IMMUNOLOGIC STUDIES IN HUMAN ORGAN TRANSPLANTATION

I. Observation and Characterization of Suppressed Cutaneous Reactivity in Uremia*

By CHARLES H. KIRKPATRICK,‡ M.D., W. E. C. WILSON,§ M.D., and DAVID W. TALMAGE, M.D.

(From the Department of Medicine, University of Colorado Medical Center, Denver)

(Received for publication, December 10, 1963)

Following sensitization, the cutaneous response to an antigen may be manifested by an immediate wheal and erythema or by a delayed induration. While the pathogenesis of these reactions is incompletely understood, certain characteristics demonstrate that they represent distinct expressions of immunologic capability. Within any species, the capacity for the wheal and erythema response can be passively transferred by serum, but delayed sensitivity can be passively transferred only by immunologically competent cells or, in man, by extracts of these cells. Important advances in the understanding of immunologic reactivity have accrued from the recognition and study of its impairment by a variety of drugs and disease states. The present report describes a decreased cutaneous reactivity found in uremia.

Human renal transplantation has provided an opportunity for investigation of the mechanism of impaired expression of immunological potential in chronic renal disease. Not only does this procedure correct the metabolic derangement; but the correction involves the passive transfer of immunologically competent tissue and the administration of purine analogues and adrenal steroids, thus affording a unique model in which to study the interrelationships between a number of factors influencing the immunologic response.

The capacity of patients with chronic renal failure to react to a panel of antigens expected to elicit immediate and delayed responses was compared to the reactivity of a normal group. Since these normal subjects were being evaluated as kidney donors, in most instances there existed a genetic relationship between members of the control and experimental groups. Furthermore, the most frequent unrelated donor was the patient's wife and therefore there was control for environmental exposure. In the postoperative period the kidney

* Supported by United States Public Health Service Research Grant No. AI-04152.

‡ Special Post-Doctoral Fellow, United States Public Health Service.

§ Research Fellow of the American College of Physicians, 1962–65.

recipients were retested for delayed sensitivities so that factors influencing passive transfer of the reactivities from the kidney donor could be evaluated.

Materials and Methods

The 28 subjects of this study were patients with chronic renal diseases and advanced uremia who were being considered for renal transplantation. The majority of the patients had chronic glomerulonephritis although several other diseases were represented. The salient

TABLE I

Description of Donor and Recipient Groups

Recipients						Donors			
Patient	Initials	Age	Sex	Diagnosis*	BUN	Initials	Age	Sex	Relationship
		yrs.			*mg per cent*				
1	S. W.	15	F	Polycystic	116	B. W.	41	F	Mother
2	T. E.	16	M	Polycystic	99	B. G.	39	F	Mother
						V. W.	25	M	None
3	R. C.	46	M	Polycystic	140	G. C.	43	M	Brother
						W. W.	22	M	None
4	L. C.	42	M	CPN	285	D. C.	38	M	Brother
5	R. W.	42	M	Gout. neph.	190	W. B.	37	M	None
6	A. C.	10	F	H-S P	137	P. C.	30	F	Mother
7	L. S.	6	M	CGN	53	M. S.	36	F	Mother
8	M. S.	15	M	CGN	122	M. S.	34	F	Mother
9	D. S.	15	F	CGN	74	M. S.	37	F	Mother
10	L. M.	16	F	CGN	55	M. M.	49	M	Father
11	F. R.	18	M	CGN	198	F. R.	18	M	Ident. twin
12	N. W.	18	F	CGN	118	W. W.	40	F	Mother
13	M. C.	19	F	CGN	172	R. K.	21	M	None
14	T. S.	20	M	CGN	132	F. S.	45	F	Mother
15	M. H.	21	M	CGN	116	A. H.	23	M	Brother
16	L. L.	22	M	CGN	140	M. L.	28	M	Brother
17	J. R.	26	F	CGN	135	L. H.	45	F	Mother
18	G. S.	33	M	CGN	105	G. S.	29	M	Brother
19	B. W.	36	M	CGN	180	J. M.	24	M	None
20	D. R.	39	M	CGN	38	B. B.	43	F	Sister
21	P. H.	40	M	CGN	166	H. B.	28	M	None
22	H. O.	41	M	CGN	116	I. O.	37	F	Wife
23	S. H.	43	M	CGN	118	M. H.	41	F	Wife
24	L. C.	44	F	CGN	166	R. C.	46	M	Brother
25	L. C.	46	M	CGN	178	V. W.	39	F	Sister
26	C. M.	47	M	CGN	41	E. M.	53	M	Brother
27	J. H.	49	M	CGN	132	E. C.	42	F	Sister
28	H. M.	54	M	CGN	204	M. M.	56	F	Wife
						F. H.	36	M	None

* The abbreviations used are: CPN, chronic pyelonephritis; gout. neph., gouty nephropathy; H-S P, Henoch-Schoenlein purpura; CGN, chronic glomerulonephritis.

clinical and laboratory findings in this group of 28 patients are summarized in Table I. Male patients predominated, and the ages of the subjects ranged from 6 years to 54 years with a mean age of 30.0 years. The blood urea nitrogen (BUN) values given in the table were obtained on the day of initial intradermal testing. The low values were the result of recent dialyses. At the time of the initial study no patients were receiving drugs known to alter cutaneous reactivity with the exception of patient 21 who had received 5 to 10 mg of prednisone daily for 1 year. For each patient at least one kidney donor was also studied. The age, sex, and relationship of the donors are summarized on the right side of Table I. This group contained 15 females and 16 males; their mean age was 36.3 years.

The antigens used for these studies were commercial preparations.[1] The immediate type of skin reactivity was evaluated in 25 patients with the following antigenic extracts: mixed tree pollens, mixed grass pollens, mixed chenopod pollens, and mixed ragweed and sage pollens in 1:10,000 dilutions, mixed molds in 1:200 dilution, and purified extracts of *Candida albicans* and *Trichophyton inguinale* in 1:100 dilution. These antigens were given in doses of 0.03 ml intradermally, and the wheal and erythema response was measured in 15 minutes. Delayed reactions to these antigens were assessed 24 hours later. Delayed hypersensitivity was also studied with intermediate strength purified protein derivative, histoplasmin, blastomycin, coccidioidin, and mumps antigen diluted according to the manufacturer's directions. These antigens were given intradermally in a 0.1 ml inoculum and the responses were measured at 24 or 48 hours. Reactions to immediate type antigens were considered to be positive when the wheal and erythema exceeded 1 cm in diameter. A positive delayed reaction developed at least 0.5 cm of induration.

The patients approved for renal transplantation were given as a routine oral azathioprine (imuran, Burroughs Wellcome and Company, Inc., Tuckahoe, New York), usually 3 to 5 mg/kg daily and then subjected to bilateral nephrectomy, splenectomy, and renal transplantation as described by Starzl *et al.* (1). The azathioprine was continued in the postoperative period and adrenal corticoids were added when kidney rejection became apparent. Of the patients studied preoperatively, 18 were available for retesting at least once during the interval between transplantation and the institution of adrenal steroid therapy. An additional 3 patients (patients 7, 16, and 23) were studied even though adrenal steroids were being given. One patient (patient 3) showed evidence of immediate rejection of a transplanted kidney which was removed after 4 hours. He was retested following this procedure and again following a successful transplant from a second donor.

The blood urea nitrogen values and peripheral blood leukocyte and differential counts were obtained on the day that the postoperative tests were read. Representative biopsies were obtained at various times during the evolution of the cutaneous reaction.

---

[1] The pollen mixture, mold mix, *Candida albicans* and *Trichophyton inguinale* antigens were purchased from Hollister-Stier Laboratories, Los Angeles. The tree pollen mix contained extracts from ash, aspen, birch, box elder, cottonwood, native elm, Chinese elm, maple, and willow. The mixed grass pollens contained June grass, timothy, orchard, redtop, and rye. The chenopods mixture contained *Kochia*, lamb's quarters, pigweed, Russian thistle, western waterhemp, and shadscale, and the ragweed-sage mixture contained giant ragweed, short ragweed, false ragweed, western ragweed, marshelder, western sage, mountain sage, and sagebrush. The mold mixture contained *Alternaria*, *Aspergillus* species, *Chaetomium*, *Fusarium*, *Helminthosporium*, *Hormodendrum*, *Mucor*, *Penicillium* species, *Pullalaria*, *Phoma betae*, *Rhizopus nigri*, grain dust and mixed smuts, and yeasts.

Intermediate strength purified protein derivative, histoplasmin, and blastomycin were manufactured by Parke, Davis and Co., Detroit. The mumps antigen was the product of Eli Lilly and Co., Indianapolis, and the coccidioidin was prepared by Cutter Laboratories, Berkeley.

RESULTS

Immediate Hypersensitivity.—The frequency of immediate skin reactions to the panel of antigens was markedly reduced in the uremic subjects. In instances where more than one donor was studied for a single patient, the candidate having the closest genetic and environmental relationship to the recipient was selected for this comparison. As shown in Table II a total of 14 positive reactions was seen in 25 uremic patients compared to 59 positive reactions in their normal donors.

In the normal donor population *Candida* was the antigen found to give the most frequent positive responses. Fifteen of these 25 subjects had a positive reaction to this antigen. These responses represented 25 per cent of the total number of positive immediate reactions. Reactions to *Trichophyton* and mixed molds were frequently observed; however, responses to the pollen antigens were relatively uncommon.

Although the incidence of positive wheal and erythema reactions was low in the group of patients with chronic kidney disease, the most frequent positive reactions were again seen with *Candida*. Five of the uremic patients reacted to this antigen, and these reactions represented 36 per cent of the number of positive tests in this group.

Delayed Hypersensitivity.—The incidence of delayed skin reactions among the 28 uremic subjects and their donor candidates prior to kidney transplantation is summarized in Table III. Twenty-five of the donor-recipient pairs in this study were identical with the subjects recorded in Table II. It is apparent that the incidence of positive reactions was much lower among the uremic patients than the healthy donors, although the ability to respond to the antigens was well preserved in several instances (patients 10, 14, 19, 22, 23, and 28). Of a total of 177 tests performed only 22 positive responses were noted in the uremic group while the corresponding group of donors had 73 positive reactions to the same antigens. All but one of the donor candidates had a positive delayed reaction to *Candida albicans*. The incidence of positive reactions to *Trichophyton inguinale* and mumps antigen was 83 per cent and 82 per cent respectively. As illustrated in the table, although delayed responsiveness was less frequent in the uremic group, these three antigens were responsible for 95 per cent (21/22) of the positive reactions observed.

Passive Transfer.—To detect the transfer of immunologically competent tissue from donor to recipient at the time of renal transplantation, the delayed cutaneous responses to the panel of antigens were restudied postoperatively in 18 patients who were available prior to the institution of adrenal steroid therapy. The results of this investigation are summarized in Table IV. Blastomycin was deleted from this table because there were no reactions to this antigen in either the donor or recipient groups. With respect to patients 2 and 28, the relatives studied preoperatively were not accepted as donors and unrelated

TABLE II

*Preoperative Immediate Hypersensitivity Responses of Recipients and Their Donor Candidates**

			ANTIGEN						
PATIENT	RECIPIENT	DONOR	MOLDS	TREES	CHENOPODS	RAGWEED	GRASSES	CANDIDA	TRICHOPHYTON ‡
1	S.W.	B.W.	▨	▨	▨	▨	▨	▨	N.D.
2	T.E.	B.G.	▨					▨	N.D.
3	R.C.	G.C.	▨						
4	L.C.	D.C.		▨		▨		▨	N.D.
5	R.W.	W.B.	▨					▨	N.D.
6	A.C.	P.C.	▨					▨	▨
7	L.S.	M.S.	▨	▨				▨	N.D.
8	M.S.	M.S.				≡		≡	N.D.
9	D.S.	M.S.							N.D.
10	L.M.	M.M.	▨					▨	▨
11	F.R.	F.R.	▨		▨	▨		▨	N.D.
12	N.W.	W.W.	▨			▨	▨		
14	T.S.	F.S.							▨
15	M.H.	A.H.	≡				▨	≡	≡
16	L.L.	M.L.							N.D.
17	J.R.	L.H.	▨	≡	▨		▨	▨	N.D.
18	G.S.	G.S.	▨					▨	N.D.
19	B.W.	J.M.						≡	≡
20	D.R.	B.B.	▨				▨	▨	N.D.
21	P.H.	H.B.	▨					▨	▨
22	H.O.	I.O.	≡			▨	▨	▨	N.D.
23	S.H.	M.H.						≡	≡
25	L.C.	V.W.	▨					▨	▨
27	J.H.	E.C.	▨	▨	▨	▨		▨	▨
28	H.M.	M.M.	≡					≡	≡

* DONOR RESPONSE POSITIVE ▨

RECIPIENT RESPONSE POSITIVE ≡

‡ N.D., TEST NOT DONE.

TABLE III

*Preoperative Delayed Hypersensitivity Responses of Recipients and Their Donor Candidates**

PATIENT	RECIPIENT	DONOR	PPD	HISTO.	COCCIDIO.	MUMPS	CANDIDA	TRICHOPHYTON
1	S.W.	B.W.						N.D.
2	T.E.	B.G.						N.D.
3	R.C.	G.C.						
4	L.C.	D.C.						N.D.
5	R.W.	W.B.						N.D.
6	A.C.	P.C.						
7	L.S.	M.S.						N.D.
8	M.S.	M.S.						N.D.
9	D.S.	M.S.						N.D.
10	L.M.	M.M.						
11	F.R.	F.R.						N.D.
12	N.W.	W.W.						
13	M.C.	R.K.					N.D.	N.D.
14	T.S.	F.S.						
15	M.H.	A.H.						
16	L.L.	M.L.						N.D.
17	J.R.	L.H.						N.D.
18	G.S.	G.S.						N.D.
19	B.W.	J.M.						
20	D.R.	B.B.						N.D.
21	P.H.	H.B.						
22	H.O.	I.O.						N.D.
23	S.H.	M.H.						
24	L.C.	R.C.					N.D.	N.D.
25	L.C.	V.W.						
26	C.M.	E.M.					N.D.	N.D.
27	J.H.	E.C.						
28	H.M.	M.M.						

* DONOR RESPONSE POSITIVE

RECIPIENT RESPONSE POSITIVE

TABLE IV

Delayed Hypersensitivity Skin Tests of Recipients Postoperatively

Patient	Recipient	Donor	Postop.	Rejection date	BUN	W.B.C. x 10^3 per mm^3	Lymph per mm^3	Azathioprine	Prednisone	PPD	Histo.	Coccidio.	Mumps	Candida	Trichophyton
			day		mg per cent			days	days						
1	S.W.	B.W.	5	21	15	13.8	2900	8	0						N.D.
			22	21	29	11.0	1210	25	1						N.D.
2	T.E.	V.W.	9	10	10	4.8	1320	11	0						N.D.
3	R.C.	G.C.	0	0	85	6.1	550	10	0						
		W.W.	2	15	20	8.6	1120	5	0						
6	A.C.	P.C.	1	5	10	12.6	1260	10	0						
7	L.S.	M.S.	1	1	16	25.3	3290	12	2						
8	M.S.	M.S.	12	22	90	9.4	1030	22	0						
9	D.S.	M.S.	1	33	12	7.3	1900	18	0						
			11	33	19	5.7	2050	28	0						
12	N.W.	W.W.	1	5	7	14.8	1180	9	0						
			6	5	16	12.5	1250	14	1						
13	M.C.	R.K.	0	14	23	3.3	590	2	0						
			4	14	8	10.3	2270	6	0						
14	T.S.	F.S.	1	9	18	11.8	240	11	0						
			3	9	13	9.8	300	13	0						
15	M.H.	A.H.	1	12	82	12.0	360	11	0						
			6	12	20	7.4	960	16	0						
16	L.L.	M.L.	7	2	178	5.8	120	20	6						N.D.
18	G.S.	G.S.	1	31	39	17.8	710	8	0						N.D.
			22	31	32	5.7	570	29	1						N.D.
20	D.R.	B.B.	1	9	38	11.1	2000	13	0						N.D.
			7	9	51	10.3	820	19	0						N.D.
21	P.H.	H.R.	1	6	47	12.5	500	5	0						
22	H.O.	I.O.	1	4	66	5.2	360	26	0						N.D.
23	S.H.	M.H.	1	0	76	17.2	170	6	1						
24	L.C.	R.C.	3	7	20	6.1	370	12	0					N.D.	N.D.
25	L.C.	V.W.	1	0	123	11.5	810	6	0						
27	J.H.	E.C.	1	14	24	10.2	610	6	0						
			6	14	20	7.8	940	11	0						
28	H.M.	F.H.	1	5	86	6.2	310	15	0						

Recipient positive preoperatively ≡ Donor positive preoperatively ▨ Recipient acquired postoperative response ▩

individuals were substituted. During the postoperative period, each of the 18 recipients not on adrenal steroids was found to have a delayed hypersensitivity reaction to at least one antigen to which he had been previously non-reactive. In each instance this reactivity had been demonstrated in the patient's donor preoperatively. The donor group was reactive in a total of 49 tests to which the recipients were non-reactive, and at some time during the postoperative period 40 of these reactivities appeared in the recipient group. Fifteen patients with no preoperative response to the mumps antigen received kidneys from donors who were reactive to this preparation. In each instance a positive

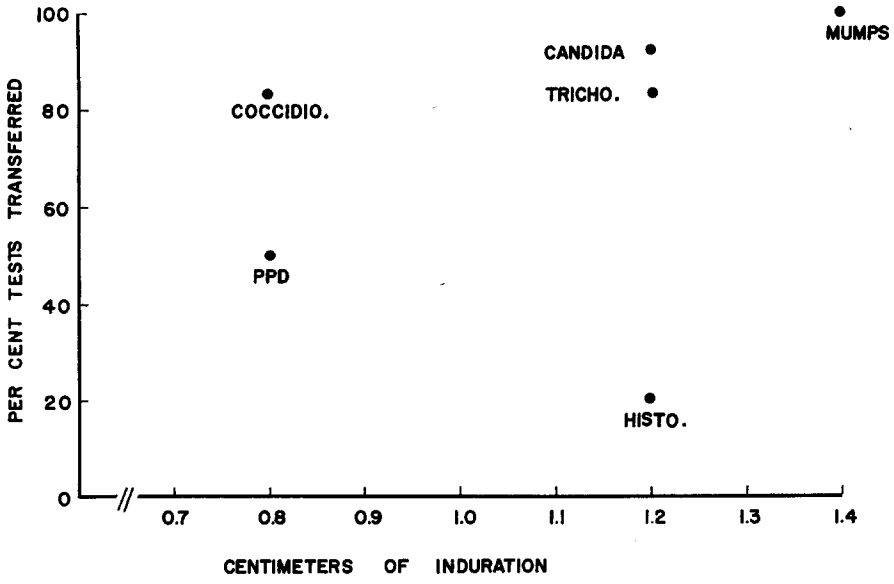

FIG. 1. The relationship of the intensity of donor reactivity to the success of passive transfer of delayed hypersensitivity.

response was found in the recipients at some time during the early postoperative period.

In the donor group the mean diameters of the induration of the delayed reactions were greatest to mumps, *Trichophyton*, and *Candida*. New postoperative reactions were seen most frequently to these three antigens, and in general the frequency of acquired response in the recipient correlated with the mean diameter of reaction to each antigen in the donor (Fig. 1).

Medications and Peripheral Blood Counts.—The number of leukocytes and the absolute lymphocyte count in the peripheral blood were not related to the delayed responses observed in the postoperative kidney recipients. The total leukocyte counts ranged from 3300 to 25,300 and the absolute lymphocyte

count from 120 to 2900 cells per mm^3. The purine analogue, azathioprine, was administered to all recipients for intervals of 2 to 29 days prior to postoperative skin testing. The skin tests which were positive in the recipients prior to surgery remained positive during azathioprine therapy. In the prednisone-treated patients, however, the incidence of positive transferred reactions was reduced (29 per cent). There were no positive reactions in patients who had been receiving prednisone for more than 24 hours. Because all patients were treated with uniformly high doses of this drug (approximately 200 mg daily), therapy is recorded as days of treatment.

Time of Testing.—The majority of the 20 patients was tested during the first 24 hours after surgery and all were studied by the 12th day. Nine members of this group were tested more than once at intervals ranging from 4 to 22 days after transplantation. One patient (patient 3) rejected the transplanted kidney immediately and the graft was removed after 4 hours. It is of interest that he demonstrated no reactions when restudied immediately following this procedure; however, new reactions, identical with those of the second donor, appeared following a successful transplant.

Two patients (patients 13 and 9) showed no reactivity when tested a few hours following transplantation but were reactive when studied 4 and 11 days later. Similar observations were made in 5 other patients (patients 1, 12, 15, 18, and 20). Each of these recipients showed one or more new positive reactions at the time of the second testing. Patient 27 became completely unreactive at his second testing and patient 15 lost one acquired test but gained two positive tests at the time of retesting. Parenthetically, patient 27 was shortly thereafter found to have active pulmonary tuberculosis.

Renal Insufficiency.—Because chronic renal insufficiency appeared to reduce the reactivity of the patients at the time of the preoperative studies, it was of interest to study the effect of postoperative uremia. In most patients the blood urea nitrogen level declined promptly after implantation of the new kidney. However, in those patients in whom the BUN remained elevated postoperatively, (patients 8, 15, 21, 22, and 25) new reactions appeared and some patients tested a second time when the BUN was rising in association with the rejection crisis (patients 1 and 20) showed an increased number of new reactions.

Biopsy Studies.—Twenty-five cutaneous reactions were biopsied at various times following injection of the antigen. In two individual tests a typical histological lesion was found in the absence of a macroscopic reaction. The results of the tissue studies are the subject of a separate communication (2).

DISCUSSION

Immediate Hypersensitivity.—The chronically uremic patients comprising this study were remarkably less reactive to the panel of antigens expected to elicit wheal and erythema than their controls. The incidence of positive responses in

the control group agrees with figures published by other investigators (3). Eighteen of the 25 donor-recipient combinations were genetically related. Forty-nine positive reactions were recorded in these 18 donors but only 6 in their uremic relatives. Because of the recognized importance of heredity in the predisposition to sensitization a much higher incidence would be expected in the patients with chronic renal disease.

The immediate wheal and erythema reaction is a vascular response mediated primarily by histamine which is released as a consequence of the union of an antigen with a B_{2A}-globulin antibody (reagin) bound to unknown sites within the skin. The immediate reaction could be interrupted by a reduction in the quantity of reagin, an interference with the interaction of antigen and antibody, a reduction of the histamine contained or released by the mast cells of the skin, or a failure of the cutaneous vessels to respond to the chemical mediators.

Patients with malignant lymphomas may have a similar type of cutaneous unresponsiveness of the immediate type (4). Because diseases of the reticuloendothelial system do not prevent normal cutaneous responsiveness to exogenous histamine and to endogenous histamine released by a chemical liberator (4) and by passive cutaneous anaphylaxis (5), the failure to respond in these diseases is believed to be more central in origin. There are meager and conflicting data regarding the production of humoral antibody in Hodgkin's disease (6–8). The mechanism of unresponsiveness of the immediate type is currently under study in this group of uremic patients.

Delayed Hypersensitivity.—Cutaneous induration, developing within 48 hours of antigen injection, indicates previous sensitization to this antigen. The pathogenesis of delayed immunologic responsiveness has not been completely characterized. One concept regards its origin to reside in a small number of cells which have an inherent potential to recognize the antigen. Primary exposure to the antigen induces an expansion of this small population of cells (induction), and some of these specifically sensitized cells circulate in the peripheral blood. Expression of the presence of these cells occurs when the antigen is then introduced into the skin. They are sequestered as they traverse the antigen depot and an interaction between the antigen and "antibody," either produced or carried by the cell, ensues. Presumably cell products are released by this reaction which in turn serve as a stimulus for infiltration of the area by nonsensitized inflammatory cells (9).

Comparison of the uremic patients with the control group has revealed a decreased incidence of reactivity of the delayed type in chronic renal insufficiency. In both series the most frequently positive tests were seen with the mumps, *Trichophyton*, and *Candida* antigens. The incidence of reactivity to these antigens was 82, 83, and 89 per cent, respectively, figures which are similar to those of others (10, 11). In contrast the uremics had approximately a 30 per cent incidence of positive reactions to each of these antigens.

A non-specific decrease in tuberculin-type reactivity has been described in the very young (12), the aged (13), with cachexia (14), corticosteroid administration (15), reticuloendothelial diseases such as sarcoidosis (10), Hodgkin's disease (11, 16), and other malignant lymphomas (4), but not with chronic renal failure. The unresponsiveness in sarcoidosis can be circumvented by passive transfer of immunologically competent cells (17). This is not the case, however, with infants, patients with Hodgkin's disease, or recipients of adrenal steroids. The cutaneous reactivity may reappear when Hodgkin's disease is in remission (16).

Even in the absence of suppressive therapy, survival of allogenic skin grafts is prolonged in Hodgkin's disease (18) and in acute and chronic renal insufficiency (19, 20). In one study the patients with Hodgkin's disease, demonstrating most prolonged acceptance of the skin grafts, were completely unreactive to the panel of delayed hypersensitivity antigens (18). In an investigation of impaired skin graft rejection in uremia Dammin *et al.* concluded that delayed hypersensitivity was not impaired (21). Concerning the patients reported here, the relationship between the suppression of delayed cutaneous reactivity and the eventual fate of the kidney allograft cannot be assessed at present. Treatment with azathioprine alone did not prevent kidney rejection and did not diminish the delayed response, whether actively or passively acquired. However, addition of adrenal steroids reversed rejection and markedly suppressed delayed sensitivity. Thus a relation between kidney allograft rejection and delayed skin reactivity seems apparent.

Passive Transfer of Delayed Hypersensitivity.—The appearance of new delayed skin reactions in the postoperative period is interpreted to indicate transfer of specific immunologic competence from the kidney donor to the recipient. Since the recipients acquired no reactivities to antigens to which their donors were unresponsive, several alternative conclusions are excluded. Improvement in the uremic syndrome is not a tenable explanation for these observations because new reactivity appeared even in the presence of continuing uremia. Malnutrition was not corrected at these early dates, therefore improved nutrition cannot be an important factor. The parallelism between donor and recipient reactivities precludes leukocytes derived from blood transfusions as possible agents.

The operative procedure provides at least two sources of immunologically competent cells. One is the vasculature of the transplanted kidney within which donor blood is transfused to the recipient. In addition the patient receives the lymphatic tissue surrounding the kidney and ureter, and this is a second source of cells which may confer new skin responsiveness to the host. Since the majority of the recipients demonstrated acquired responsiveness on the 1st postoperative day, the donor cells clearly had ready access to the recipient's circulation. In those patients in whom the conversion was delayed, the contribution from the

intravascular compartment was presumably insufficient and for this reason reactivity did not appear until lymphatic continuity was reestablished.

In addition to the classical method of passive transfer of delayed hypersensitivity requiring viable lymphoid cells, Lawrence has demonstrated, in man, that delayed reactions can be conveyed by a "transfer factor" derived from disrupted peripheral blood leukocytes obtained from highly sensitized donors (22). Since injection of the transfer factor requires an interval of 4 to 6 days before generalized sensitivity is demonstrable and passively transferred viable lymphocytes are effective much sooner, it is clear that the viable cells play an important role in the genesis of the observations reported here. At present we cannot assess the role played by transfer factor in the few patients with delayed appearance of reactivity.

The successful transfer of delayed hypersensitivity in man is dependent upon the degree of sensitivity of the leukocyte donor and the volume of cells used. A large number of cells from an insufficiently sensitive donor or an inadequate number of cells from a highly sensitized donor will not achieve transfer (22). Since the design of this investigation precluded control of these variables, an explanation for our failure to observe transfer of all of the donor reactivities is apparent. Some support for this conclusion is obtained from the demonstration of a histologic lesion in a few instances in the absence of a typical macroscopic response. In addition, reactivities to mumps and *Candida*, which initiated the greatest reactions in the donor, were transferred most frequently. The mean reaction diameter to histoplasmin was larger than those to coccidioidin and purified protein derivative and yet histoplasmin reactivity was transferred less frequently. This may indicate introduction of an insufficient number of donor cells or a peculiarity specific to histoplasmin in passive transfer. The passive transfer of histoplasmin reactivity in man has not been previously reported.

In one recipient, patient 3, no contribution from extravascular cells could be expected because the new kidney was in continuity with the circulation for only 4 hours. The recipient acquired no reactivity from the donor of this kidney. His subsequent positive responses to the same antigens following a second transplant attest to his suitability as a recipient for passive transfer studies. Another patient, patient 25, was in many ways comparable to patient 3 because his transplanted kidney did not function. This kidney, however, was left in place. Reactions of donor specificity were observed indicating that a sufficient number of immunologically competent cells, but not kidney function, is required for successful transfer.

Lawrence has reported that the duration of passively transferred reactivity is also related to the degree of donor sensitivity and the number of cells used (23). Either factor may be the explanation for the loss of acquired reactivity observed in 2 patients who were tested for a second time in the postoperative

period. Seven other patients similarly retested demonstrated persisting reactivity.

In experimental animals, passive transfer can be demonstrated to persist until rejection of the allogenic cells occurs (24). In this series of patients, the therapeutic protocol dictates institution of adrenal steroid therapy when rejection of the kidney is recognized. However, 3 patients were studied within 24 hours following the recognized onset of kidney rejection and acquired reactivity was present. More information concerning the duration of the acquired reactivity will be obtained when the steroid therapy is discontinued and skin testing can be repeated.

Influence of Therapy on Delayed Response and Passive Transfer.—In laboratory animals, induction of the delayed type of immunologic responsiveness is unsuccessful when accompanied by the administration of alkylating agents or x-irradiation (24). After sensitization, manifestation of the delayed reaction is impeded by these cytotoxic agents and by adrenal steroids. Although azathioprine has not been investigated with respect to classical delayed hypersensitivity, it does retard allogenic graft rejection (25). In those instances in which x-ray, adrenal steroids, and the radiomimetic drugs have been studied, they also impair reactivity following passive transfer (24, 26, 27).

Splenectomy has not been reported to impair established or passively transferred delayed hypersensitivity. All of the recipients in this study were splenectomized. This was followed by no change from preoperative reactivity nor did the procedure prevent demonstration of acquired reactivity.

Delayed hypersensitivity passively transferred to previously irradiated recipient animals is impaired (27). The mechanism of this impairment is not understood but it may represent interference with the non-specific component of the delayed reaction. Because of the similarity of response to irradiation and azathioprine administration, impairment of response following passive transfer might be expected in our patients. The fact that this was not observed even after 29 days of therapy is more likely related to the dose of azathioprine rather than a fundamental difference between the two. The azathioprine therapy did not depress the peripheral leukocyte count in the majority of the kidney recipients and the absolute lymphocyte count was frequently normal. Administration of azathioprine for as long as 26 days did not significantly decrease the frequency or intensity of those reactions present preoperatively.

Adrenal steroids may not prevent induction, but hamper demonstration of delayed sensitivity acquired either actively or passively (26). We have noted that treatment of our patients with prednisone for more than 24 hours usually resulted in failure to elicit reactions macroscopically.

Mechanisms of Suppression of Delayed Hypersensitivity.—The evolution of the delayed cutaneous response is susceptible to interference by a number of distinct mechanisms. The initial expansion of the cells with specific potential

which occurs during induction can be prevented by simultaneous irradiation. A decrease in either the capacity of the cells to transport or produce antibody, either qualitatively or quantitatively, would be expected to impair the response. The abnormality observed in sarcoidosis and cachexia presumably resides here. Local factors could interfere with the union of antigen and antibody in the skin or such a union might not initiate the non-specific reaction. Hodgkin's disease and adrenal steroid suppression are examples of interference with these final steps in the development of the delayed reaction.

The pathogenesis of the unresponsiveness demonstrated in chronic renal insufficiency cannot be completely clarified at present. The ability of the skin to react following passive transfer excludes a defect in the later stages of the reaction. The abnormality must therefore lie in the preparative phases but definition within this area is difficult. The predominantly aregenerative nature of the anemia of chronic renal failure might indicate a failure in cell proliferation (28). However, the marked wasting of protein characteristic of advanced uremia suggests a generalized disorder of nitrogen metabolism. This is a possible explanation for our observations in delayed sensitivity which would encompass the abnormality noted in immediate hypersensitivity as well.

SUMMARY

Twenty-eight patients with chronic renal diseases and uremia were investigated with respect to their cutaneous responsiveness to a panel of antigens expected to elicit immediate and delayed hypersensitivity reactions. Compared to a control group, there was a marked decrease in the incidence of responses of both types.

Eighteen patients received renal allografts from members of the control group and were available for restudy in the postoperative period prior to the institution of adrenal steroid therapy. Each recipient acquired delayed responsiveness with specificity identical with that of the kidney donor. The donor group was reactive to 49 antigens to which the recipients were non-reactive preoperatively. Postoperatively, 40 of these reactivities were observed in the recipients.

This successful demonstration of the transfer of immunologically competent tissue in association with renal transplantation indicates that the cause of depressed cutaneous hypersensitivity in uremia is not an inability of the skin *per se* to react.

We would like to thank Dr. T. E. Starzl, Dr. T. L. Marchioro, Dr. W. R. Waddell, Dr. M. P. Hutt, and Dr. J. H. Holmes for permission to study their patients.

BIBLIOGRAPHY

1. Starzl, T. E., Marchioro, T. L., and Waddell, W. R., The reversal of rejection in human renal homografts with subsequent development of homograft tolerance, *Surg., Gynec. and Obst.*, 1963, **117**, 385.

2. Rowlands, D. T., Jr., Wilson, W. E. C., and Kirkpatrick, C. H., Immunologic studies in human organ transplantation. II. The histology of passively transferred delayed hypersensitivity, *J. Allergy*, 1964, in press.
3. Curran, W. S., and Goldman, G., The incidence of immediately reacting skin tests in a "normal" adult population, *Ann. Int. Med.*, 1961, **55,** 777.
4. Rostenberg, A., Jr., and Bluefarb, S. M., Cutaneous reactions in the lymphoblastomas, *Am. Med. Assn. Arch. Dermat. Syph.*, 1954, **69,** 195.
5. Rostenberg, A., Jr., McCraney, H. C., and Bluefarb, S. M., Immunologic studies in the lymphoblastomas. II. The ability to develop eczematous sensitization to a simple chemical and the ability to accept passive transfer antibody, *J. Inv. Dermat.*, 1956, **26,** 209.
6. Hoffman, G. T.,and Rottino, A., Studies of immunologic reactions of patients with Hodgkin's disease, *Arch. Int. Med.*, 1950, **86,** 872.
7. Dubin, I. N., The poverty of the immunological mechanism in patients with Hodgkin's disease, *Ann. Int. Med.*, 1947, **27,** 898.
8. Geller, W., A study of antibody formation in patients with malignant lymphomas, *J. Lab. and Clin. Med.*, 1953, **42,** 232.
9. Feldman, J. D., and Najarian, J. S., Dynamics and quantitative analysis of passively transferred tuberculin hypersensitivity, *J. Immunol.*, 1963, **91,** 306.
10. Sones, M., and Israel, H. L., Altered immunologic reactions in sarcoidosis, *Ann. Int. Med.*, 1954, **40,** 260.
11. Schier, W. W., Roth, A., Ostroff, G., and Schrift, M. H., Hodgkin's disease and immunity, *Am. J. Med.*, 1956, **20,** 94.
12. Warwick, W. J., Good, R. A., and Smith, R. T., Failure of passive transfer of delayed hypersensitivity in the newborn human infant, *J. Lab. and Clin. Med.*, 1960, **56,** 139.
13. Johnston, R. N., Ritchie, R. T., and Murray, I. H. F., Declining tuberculin sensitivity with advancing age, *Brit. Med. J.*, 1963, **2,** 720.
14. Good, R. A., Kelly, W. D., Rötstein, J., and Varco, R. L., Immunological deficiency diseases, *Prog. Allergy*, 1962, **6,** 187.
15. Raffel, S., Immunity, New York, Appleton-Century-Crofts, 1961.
16. Sokal, J. E., and Primikirios, N., The delayed skin test response in Hodgkin's disease and lymphosarcoma, *Cancer*, 1961, **14,** 597.
17. Urbach, F., Sones, M., and Israel, H. L., Passive transfer of tuberculin sensitivity to patients with sarcoidosis, *New England J. Med.*, 1952, **247,** 794.
18. Kelly, W. D., Lamb, D. L., Varco, R. L., and Good, R. A., An investigation of Hodgkin's disease with respect to the problem of homotransplantation, *Ann. New York Acad. Sc.*, 1960, **87,** 187.
19. Smiddy, F. G., Burwell, R. G., and Parsons, F. M., The effect of acute uremia upon the survival of skin homografts, *Brit. J. Surg.*, 1960, **48,** 328.
20. Morrison, A. B., Maness, K., and Tawes, R., Skin homograft survival in chronic renal insufficiency, *Arch. Path.*, 1963, **75,** 139.
21. Dammin, G. J., Couch, N. P., and Murray, J. E., Prolonged survival of skin homografts in uremic patients, *Ann. New York Acad. Sc.*, 1957, **64,** 967.
22. Lawrence, H. S., Delayed hypersensitivity and the behavior of the cellular transfer system in animal and man, *in* Mechanisms of Hypersensitivity, (J. H.

Shaffer, G. A. Lo Grippo, and M. W. Chase, editors), Boston, Little, Brown and Co., 1959, 453.

23. Lawrence, H. S., The transfer of hypersensitivity of the delayed type in man, *in* Cellular and Humoral Aspects of the Hypersensitive States, (H. S. Lawrence, editor), New York, Hoeber-Harper, 1959, 279.
24. Crowle, A. J., Delayed Hypersensitivity in Health and Disease, Springfield, Illinois, Charles C Thomas, 1962.
25. Murray, J. E., Merrill, J. P., Harrison, J. H., Wilson, R. E., and Dammin, G. J., Prolonged survival of human kidney homografts by immuno-suppressive drug therapy, *New England J. Med.*, 1963, **268,** 1315.
26. Cummings, M. M., and Hudgins, P. C., The influence of cortisone on the passive transfer of tuberculin hypersensitivity in the guinea pig, *J. Immunol.*, 1952, **69,** 331.
27. Cummings, M. M., Hudgins, P. C., Patnode, R. A., and Bersack, S. R., The influence of X-irradiation on the passive transfer of tuberculin hypersensitivity in the guinea pig, *J. Immunol.*, 1955, **74,** 142.
28. Loge, J. P., Lange, R. D., and Moore, C. V., Characterization of the anemia associated with renal insufficiency, *Am. J. Med.*, 1958, **24,** 4.