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Immunologic Status of Uremic Patients

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The immunologic status of 25 uremic patients was studied with a battery of tests evaluating the humoral and cytological aspects of immunity. The individual's humoral immune status was evaluated as follows: quantitation *of the three major serum immunoglobulins (IgG, IgA and IgM) expressed in mg/100 ml of serum and compared to established normal clinical standards (±2 SD/mean);* qualitation *of IgG and IgM evaluated by specific virus antibody titers and antitoxin values associated with IgG and by isoagglutinin titers of the ABO blood groups associated with IgM. Immunoglobulin status is grouped into hyper- and hypo-immunoglobulin variations from normal and correlated with serum complement values ($Beta_{1C}$ / $_{1A}$ and hemolytic activity). The* cytological status *in the evaluation consists of assaying the individual's ability to produce interferon by the Sendai-Sindbis virus system in peripheral blood leukocytes. Results of the study emphasize the need for individual evaluation of uremic patients to enable more effective immuno-depressive therapy before and after renal transplantation.*

Some investigators have suggested that long-standing uremia depresses the lymphoreticular system with resulting immuno-suppression.[3,4] Understanding the nature and degree of such immuno-depression may be critically important in the control of renal allograft rejection reactions. In order to differentiate between graft rejections and super infections, there should be precise evaluation of the patient's immunologic status before renal allograft.[5]

Our approach to what is termed "immunologic evaluation of the individual" is an attempt to measure the biologic status of the lymphoreticular system quantitatively and qualitatively. The quantity of the three major serum immunoglobulins is determined by methods, clinical standards and criteria reported elsewhere.[1,2,6] In this paper we report the degrees and variations in immunologic factors found in 25 uremic patients. The findings offer some interesting aspects to organ transplantation problems and suggest a different view in the management of the patient, both before and after organ transplantation.

Patients and Methods

Twenty-five patients in the acute and chronic stages of uremia were evaluated. Although multiple evaluations were made, the tables have been simplified to show only one evaluation for each patient. The parameters employed in evaluating individual immunologic

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status are shown in Figure 1. The humoral immune status is reflected in the quantitation and qualitation of the major immune factors in the serum. The cytological immune status is partially reflected in the interferon response in peripheral blood leukocytes. In this report quality of the IgG class is indicated by the antibody responses to 12 enteric viruses,[1,7] four upper respiratory viruses and two antitoxins. IgM is qualitated by the ABO blood group isoagglutinin titers in serum when applicable. IgA cannot be satisfactorily qualitated at this time.

Quantitative Determination:

Serum immunoglobulins (IgG, IgA and IgM) were determined by a micro-double diffusion in agar technic developed and standardized in this laboratory.[6] Normal adult serum immunoglobulin levels have been found to range (mean ± 2 standard deviations) as follows: 600-1400 mg/100 ml for IgG; 30-135 mg/100 ml for IgA and 40-120 mg/100 ml for IgM. Serum globulin ($Beta_{1C}/_{1A}$) complement was quantitated by the same micro-double diffusion technic as employed for the Igs. Normal serum level by this procedure was found to be 100-200 mg/100 ml serum (± 2 SD from mean value).

Serum Neutralizing Antibody Titers:

Virus neutralizing antibody (VNAT) titers were determined for the enteric viruses. These were carried out in plastic plates rather than the usual tissue culture tube method.[1] Twelve strains

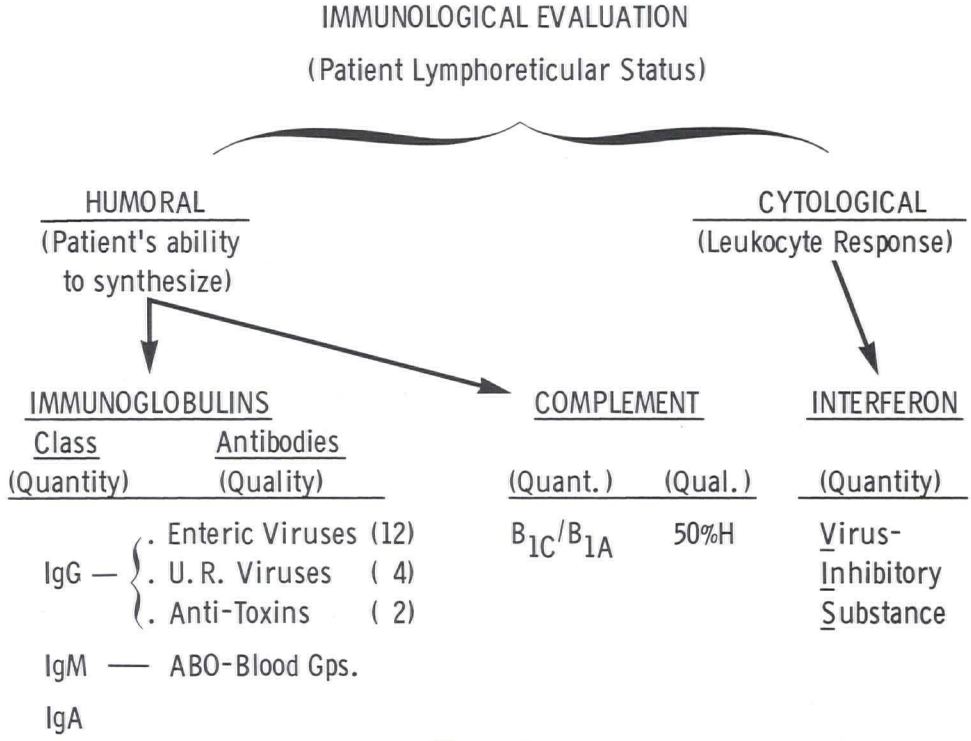

Figure 1

of entero viruses are routinely used to evaluate the antibody quality associated with IgG. These include: poliomyelitis prototypes 1, 2 and 3; Coxsackie A9, B-1, B-2, B-3, B-4, B-5 and Echo 6, 9 and 14. Enteric virus antibody titers generally are consistently present throughout the year and are more reliable measurement for individual evaluation studies. In addition, the enteric viruses selected for antibody evaluation in individual patients are those virus strains which are consistently present in blood bank plasma pools throughout the year.[1]

Hemagglutination Titration:

The hemagglutination procedures used for diptheria and tetanus antitoxins associated with IgG were essentially the method of Levine et al,[8] but adapted to microtiter technics in plastic micro plates.[9] Serum dilution titers were converted to antitoxin units per ml of sera by parallel tests conducted with known standard antitoxins. Diphtheria and tetanus antitoxin values $\leq$0.04 units/ml are considered adequate serum residual levels for a competent immunoglobulin-G system. Lesser quantities are not detectable and warrant challenge with toxoid doses for anamnistic responses. Qualitation of IgM was determined by isoagglutinin titers to the ABO blood groups by standard blood bank procedures.

In Vitro Interferon Response of Peripheral Blood Leukocytes:

The individual's leukocyte immune status in regard to viruses (ie his ability to respond with interferon production) was determined *in vitro*. In a comparative study between humoral immune status and interferon response in health and disease we found no interferon titer of 1:8 or less among healthy individuals fulfilling our criteria.[2] Therefore, titers of 1:32 or greater are considered "good responses" in this test system; a titer of 1:16 is a "low borderline response" and a titer of 1:8 or less, a "poor response."

Serum Complement

Complement activity was determined at the 50% hemolytic end point as described by Heidelberger.[10,11] We modified this procedure slightly in the dilutions used and determined the hemolytic activity by optical density at 545 millimicrons in a Coleman Jr. Spectrophotometer. Complement activity was calculated and expressed as 50% hemolytic units per ml of serum. The mean normal value for this procedure is 175 ($\pm$1 SD) with a range of 150 to 200 units/ml. However, for practical reasons we consider 2 SD as meaningful and the values ($\pm$2 SD), which range from 125 to 225 units/ml, as more significant. See Tables I-IV.

Results

The data on the 25 uremic patients have been grouped into five tables (I to V) according to immunoglobulin levels. Because the immune status for individuals with serial studies did not vary with time and repeated evaluations, only one evaluation is given for each patient. Arrows indicate those values above or below two standard deviations from the mean as established in this laboratory.[6] The remaining three parameters (antibody response, leukocyte interferon yield, and complement factors) are compared with each of the immunoglobulin find-

Table I

IMMUNOLOGIC STATUS OF UREMIC PATIENTS

IgG-Group

Pt.	IMMUNOGLOBULINS: (Ig-Quantity)*			ANTIBODY RESPONSE: (IgG-Quality)		VIS (WBC)	COMPLEMENT 50%H U/ml	COMPLEMENT $\beta_{1C/1A}$ mg%
	IgG	IgA	IgM	12 Vir's**	2-Tox's***	1:32	150-200	100-200
U-8	420↓	19↓	18↓	7/10	1/2	64	91	167
U-10	309↓	61	28↓	6/10	0/2	NT	132	101
U-11	304↓	99	106	4/10	2/2	NT	137	132
U-17	370↓	72	138↑	4/10	2/2	32	93	155
U-21	370↓	24↓	61	7/10	2/2	32	76	29
U-22	404↓	24↓	14↓	3/10	1/2	64	153	126
U-7	1777↑	217↑	104	5/10	2/2	NT	187	195
U-24	1564↑	98	161↑	7/10	2/2	8	111	117

*Ig Values (2SD) ** $\geq$ 1:16 titer *** $\geq$ 0.04 u/ml

NT = Not Tested

ings. In table I, the eight uremic patients who showed variations from normal in IgG are grouped together. Six of the eight showed low IgG quantity, and two showed greater than two standard deviations above the normal serum levels for IgG. Although IgG values are low in quantity in six of these patients, the quality is good. This is reflected in the high serum antibody titers found for 12 enteric viruses and two toxins tested; the numerators represent the number of viruses having antibody titers equivalent to or greater than 1:16 dilution. In antitoxin values, the numerator indicates serum levels $\geq$0.04 units/ml, the minimal detectable amount. In the third column labeled VIS (Virus Inhibitory Substance) a level above 1:32 dilution is found in all but one of the patients tested. In the last column, the serum complement values show a significant decrease in hemolytic activity, and in one instance a decrease in the quantitation of $Beta_{1C/1A}$. Of the two hyper-immunoglobulin-G patients, one shows low hemolytic activity associated with normal quantity of $Beta_{1C/1A}$ component while the other has normal complement values.

Table II includes those patients who had elevated IgA levels. Eight of the 25 patients with uremia fell into this group, showing elevations of IgA above two standard deviations from normal mean value. Since the biologic activity of IgA cannot be adequately assayed at this time, its quality cannot be determined. Antibody responses associated with IgG were good and interferon responses were found to be low in two of the five patients tested. Decreased complement values were found in six of the eight patients. We are not certain whether the low complement

Table II

IMMUNOLOGIC STATUS OF UREMIC PATIENTS

IgA-Group (↑)								
Pt.	IMMUNOGLOBULINS: (Ig-Quantity)*			ANTIBODY RESPONSE (IgG-Quality)		VIS (WBC)	COMPLEMENT 50%H U/ml	COMPLEMENT $\beta_{IC/IA}$ mg%
	IgG	IgA	IgM	12 Vir's**	2-Tox's***	1:32	150-200	100-200
U-1	1080	145↑	80	4/11	2/2	NT	128	95
U-2	1392	165↑	70	7/11	2/2	32	100	150
U-7	1777↑	217↑	104	5/10	2/2	NT	187	195
U-9	948	168↑	94	10/10	2/2	16	0	138
U-13	856	231↑	30↓	4/10	NT	NT	119	86
U-19	949	182↑	53	6/10	2/2	28	90	95
U-23	1330	150↑	115	7/10	1/2	4	221	177
U-25	1023	254↑	98	10/10	2/2	32	114	68

* Ig Values (2SD) ** $\geq$ 1:16 titer *** $\geq$ 0.04 u/ml

NT = Not Tested

Table III

IMMUNOLOGIC STATUS OF UREMIC PATIENTS

IgA-Group (↓)

Pt.	IMMUNOGLOBULINS: (Ig-Quantity)*			ANTIBODY RESPONSE: (IgG-Quality)		VIS (WBC)	COMPLEMENT 50%H U/ml	COMPLEMENT $\beta_{IC/IA}$ mg%
	IgG	IgA	IgM	12 Vir's**	2-Tox's***	1:32	150-200	100-200
U-5	902	25↓	28↓	5/12	1/2	64	256	339
U-8	420↓	19↓	18↓	7/10	1/2	64	91	167
U-18	1226	29↓	46	4/9	1/2	32	93	83
U-21	370↓	24↓	61	7/10	2/2	32	76	29
U-22	404↓	24↓	14↓	3/10	1/2	64	153	126

*Ig Values (2SD) ** $\geq$ 1:16 titer *** $\geq$ 0.04 u/ml

Table IV

IMMUNOLOGIC STATUS OF UREMIC PATIENTS

IgM-Group

Pt.	IMMUNOGLOBULINS: (Ig-Quantity)* IgG	IgA	IgM	ANTIBODY RESPONSE: (IgG-Quality) 12 Vir's**	2-Tox's***	VIS (WBC) 1:32	COMPLEMENT 50%H U/ml 150-200	$\beta_{1C/1A}$ mg% 100-200
U-8	420↓	19↓	18↓	7/10	1/2	64	91	167
U-5	902	25↓	28↓	5/12	2/2	64	256	339
U-6	805	109	17↓	4/12	1/2	256	140	108
U-13	856	231↑	30↓	4/10	2/2	16	119	86
U-22	404↓	24↓	14↓	3/6	1/2	64	153	126
U-10	309↓	61	28↓	6/10	0/2	NT	132	101
U-4	942	85	151↑	5/12	0/2	128	217	167
U-17	370↓	72	138↑	4/10	2/2	32	93	155
U-24	1564↑	98	161↑	7/10	2/2	8	111	117

* Ig Values (2SD) ** $\geq$ 1:16 titer *** $\geq$ 0.04 u/ml

NT = Not Tested

values indicate the individual's inability to synthesize this component, or whether increased utilization is taking place from antigen-antibody reactions.

Table III shows five uremic patients with IgA values below normal. It will be noted that two of these (patient U-8 and U-22) are below normal in all three immunoglobulin classes. In spite of this the antibody responses are considered good in terms of specific neutralizing antibody titers associated with IgG. Leukocyte interferon responses are within normal range of activity, and complement factors are well below normal values in three of the five patients.

Table IV gives the data on patients who show alterations from normal in the IgM class; six show low values and three show IgM values above two standard deviations from the mean. The viral antibody titers for all nine patients in this group were good, even though four of the nine patients had below standard serum level values for IgG. All patients with IgM values below normal who were tested for leukocyte interferon showed normal responses, whereas one of three patients with IgM values above normal showed "poor" interferon response in peripheral blood leukocytes. Complement values varied in this group without a consistent trend. The IgM group was similar to the IgG and IgA groups in regard to variations from normal and lack of correlation between complement factors and interferon responses.

Table V shows that six of the 25 patients had normal values for all three immunoglobulin classes. In this group all six had good virus serum antibody levels, all but one showed normal titers against bacterial toxins, all had normal interferon responses, and only one pa-

Table V

IMMUNOLOGIC STATUS OF UREMIC PATIENTS

	IMMUNOGLOBULINS: (Ig-Quantity)*			ANTIBODY RESPONSE: (IgG-Quality)		VIS (WBC)	COMPLEMENT 50%H U/ml	COMPLEMENT $\beta_{1C/1A}$ mg%
Normals	IgG	IgA	IgM	12 Vir's**	2-Tox's***	1:32	150-200	100-200
U-3	565 ±	42	47	6/12	1/2	32	69	131
U-12	645	94	59	10/10	0/2	NT	195	104
U-14	1179	86	87	3/10	2/2	128	149	93
U-15	796	126	54	8/10	2/2	>128	182	101
U-16	770	131	111	4/10	2/2	64	145	119
U-20	978	56	98	8/10	2/2	32	120	132

* Ig Values (2SD) ** $\geq$ 1:16 titer *** $\geq$ 0.04 u/ml

NT = Not Tested

tient showed low complement activity.

The isoagglutinin titers associated with the IgM of all patients studied with ABO blood group types were found to be greater than 1:16 in serum dilution. To simplify the data these results were not included in the tables.

Discussion

The degrees and variations in the immunologic status of 25 uremic patients were considerable. Although both serum immunoglobulin deficiencies and excesses were found, immunoglobulin dyscrasias, ie, normal Ig values without biologic activity,[1] were not detected among the 25 patients studied. The quality of specific antibody responses associated with IgG and IgM are adequate in uremic patients as reflected in the virus neutralizing antibody titers, antitoxin values and isoagglutinin titers to the ABO Blood Groups. Suitable qualitation associated with IgA is not available at this time. In regard to the cellular defense system, leukocyte interferon responses were found to be "poor" in two patients and "low borderline" in two others. All of the remaining patients tested showed good leukocyte interferon responses. Our interpretation of the poor and low responses suggests a leukocyte inefficiency in the mass population of the peripheral blood leukocytes or an inability of the leukocytes to respond with interferon production by some physiologic block. What this means to the host's defense mechanism warrants further investigation.

The measurements on serum complement quantity ($Beta_{1C/1A}$) and associated hemolytic activity vary from normal status to persistently low values in some patients. No consistent correlation could be found between serum complement values and the other immune factors studied.

The immunologic variations in 25 uremic patients indicate that few generalizations can be made and that individual appraisal of each patient's immune status may be required. In addition, more adequate clinical interpre-

tation and qualification may be necessary if immmuno-depressive therapy is to be applied more rationally before and after kidney transplantation in uremic patients. Individuals with immunoglobulin excesses may necessitate and tolerate larger doses of immunodepressive drug therapy than individuals with immunoglobulin deficiencies. Moreover, patients with immunoglobulin deficiences may warrant passive immunity therapy (similar to agammaglobulinemic patients) prior to surgery in the form of commercial gammaglobulin, hepatitis-free pooled plasma[13,14] or both. Current practice in the management of homotransplantation tends to follow a pattern of dosage of immunosuppressive agents varying only with time and evidence of graft rejection. The results reported here suggest that therapy should be based on the individual's immune status. Obviously "rules of thumb" are to be avoided in the prophylaxis of transplant rejection and we suggest the same considerations for *any* individual being evaluated for *any* organ transplantation.

The changes in immunologic status following nephrectomy and kidney transplantation have been studied in more detail and are planned for a subsequent publication.

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