

Serum IgG and Renal Transplantation

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Summary

In 57 patients with renal allografts the prolonged administration of prednisolone ≥ 1 mg/kg/day and azathioprine ≥ 3 mg/kg/day caused a significant and persistent fall in serum IgG at all levels of creatinine clearance. The fall in IgG was more striking when creatinine clearance was below 25 ml/min. At lower doses of azathioprine and prednisolone serum IgG fell when the creatinine clearance was less than 35 ml/min, the degree of recovery towards normal being dependent on creatinine clearance and dosage. Post-transplant haemodialysis decreased the depression of IgG, and patients with immediately functioning grafts had minimal IgG depression. An inverse relation between IgG and IgM was observed in some patients. Severe infections and toxicity were associated with the greatest reduction in IgG; leucopenia and thrombocytopenia were not consistently reliable guides to toxicity. The deaths of four patients (7%) were associated with severe infections. Falls in IgG were not related to the rejection process. IgG measurement should be used as a guide to immunosuppression and toxicity in renal allograft patients.

Introduction

The lack of specificity of the immunosuppressive agents in patients with renal allografts often gives rise to fatal infections and toxicity (Hill *et al.*, 1967). The toxic effects of azathioprine are generally monitored by the peripheral white blood and platelet counts. However, the concomitant use of prednisolone, with its effects on the bone marrow, may make the circulating white cell count difficult to interpret (Woodruffe *et al.*, 1969). The finding of hypogammaglobulinaemia and *Pneumocystis carinii* infection in a renal allograft patient prompted a serial study of serum immunoglobulins in other renal transplant patients. Previous studies of immunoglobulins in patients with renal allografts over the short term showed inconsistent results (Zuhke *et al.*, 1967; Levine *et al.*, 1970; Rodriguez-Iturbe *et al.*, 1971; Wells and Jeremy, 1971). In the present study the follow-up of serum immunoglobulins was prolonged. The pattern of change was evaluated and related to the dosage of immunosuppressive agents, the creatinine clearance, the white cell and platelet counts, and the clinical events including rejection, infection, and other toxic manifestations.

Patients and Methods

Fifty-seven patients on the renal failure programme (Moorhead *et al.*, 1970) were selected for cadaveric renal allografts. Only

one patient did not receive maintenance haemodialysis before transplantation. No patient had a splenectomy or thymectomy. Of the 57 patients 25 were selected for study on the basis of completeness of follow-up and graft function for more than one month. Twenty patients had first, and five had second grafts. There were 11 men and 14 women (age range from 20 to 57 years). The duration of follow-up varied from two to 36 months (mean 17.4 months) (table I). In cases 2, 3, 6, 12, and 13 pre-transplant serum immunoglobulins were determined in retrospect from stored sera; post-transplant studies were started from the eighteenth, seventeenth, twelfth, tenth, and eighth month after operation respectively.

Donor selection, preservation of donor kidneys, and histocompatibility testing were consistent, and have been previously described (Festenstein *et al.*, 1971). Post-transplant haemodialysis was carried out in all these patients with Kiil dialysers. The number of haemodialyses that the patients required before the creatinine clearance reached 10 ml/min varied from 1 to 41, and the time taken to achieve this level of renal function was up to 78 days (tables I and II). Haemodialysis frequency varied between 1 and 5 times a week, depending on the clinical state of the patient, each session lasting from six to eight hours.

TABLE I—Serum IgG Range in 25 Cadaveric Renal Allograft Patients

Series No.	Case No.	Age	Sex	Lowest Serum IgG in mg/100 ml ("., of Pretransplant Level*)	Highest Serum IgG in mg/100 ml ("., of Pretransplant Level*)	Duration of Follow-up (months)
1	2	48	F.	750 (51)	1,950 (123)	25
2	3	41	M.	620 (41.6)	1,300 (87)	26
3	6	30	M.	500 (33)	1,050 (78)	28
4	12	26	M.	780 (53)	1,800 (121)	34
5	13	37	F.	480 (31)	980 (66)	24
6	15	30	F.	560 (38)	1,170 (79)	36
7	17	33	M.	800 (73)	1,700 (156)	30
8	18	43	F.	700 (64)	1,500 (138)	26
9	21	57	M.	640 (51)	1,200 (96)	19
10	23	23	F.	720 (41)	1,400 (80)	25
11	24	43	M.	840 (47)	1,850 (102)	15
12	27	29	F.	450 (45)	900 (90)	12
13	28	23	M.	520 (47)	1,500 (136)	24
14	29	35	M.	720 (80)	1,400 (155)	21
15	30	43	F.	500 (50)	2,400 (240)	18
16	31	41	F.	460 (19)	2,000 (83)	18
17	33	42	M.	680 (57)	1,650 (138)	18
18	35	48	F.	580 (50)	1,400 (121)	15
19	36	20	F.	1,100 (48)	1,600 (70)	12
20	39	45	M.	760 (49)	1,600 (103)	16
21	43	46	M.	500 (33)	1,130 (75)	6
22	44	46	F.	740 (74)	1,700 (170)	12
23	54	25	F.	315 (29)	900 (83)	2
24	55	39	F.	430 (44)	1,120 (114)	3
25	56	50	F.	410 (41)	950 (95)	3

*Pretransplant IgG = 100%.

IMMUNOSUPPRESSION

Bolus intravenous injections of azathioprine 200 mg and prednisolone 200 mg were given postoperatively. Thereafter, the patients were given azathioprine by mouth. The dose of azathioprine was controlled in the last 43 patients (table III, group B) by keeping serum IgG above 500 mg/100 ml, the white blood count above 5,000 mm³, and platelet count above 150,000 mm³. The maintenance dose of azathioprine three months after the transplantation was 50-150 mg/day. Prednisolone was given by mouth in a dose of 100 mg/day, reducing to 30 mg/day by the end of the first month. The dose was further reduced by 2.5 mg weekly until a maintenance dose of 10-15 mg/day was reached. Antilymphocyte globulin was not used.

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TABLE II—Outcome of 57 Consecutive Renal Allografts from March 1968 to February 1973

Case No.	Date of Transplant	Outcome	Plasma Creatinine (mg/100 ml)	Case No.	Date of Transplant	Outcome	Plasma Creatinine (mg/100 ml)
1	21/3/68	Non-function due to cortical necrosis. Re-transplanted (case 29)		30*	12/5/71	Second graft. Alive and well	2.8
2*	8/10/68	Died of unknown cause after 46 months		31*	5/6/71	Alive and well	1.8
3*	15/10/68	Alive and well	1.8	32†	12/6/71	Died of intracranial haemorrhage at four months	
4	21/11/68	Non-function due to renal artery thrombosis		33*	22/6/71	Alive and well	2.5
5	27/1/69	Rejection at 10 months		34	17/7/71	Non-function due to renal vein thrombosis	
6*	22/3/69	Rejection at 36 months		35*	15/8/71	Alive and well	1.8
7	8/4/69	Non-function due to renal artery stenosis		36*	21/8/71	Second graft. Alive and well	1.5
8†	26/4/69	Died of <i>Pneumocystis carinii</i> pneumonia and <i>Escherichia coli</i> septicaemia at 10 months		37	29/8/71	Non-function due to renal artery thrombosis	
9	28/4/69	Rejection at five months		38	4/9/71	Non-function due to rejection	
10†	2/5/69	Rejection at six months. Died of air embolism after return to haemodialysis		39*	15/9/71	Alive and well	
11	29/5/69	Rejection after 26 months. Re-transplanted (case 55)		40†	4/11/71	Died of debilitation and <i>Staphylococcus aureus</i> septicaemia after return to haemodialysis	
12*	17/6/69	Alive and well	1.6	41	6/11/71	Rejection at four months	
13*	14/7/69	Alive and well	1.2	42†	5/12/71	Died of cardiac arrest of unknown cause on first day	
14†	19/7/69	Died of intracranial haemorrhage, <i>Pseudomonas</i> lung abscess, and septicaemia at two months		43†	11/1/72	Died of transplant lung at five months	
15*	20/1/70	Alive and well	1.5	44*	21/1/72	Rejection and ureterovesical obstruction at 13 months	
16	17/3/70	Non-function due to rejection. Re-transplanted (case 28)		45†	17/2/72	Rejection at two months. Died of pulmonary embolism and <i>Klebsiella</i> septicaemia	
17*	19/4/70	Alive and well	1.0	46	27/3/72	Alive and well	1.3
18*	30/4/70	Alive and well	1.4	47	13/4/72	Chronic rejection	3.5
19	14/5/70	Non-function due to renal artery and vein thrombosis. Re-transplanted (case 30)		48	4/5/72	Alive and well	1.1
20	24/5/70	Non-function due to rejection. Re-transplanted (case 36)		49	16/5/72	Non-function due to cortical necrosis	
21*	11/10/70	Alive and well	2.6	50	22/5/72	Alive and well	1.6
22	9/11/70	Non-function due to rejection		51†	12/6/72	Non-function due to cortical necrosis. Died of cerebrovascular accident after return to haemodialysis	
23*	27/11/70	Rejection at 28 months		52	17/6/72	Alive and well	1.2
24*	9/12/70	Alive and well	2.3	53	24/9/72	Transplant nephrectomy for uncontrollable haemorrhage from graft bed on second day	
25*	2/1/71	Died of accidental endotracheal tube extubation on third day		54*	3/11/72	Graft nephrectomy for transplant lung at two months	
26	30/1/71	Non-function due to rejection		55*	2/2/73	Second graft. Alive and well	3.4
27*	14/4/71	Alive and well	1.0	56*	6/2/73	Alive and well	1.8
28*	28/4/71	Second graft. Alive and well	1.4	57	26/2/73	Alive and well	2.1
29*	8/5/71	Second graft. Alive and well	1.9				

*Patients included in this study.

†Died.

TABLE III—Summary of Outcome of 57 Consecutive Renal Allografts from March 1968 to February 1973

Outcome	Group A (Cases 1-14)	Group B (Cases 15-57)
Alive and well	3	22
Failure	11	21
Causes:		
Rejection	5	8
Sepsis	2	3
Thrombosis of graft vessels	2	2
Cortical necrosis	1	2
Transplant lung	—	4
Miscellaneous	1	4

During the initial 18 months of the transplantation programme azathioprine 200-300 mg and prednisolone up to 200 mg daily was used in the first 14 patients (table III, group A) for two weeks or longer during obvious or suspected rejection episodes. These doses were not controlled by IgG monitoring.

In the last 43 patients (table III, group B), during a rejection episode, prednisolone was increased to 60 mg/day until the episode was controlled and then gradually reduced to the maintenance level over four weeks. One or more of the following were given additionally to both groups of patients: intravenous hydrocortisone 100 to 400 mg/day; actinomycin C 200 µg/day intravenously for a maximum of three doses; and local graft irradiation, 200 rads for not more than three times. The dose of azathioprine was not altered during a rejection episode except in relation to the current serum IgG level, white blood cell count, and platelet count.

POST-TRANSPLANT FOLLOW-UP AND ANALYSIS

Serum IgG, IgA, and IgM were measured by the radial immunodiffusion technique (Mancini *et al.*, 1965). Serum creatinine and creatinine clearance were measured by the Technicon AutoAnalyzer, and white blood cell and platelet

counts by the Coulter counter. These tests were carried out daily for two weeks, on alternate days for the next two weeks, and at progressively longer intervals thereafter. Plasma urea and electrolytes, packed cell volume, liver function tests, 24-hour urinary protein, and other relevant tests were performed often. Patients were on a particular dosage of prednisolone and azathioprine for a minimum of three days preceding these tests. In cases where frequent alterations of immunosuppressive drugs were required the average dosages of the preceding three days were calculated. Serum immunoglobulin results were expressed as percentages of the pretransplant values. Prednisolone and azathioprine doses were classified into five groups (figs. 1 and 3), and a correlation was made between serum IgG

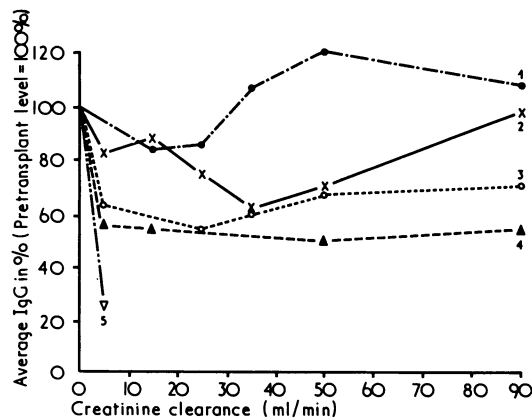


FIG. 1—Correlation of azathioprine and prednisolone dosages, creatinine clearance, and mean serum IgG in 25 renal allograft patients no longer requiring haemodialysis. Group 1 = Prednisolone 0.25 mg/kg/day and azathioprine 1 mg/kg/day. Group 2 = Prednisolone 0.5 mg/kg/day and azathioprine 2 mg/kg/day. Group 3 = Prednisolone 1 mg/kg/day and azathioprine 3 mg/kg/day. Group 4 = Prednisolone 1.5 mg/kg/day and azathioprine 4 mg/kg/day. Group 5 = Prednisolone 2 mg/kg/day and azathioprine 5 mg/kg/day.

and the dosage in each group. The IgG results were obtained and plotted as the mean for each dosage group (fig. 3). Correlation with creatinine clearance was performed when patients no longer required post-transplant haemodialysis. The IgG results, classified on the basis of the prednisolone and azathioprine dosage, were grouped according to creatinine clearance: the mean IgG was then plotted for each level of creatinine clearance (fig. 1).

A longitudinal study was made of 15 patients (cases 6-20, table I) during the first 12 months after transplantation. The mean creatinine clearance and IgG is shown in fig. 2, and the coefficient of linear correlation and level of significance were calculated. Correlations of clinical events and serum immunoglobulin changes were made.

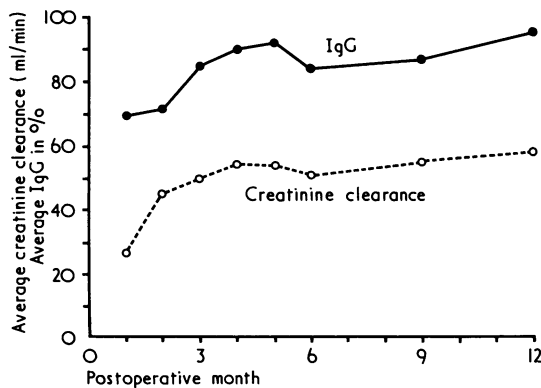


FIG. 2—Mean serum IgG and creatinine clearance in 15 renal allograft patients followed up for 12 months.

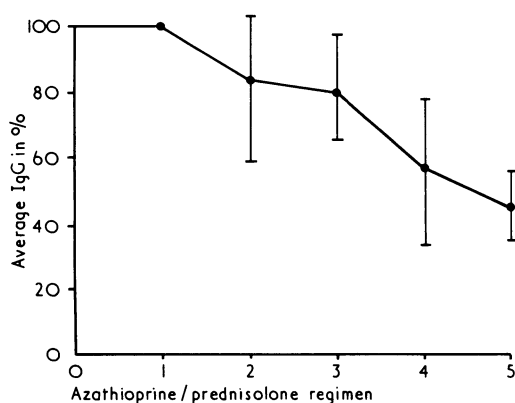


FIG. 3—Correlation of azathioprine and prednisolone dosages (mean serum IgG \pm 2 S.D.) in 25 renal allograft patients during period of post-transplant haemodialysis.

Results

The results of 57 consecutive renal cadaveric allografts are shown in tables II and III. Serum IgG, invariably depressed at some point in the postoperative course of all the patients studied, varied from 80% to 90% of pretransplant values (table I). The changes in IgG in patients who no longer required haemodialysis in the postoperative period are shown in fig. 1.

GROUP 1

Patients in group 1 (prednisolone 0.25 mg/kg/day and azathioprine 1 mg/kg/day) had a small fall of 17% in the average serum IgG levels when the creatinine clearance was less than 25 ml/min. IgG concentration returned to pretransplant level and above when the creatinine clearance exceeded 35 ml/min.

GROUP 2

In group 2 (prednisolone 0.5 mg/kg/day and azathioprine 2 mg/kg/day) the maximal depression was 40%, when the creatinine clearance was below 35 ml/min. As in group 1 improvement in creatinine clearance was followed by a gradual rise of serum IgG to the pretransplant levels.

GROUP 3

In group 3 (prednisolone 1 mg/kg/day and azathioprine 3 mg/kg/day) serum IgG depression was more than 30%, and this persisted at all levels of glomerular filtration rate.

GROUP 4

This phenomenon was even more obvious in patients in group 4 (prednisolone 1.5 mg/kg/day and azathioprine 4 mg/kg/day) when the depression approximated 50%, though creatinine clearance levels reached 90 ml/min.

GROUP 5

A profound fall of more than 70% was observed in patients in group 5 (prednisolone 2 mg/kg/day and azathioprine 5 mg/kg/day).

The overall pattern of IgG change is exemplified by case 56 (fig. 4), where IgG fell to 40% (410 mg/100 ml) after pulse immunosuppressive therapy was given for the first rejection episode. The fall was less precipitous (IgG 580 mg/100 ml) after the second rejection when the creatinine clearance was 21 ml/min. This patient showed an inverse relation between IgG and IgM. The fall in IgG during immunosuppression was accompanied by a rise in IgM. Recovery of creatinine clearance plus reduction of immunosuppression was followed by a rise in IgG and a concomitant fall in IgM (fig. 4).

Escherichia coli urinary infection was present at the height of the IgG depression, but the association could be fortuitous. Leucopenia or thrombocytopenia was, however, absent.

The relation between the depression of serum IgG and mean creatinine clearance is also shown in fig. 2. Both these parameters were significantly related ($r=0.8663$, $P<0.01$). Improvement of IgG followed gradual reduction of immunosuppressive drugs and was maximal when maintenance doses were reached towards the end of the 12-month period. IgG depression was minimal in patients whose renal graft "worked on the table." This is illustrated by cases 17 and 45 (table II) in whom the creatinine clearance was 51.4 ml/min and 86.4 ml/min respectively on the first postoperative day. The maximal IgG depression during that period was 12% for the first patient and nil for the second.

EFFECT OF HAEMODIALYSIS

At each dosage level of prednisolone and azathioprine in haemodialysed patients (groups 1 to 5) the mean serum IgG depression was much less than the corresponding value in transplanted patients with creatinine clearances of less than 10 ml/min (fig. 1) who no longer required haemodialysis. In group 5 the mean serum IgG was 55% of pretransplant values in patients during the haemodialysis period compared with the 25% in those who no longer required haemodialysis.

IGG AND INFECTION

In the first 14 grafts high doses of immunosuppressive drugs were used (table III, group A). Two patients (14.3%) died of sepsis (cases 8 and 14, table II). In the last 43 cases (table III,

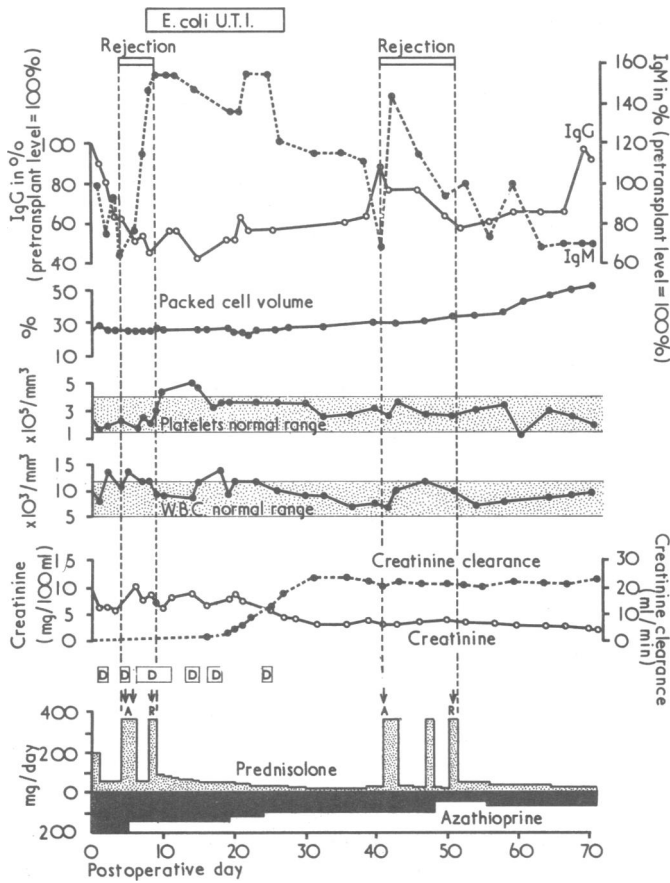


FIG. 4—Post-transplant course of case 54 (chronic glomerulonephritis). A=Actinomycin. R=Deep x-ray graft irradiation. D=Haemodialysis. U.T.I.=Urinary tract infection.

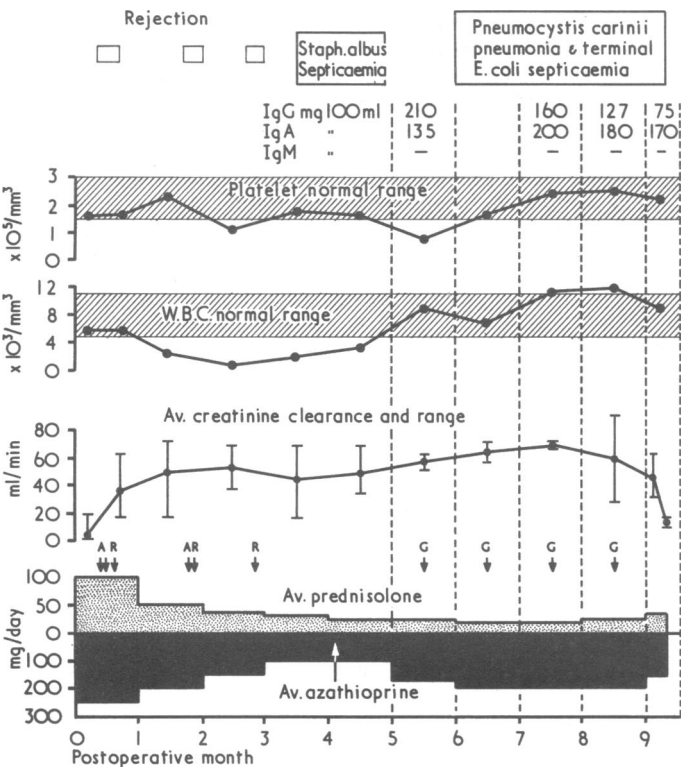


FIG. 5—Post-transplant course of case 8 (congenital hypoplastic kidneys). Association of hypogammaglobulinaemia and *Pneumocystis carinii* pneumonia. G=Gammaglobulin administration.

group B), two patients (4.7%) (cases 40 and 45) also died of sepsis. One (case 40) had gross debilitation and *Staphylococcus aureus* septicaemia (table II). The IgG level was 340 mg/100 ml. The other (case 45) died from pulmonary embolism and *Klebsiella* septicaemia, the IgG level being 440 mg/100 ml (table II).

A low serum IgG was associated with *Pneumocystis carinii* pneumonia, diagnosed by lung biopsy, in case 8 (fig. 5). In this patient the average azathioprine dose was more than 3 mg/kg/day and prednisolone more than 0.5 mg/kg/day. The creatinine clearance remained at 50 ml/min until two days before death from *E. coli* septicaemia and respiratory failure. Total immunoglobulins were less than 500 mg/100 ml, IgA was normal, IgM was not measurable, IgG was very low at 210 mg/100 ml and continued to decrease until it was 75 mg/100 ml, in spite of the administration of gammaglobulin by injection at monthly intervals. White cell count was normal during this period.

In case 54 (fig. 6) low IgG was associated with "transplant" lung. IgG depression after the first rejection was further aggravated after a second rejection episode. Coinciding with the maximum depression of IgG (310 mg/100 ml) the patient developed a combination of pyrexia, extreme muscular weakness, tachypnoea, dyspnoea on the slightest exertion, hypoxia, and fluctuating renal function. Chest auscultation and radiography were uninformative. Respiratory function tests showed a restrictive type of defect with reduction in transfer factor. No systemic bacterial, viral, or fungal infection could be identified. Leucopenia was present. Cessation of immunosuppression, graft nephrectomy, and return to haemodialysis were resorted to. Her symptoms improved spontaneously. Gammaglobulin was given and serum IgG returned to normal.

REJECTION AND MONITORING OF IMMUNOSUPPRESSION BY SERUM IgG

Rejection caused loss of graft function in five of the 14 cases (36%) in group A patients who received high dose immunosuppression initially and in eight of the 43 cases (19%) in group B (table III). Thus lower doses of immunosuppressive drugs did not cause an increased incidence of graft rejection in the latter group.

Discussion

Various patterns of change in immunoglobulins have been described in renal allografts (Zuhke *et al.*, 1967; Levine *et al.*, 1970; Rodriguez-Iturbe *et al.*, 1971; Wells and Jeremy, 1971). In this study the only consistent trend detected was IgG depression. High dosage of immunosuppressive drugs, low levels of creatinine clearance, and haemodialysis were the major influencing factors. When the creatinine clearance was less than 25 ml/min depression of IgG was common at any dosage level of the immunosuppressive drugs, and became more severe at the higher dosages. Prolonged utilization of prednisolone > 1 mg/kg/day and azathioprine > 3 mg/kg/day caused a significant fall of serum IgG and a rise in creatinine clearance not matched by improvement in IgG concentration. Though in group 5 (fig. 1) there was insufficient data to correlate IgG and creatinine clearance beyond 5 ml/min, the results in groups 3 and 4 suggest that the drastic fall would be sustained in spite of improvement in glomerular filtration.

Haemodialysis was associated with a fall in IgG which was smaller than expected. This phenomenon was conspicuous with higher doses of prednisolone and azathioprine. Ultrafiltration on haemodialysis did not cause a significant change in serum IgG level—a fact confirmed in a study of non-grafted haemodialysis patients (Z. Varghese and J. F. Moorhead, unpublished data).

Though IgG depression was most obvious in relation to graft rejection it was clear that rejection alone could not account for it, as the maximum fall of IgG occurred after the completion of

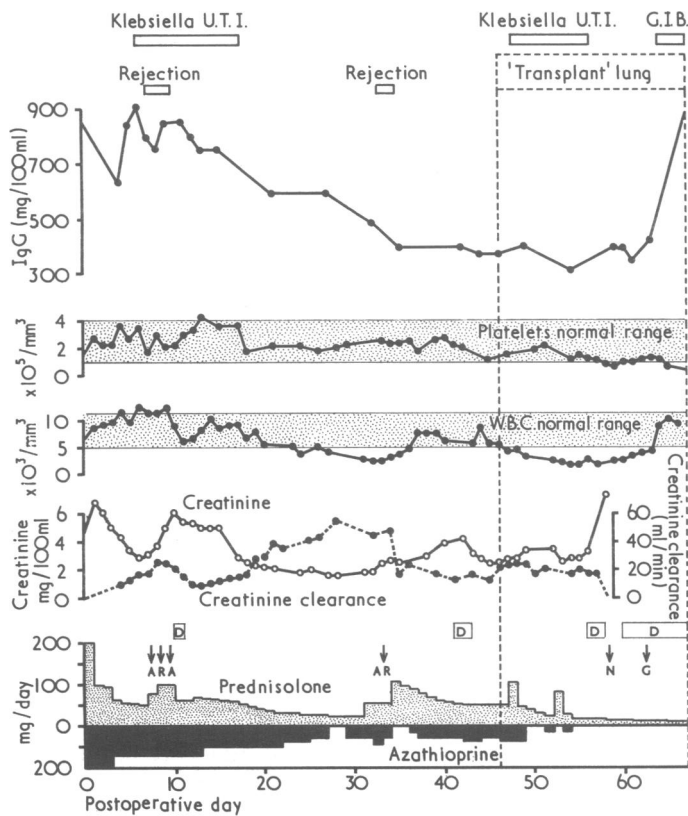


FIG. 6—Post-transplant course of case 54 (congenital hypoplastic kidneys). Association of low serum IgG and "transplant" lung. N=Graft nephrectomy. GIB=Gastrointestinal bleeding.

the pulse therapy. The degree of IgG depression appeared to be a function of the quantity and duration of immunosuppressive therapy, the current creatinine clearance, and the sensitivity of the patient to these drugs. The latter is illustrated in case 54 (fig. 6), in which IgG depression persisted in spite of improvement of creatinine clearance and reduction of immunosuppression.

Patients with established functioning renal allografts often had minimal or no IgG depression, as did those who achieved good diuresis and adequate creatinine clearance immediately after operation.

Though in cases 2, 3, 6, 12, and 13 high-dose immunosuppression was given initially, very low serum IgG was not seen since immunoglobulin studies for these patients were started from eight to 18 months after the operation—at a time when maintenance immunosuppression was used and renal function had become adequate and stable. In case 14 the patient died of sepsis, but serum IgG was not measured because its significance had not been appreciated at the time.

Haemodilution could not account for falls in serum IgG. Serial packed cell volumes, a good clinical indicator of acute changes in plasma volume, bore no relation to IgG concentration (fig. 4). Loss of immunoglobulins in the urine was found to be insufficient to account for the serum changes (Levine *et al.*, 1970). The possible effect of blood transfusion could also be discounted, as these were given infrequently, and in any case bore no time relationship to the occurrence of IgG depression.

In studies of immune response in patients with autoimmune disease Swanson and Schwartz (1967) found that 65% of their patients had a significant selective fall of IgG during treatment with azathioprine accompanied by enhanced IgM synthesis. This phenomenon had been previously reported (Sahiar and Schwartz, 1965) in rabbits treated with mercaptopurine, and the authors suggested that the fall in IgG might cause IgM synthesis to be started through activation of a negative feedback

process. Our finding of a reciprocal relation between serum IgG and IgM in some of our patients—for example, case 56 (fig. 4)—agrees with this observation. However, the fall in IgG cannot be attributed solely to azathioprine therapy, since prednisolone—a mainstay of treatment in transplantation—is capable of decreasing IgG concentrations in chronic active hepatitis (Mackay, 1968).

The effect of actinomycin C on serum IgG is not known, as it has always been used in combination with other drugs in these patients. Irradiation of the graft is unlikely to contribute to IgG change. The use of a combination of immunosuppressive drugs for conquering graft rejection made analysis of individual drugs difficult.

The association of low IgG and septic complications in renal allografts was reported by Harris *et al.* (1971) who found low IgG and leucopenia in six out of seven patients who had "late" infections after transplantation. The proximity of these to rejection and its treatment was emphasized. Anderson *et al.* (1973) found that azathioprine at 2 mg/kg/day was not associated with deaths due to infections in renal allograft patients. The data we have presented in this study show that at a dose of 2 mg azathioprine/kg/day and prednisolone 0.5 mg/kg/day serum IgG improves if creatinine clearance rises, but at a dose of azathioprine ≥ 3 mg/kg/day and prednisolone ≥ 1 mg/kg/day IgG depression persists irrespective of glomerular filtration rate.

The complexity of the clinical transplant setting makes it difficult to attribute the lower incidence of fatal infections in the last 43 patients to the monitoring of IgG concentrations alone. Increased experience and awareness in the detection and management of complications are important contributing factors.

Our data are insufficient for meaningful statistical analysis, but we suggest that monitoring of IgG—particularly in the immediate post-transplant period when IgG might be expected to be low—is useful in modifying immunosuppressive dosage. Of 466 transplant deaths in Europe in 1970-1 (Brunner *et al.*, 1972) no less than 38.1% were due to uncontrollable infection in the first two post-transplant months. The same study pointed out that 18% of cadaver kidney recipients in 1970-1 died of infections, and that infection was likely to remain the principal cause of death with present immunosuppressive regimens. Experience in the United Kingdom alone is similar, infection—the principal cause of mortality—accounted for 41% of deaths in renal allograft patients (Royal College of Physicians, 1972).

In comparison with leucocyte and platelet counts IgG measurement has proved to be a more consistent, reliable, and objective guide—probably of greater value in respect of azathioprine than prednisolone dosage.

Frequent measurements of serum IgG, in addition to haematological counts should be carried out when the creatinine clearance is less than 25 ml/min, and when high dose immunosuppression is used. Serum IgG should probably be kept above 500 mg/100 ml. Below this arbitrarily chosen concentration fatal sepsis occurred in three patients. Azathioprine of ≥ 3 mg/kg/day and prednisolone ≥ 1 mg/kg/day should not be used for long periods, and frequent monitoring of toxicity at this dose level is essential.

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Specific Lymphocyte Stimulation by Purified, Heat-inactivated Hepatitis-B Antigen

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Summary

The ability of purified, heat-inactivated hepatitis-B antigen (HB_{Ag}) to stimulate sensitized lymphocytes in vitro was investigated with the lymphocyte stimulation test on lymphocytes from three groups of individuals. Stimulation was minimal in the lymphocytes of two out of 15 normal controls, whereas lymphocytes from nine out of 12 patients who had recovered from hepatitis B showed stimulation, as did lymphocytes from five out of 12 laboratory technicians who had been regularly exposed to HB_{Ag} but who had no history of hepatitis or signs of it in the previous two years. No differences were observed in the responses to phytohaemagglutinin of lymphocytes from persons in the three groups. HB_{Ag} and HB_{Ag} inactivated by heat were shown in immunodiffusion to be immunologically identical. Inactivated HB_{Ag} stimulated antibody production in guinea-pigs. These findings suggest that not only humoral but also cell-mediated immunity might be induced by vaccination with purified, heat-inactivated HB_{Ag}.

Introduction

The role of humoral and cell-mediated immunity against hepatitis-B antigen (HB_{Ag}), formerly called Australia antigen or hepatitis-associated antigen (H.A.A.), in hepatitis B (serum hepatitis) is not at present clear. In many viral diseases elimination of the virus depends on intact cell-mediated immunity. This may also be important in the case of hepatitis B (Dudley et al., 1972 a). Cell-mediated immunity against HB_{Ag} in patients recovered from acute hepatitis B has been studied with crude preparations of HB_{Ag} (Yeung Laiwah, 1971; Dudley et al., 1972 b). In vitro lymphocyte stimulation and leucocyte migration inhibition in the presence of serum containing HB_{Ag} or of liver tissue homogenates, however, might be due to other antigens. A test for cell-mediated immunity which used purified HB_{Ag} would therefore be preferable.

Experiments have shown that HB_{Ag}-positive serum is inactivated by heat (Krugman et al., 1970), and that active immunization with heat-inactivated serum provides some protection against hepatitis B infection (Soulier et al., 1972; Krugman and Giles, 1973). But whether the protection is due to humoral or cell-mediated immunity and whether the cell-mediated immunity has been induced by the vaccination is uncertain. We report here our investigations of the antigenic properties in vitro of purified, heat inactivated HB_{Ag} in relation to humoral and to cell-mediated immunity.

Materials and Methods

In vitro lymphocyte stimulation tests were done on lymphocytes from three groups of persons seronegative for HB_{Ag}. The first group consisted of 15 persons with no history or signs of hepatitis (normal controls); the second consisted of 12 patients who had recovered from acute hepatitis B (the interval between the disappearance of HB_{Ag} from their serum and the stimulation test varied from two to 72 weeks); and the third consisted of 12 laboratory technicians who had been regularly exposed to HB_{Ag} but who had shown no signs of hepatitis during a two-year period of monthly screening for serum alanine transaminase, serum aspartate transaminase, and HB_{Ag} and who had no history of hepatitis.

HB_{Ag} (subtypes ad and ay) was isolated from pooled HB_{Ag}-positive sera by a three-stage method (Houwen et al., 1973). Sera diluted 1:3 with phosphate-buffered saline were ultracentrifuged at 350,000 g for six hours and the pellets resuspended and chromatographed on Sepharose 4B. HB_{Ag}-positive fractions were pooled and concentrated. Human serum proteins were removed from the solution by immunoabsorption, and shown by immunodiffusion to have been removed. Moreover, this purified HB_{Ag} preparation raised a monospecific antiserum against HB_{Ag} in guinea-pigs.

The purified HB_{Ag} was heated in a waterbath at 100°C for 3 min. The HB_{Ag} titre had decreased by two dilution steps after the heating, probably owing to some precipitation and aggregation. HB_{Ag} was measured by radioimmunoassay (Ausria-125, Abbot Lab.). Hepatitis B antibodies (HB_{Ab}) were identified by counterelectrophoresis and reactions of identity by immunodiffusion.

For the lymphocyte stimulation tests suspensions of lymphocyte-monocytes were used which had been isolated from defibrinated blood by Ficoll-Isopaque gradient centrifugation according to the technique of Böyum (1968). Three hundred thousand lymphocytes were cultured in 12-75 mm Falcon tubes in 1 ml MEM-Eagle with 20% autologous serum supplemented with penicillin 100 IU/ml and streptomycin 100 µg/ml. Cultures

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