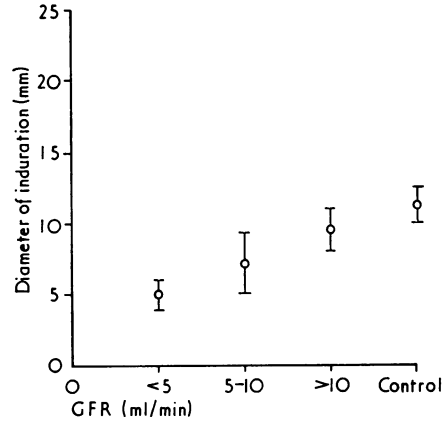

Fig 1 *Mean ± SEM of the 48-hour induration diameters after intradermal injection of tuberculin PPD,* C. albicans, *trichophytin, and streptokinase–streptodormase in the four groups studied.*

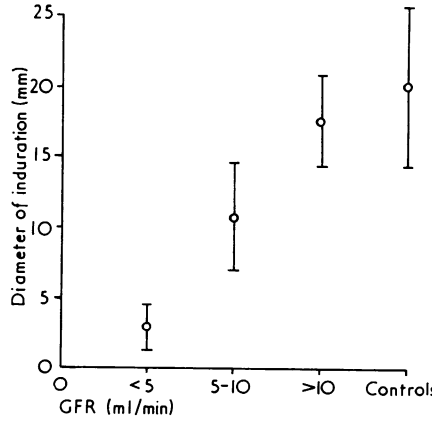

Fig 2 *The 48-hour induration diameters (mean ± SEM) to 0·05 ml 1% dinitrochlorobenzene 12 days after sensitization in the four groups studied.*

parison was made between the groups after the same incubation time significant differences were demonstrated between < 5 and > 10 and between < 5 and controls after 96 hours of culture. In this case, < 5, the group with GFR less than 5 ml/min, had the greater blastogenic response.

The diameter of induration induced by patch testing after sensitization to DNCB is shown in figure 2. Similar results were obtained with both patch test concentrations. Significant differences were found between < 5 and 5—10, < 5 and > 10, and < 5 and controls.

The blastogenic response to tetanus toxoid both before and after immunization was poor. Significant differences were not found between groups when either the pre- or post-immunization results were compared. The only difference observed was that of a non-significant decrease in index 14 days after immunization in the control group while all uraemic groups showed an increase.

Discussion

This study was designed to intercompare parameters of cellular and humoral immune reactivity in three subject groups with differing glomerular filtration rates, and also to compare the results with those obtained from a group of normal volunteers.

No attempt was made to match these patients to control subjects with respect to sex. The age range of each subject group is similar (table I).

The interpretation of responses to TABT could be complicated by inequality of previous immunological experience of these antigens, some individuals producing a primary and some a secondary response. The mean ages of the groups are similar, and significant differences in pre-immunization antibody titres to Salmonella antigens were not found. This implies that valid comparisons can be made of inducible antibody titres between the groups with respect to these antigens. The two groups with lowest GFR both had significantly less tetanus antitoxin than the others before immunization. While this could represent differences in the proportion of individuals who had previously received tetanus toxoid, a more likely explanation, based on their subsequent responses to other antigens, is that the observation reflects their lower immune potential.

The responses obtained to *Salm. paratyphi* A and B antigens were poor throughout all the groups. Although differences were demonstrable with respect to *Salm. paratyphi* A antigens, clearer trends were seen with the better immunogens tetanus toxoid and *Salm. typhi* 'O' and 'H'. In general the induced antibody titres fell with decreasing GFR. In the response to *Salm. typhi* somatic antigens significant decreases were observed between each of the four groups, while with the tetanus toxoid and *Salm. typhi* flagellar antigens the two groups with lower GFR were significantly less than both those with GFR > 10 ml/minute and the controls. The > 10 group produced antibody titres to these antigens which did not differ significantly from the controls. As antibodies to flagellar antigens and to tetanus toxoid are normally of IgG class, the finding that the same patients had impaired responses to *Salm. typhi* 'O' antigen, normally of IgM class, leads to the tentative suggestion that responsiveness in the IgM class is impaired at a slightly higher GFR than responses in the IgG class. In general, the degree of renal impairment at which a significant decrease in humoral potential begins to appear corresponds to a GFR of approximately 10 ml/min.

The results are in agreement with those of Boulton-Jones *et al* (1974) with respect to tetanus toxoid and extend those of Wilson *et al* (1965) who showed reduced inducible titres to *Salm. typhi* antigens in uraemic patients. Souhami's (1971) work in experimentally uraemic mice implies that the mice have an antigen-processing fault which reduces their immune response to soluble protein antigens, while their responses to a particulate antigen (sheep erythrocytes) remained normal. We failed to show a difference in response between the particulate Salmonella and the soluble tetanus toxoid antigens present in TABT vaccine.

The apparent controversy regarding reports for (Wilson *et al*, 1965; Souhami, 1971) and against (Stoloff *et al*, 1958; Balch, 1955) a reduced humoral immune potential in uraemia appear to be resolved by this study and that of Boulton-Jones *et al* (1974). The inability of uraemic patients to produce high-titre antibodies does not appear to be due to protein depletion but to result from a defect in one, or a number, of the steps which normally lead to the synthesis of antibody molecules. Are the antibodies produced 'avid'? The production in inbred nephritis-prone mice of antibody with subnormal average association constants after immunization with human serum albumin (Soothill and Steward, 1971) demonstrates an obvious need for similar investigations in human uraemia since the low-antibody titres we have demonstrated in response to vaccination could be due to both quantitative and qualitative defects in the antibodies produced.

The cellular immune potential of these subject groups has been investigated from several angles. Mean 48-hour induration diameters to a bank of common antigens are presented in fig 1 as a measure of pre-existing cellular immune potential. In addition, reactivity after sensitization to DNCB was used to measure the inducible component of the cellular

response. Figures 1 and 2 show the pattern of depressed cellular immune potential with decreasing glomerular filtration rate. Whether the depression of cellular reactivity in uraemia is due to poor effector mechanisms or to a reduced ability to produce sensitized T-lymphocytes after antigenic stimulation, or both, is unresolved by this work.

Lymphocyte transformation to tuberculin PPD was performed only in those subjects who demonstrated positive delayed hypersensitivity skin tests. Although there was a diminished blastogenic index in all three renal failure groups as compared with the controls the differences were not significant.

Transformation with tetanus toxoid was performed on lymphocytes from all subjects both before and after immunization with TABT. The response pattern was consistent throughout the antigen concentrations employed. Tetanus toxoid appears to be only a weak inducer of cell-mediated immunity, resulting in low blastogenic indices. The blastogenic indices did increase with increasing filtration rate but the differences between each renal failure group and the control group were not significant.

It is important to note that no significant group deviation from normal was shown in the ^{3}H thymidine uptakes of unstimulated cultures. Group differences noted in blastogenic index to the above antigens, therefore, were not wholly or partially due to inter-group control culture blastogenic variations.

The blastogenic response of uraemic lymphocytes to the non-specific mitogen PHA is a source of controversy. Kasakura and Lowenstein (1967) and Boulton-Jones *et al* (1974) showed human 'uraemic' lymphocytes to react normally to PHA, whereas Huber *et al* (1969) described a depressed response. We found a raised blastogenic response to PHA after 96 hours of culture in uraemic subjects. It is interesting to note from table III that the peak response to reagent-grade PHA in groups < 5 and > 10 appears to be delayed relative to the controls, suggesting that the time response pattern to stimulation is altered in these uraemic subjects. Such a difference in the pattern of response might account for inconsistencies in previous reports.

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