Blastogenic Suppression and Alloantibody Activity of Sera from Renal Allograft Recipients

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ABSTRACT To evaluate whether immunological enhancement plays a role in adaptation to renal allografts, we studied sera from transplant recipients to determine whether those which suppressed mixed leukocyte culture (MLC) responses in vitro contained alloantibodies reactive with donor cells. Sera from five of nine renal transplant recipients consistently and specifically suppressed autologous MLC responses to donor cells without impairing the blastogenic responses to third-party leukocytes, soluble antigens, or nonspecific mitogenic agents. In three of the five cases the suppressive acivity of the serum was striking; in two cases the effect was less marked but still readily demonstrable in studies designed to evaluate the dose of serum which provided optimal suppression of MLC responses. Serum from one of the recipients nonspecifically suppressed blastogenic activity both to donor cells and other stimuli. No alloantibody reactive with donor leukocytes was found in any of the sera which exhibited suppressive activity in MLC, whereas in one patient, serum which contained antibody reactive with donor cells did not suppress MLC response to that donor. These findings suggest that, if the serum factors which suppress MLC responses in vitro are enhancing antibodies, they are not detectable even with very sensitive techniques either because they are present in very low concentrations, belong to immunoglobulin classes other than IgA, IgG, or IgM, or are complexed with donor antigen in such a way that their ability to react with fresh donor cells in vitro is blocked.

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INTRODUCTION

Some form of immunological adaptation must occur to allow acceptance of histoincompatible renal allografts in patients receiving low doses of immunosuppressive drugs. Experiments with renal transplants in animals have suggested that immunological enhancement could be one mechanism by which this adaptation may occur (1–7, reviewed in 8 and 9). In support of this idea, several investigators have reported that sera from recipients whose allografts function well frequently contain factors which will suppress the response of recipient lymphocytes to donor leukocytes in the one-way mixed leukocyte culture (MLC)[1] (10, 11), or which suppress the ability of recipients' lymphocytes to kill donor cells in vitro (12). Other investigators have also documented that sera containing cytotoxic alloantibodies may inhibit blastogenic transformation in the one-way MLC, especially when cells from an immunized individual are cultured in his own serum using the immunizing donor's cells as antigen (13, 14). One could relate these two observations by postulating that successful renal allograft recipients adapt to their grafts in a manner similar to the way immunologically enhanced mice adapt to histoincompatible tumors, by developing an antibody to donor cell-surface antigens which suppresses the cellular immune response to the graft (1, 8, 9). However, previous experience with patients who have developed alloantibodies reactive with those of the graft had indicated that the kidney transplants in such circumstances generally succumb to uncontrollable rejection (15–18).

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; MEM, minimal essential medium; MLC, mixed leukocyte culture; PBS, phosphate-buffered saline; PHA, phytohemagglutinin-M; SLO, Streptolysin O.

Therefore, it is reasonable to postulate that if the serum factors responsible for the suppression of MLC reactivity in vitro or immunological adaptation in vivo are enhancing antibodies, these antibodies differ in some respect from the cytotoxins previously associated with early allograft rejection (15, 16). These differences conceivably could include: (*a*) a difference in the avidity of the cytotoxins and the enhancing antibodies for cell surface antigens; (*b*) differences in their concentration relative to that of available donor antigen; or (*c*) differences in the relative numbers of complement fixing and noncomplement-fixing antibodies among those which are reactive with donor antigens (8). The present investigation was undertaken to determine whether we could detect antibodies reactive with donor cells in sera which suppressed the MLC response of the recipient to these same donor cells. In addition to the highly sensitive membrane immunofluorescence technique (19, 20) we employed a new serologic method, the antiglobulin microcytotoxicity test (21). Both tests are more sensitive than those previously used to test large numbers of alloantisera and have the advantage that they will detect antibodies which do not fix complement per se.

METHODS

Selection of patients

Nine recipients of renal allografts from living related donors were studied 2 wk to 42 mo posttransplantation. Seven recipients had stable renal function with serum creatinine less than 1.6 mg/100 ml while receiving less than 60 mg prednisone and less than 200 mg azathioprine daily. One recipient whose transplant failed 6 days posttransplant (patient 8) and one whose allograft function was relatively poor but stable (patient 1) were also studied. Recipient 6, whose kidney has functioned for 42 mo, was thought to be HL-A identical with his sibling donor based on reciprocal nonstimulation in MLC and the results of HL-A phenotyping.

Adequacy of renal function was classified according to the criteria for the evaluation of the severity of established renal disease of the American Heart Association Council on the kidney in Cardiovascular Disease (22) (see Table III).

In vitro lymphocyte blastogenesis

Previously reported techniques for lymphocyte culture and measurement of lymphocyte blastogenesis were used (23). Leukocytes were obtained from defibrinated peripheral blood by dextran sedimentation and were washed twice in 40 ml minimal essential medium (MEM) without serum to prevent carry-over of residual autologous serum. Cells from each recipient were cultured in 16 × 125-mm screw-capped glass vials without stimulants and with the following mitogenic agents or antigens: 0.05 ml phytohemagglutinin-M (PHA, Difco Laboratories, Detroit, Mich.), 0.1 ml Streptolysin O (SLO, Difco Laboratories), 0.1 ml streptokinase-streptodornase (Varidase, Lederle Laboratories, Pearl River, N. Y.), and 10^6 lymphocytes in 2 ml Spinner Modified Eagle's MEM (Gibco Laboratories, Grand Island, N. Y.) supplemented with glutamine (20 mM per liter), penicillin (100 U), and dihydrostreptomycin (100 μg per ml). Each MLC incorporated 10^6 allogeneic lymphocytes (stimulator cells), irradiated with 4,000 *R*, 10^6 lymphocytes from the patient (responder cells), 2 ml MEM, and 1 ml human serum to give a final culture volume of 3 ml.

After incubation of the MLC for 7 days and other cultures for 5 days at 37°C in 5% CO_2 in air, the cells were incubated with 2 μCi of [^{3}H]thymidine (sp act, 1.9 Ci/mmol, Schwarz/Mann Div., Orangeburg, N. Y.) for 3 h. The incorporated acid-insoluble radioactivity was measured by liquid scintillation counting. The [^{3}H]thymidine incorporation was expressed as counts per minute per 10^6 responder lymphocytes after subtraction of background.

To evaluate whether sera contained factors capable of suppressing the blastogenic response, and to see whether the degree of suppression was related to the quantity of the serum factors present in the culture, replicate cultures were set up using serial dilutions of the test serum in normal donor serum or serum from a blood type AB, Rh-positive man who has never been transfused. Such cultures therefore contained 1 ml of either undiluted or 1:2, 1:3, or 1:6 dilutions of the test serum in normal human serum. Controls included MLCs set up in normal donor serum.

Cultures were set up in duplicate or triplicate when sufficient numbers of cells were available. The average standard error for triplicate cultures was 21% of the mean with a range from 10 to 30% among the nine recipients. Considering a maximum standard error of ±30% a ratio of blastogenic responses in normal serum divided by that in the patient's serum of greater than 5 was considered indicative, and a ratio of 2 to 5 considered suggestive that the patient serum contained a suppressive factor or inhibitor of lymphocyte blastogenesis.

Tests for alloantibody activity

Antiglobulin microcytotoxicity. The antiglobulin microcytotoxicity test has been described previously (21). The interaction of alloantibody with cell surface antigen is magnified by the subsequent addition of an antihuman immunoglobulin reagent specific for human γ-, α-, or μ-heavy chains thereby increasing the sensitivity of the test. Each test included a serum from a type AB, Rh-positive, untransfused male donor as a negative control.

By means of indirect immunofluorescence, sera obtained from renal transplant recipients were tested against biopsies of their donor kidneys obtained before transplantation. In this way two sera were detected which contained only antibodies of the IgA class reactive with the donor tissue. These sera were subsequently tested against lymphocytes from normal individuals using the indirect membrane immunofluorescence technique and the antiglobulin and direct microcytotoxicity tests. Antibody reactivity could be demonstrated against certain of these normal lymphocytes by the membrane immunofluorescence and antiglobulin techniques when anti-IgA reagents but not when anti-IgG or anti-IgM were used to demonstrate the reaction of the primary antiserum. Neither of the IgA antibodies could be detected by the regular microcytotoxicity test. On the basis of these findings we conclude that both the membrane immunofluorescence test and the antiglobulin microcytotoxicity test can detect antibodies which do not fix complement by themselves (21).

Direct microcytotoxicity. The microcytotoxicity test of

TABLE I

*Influence of Serum Factors on Leukocyte Incorporation of [^3H]Thymidine in Transplant Recipients**

Recipient No.	Days post transplant	Cells cultured in normal serum (antigenic stimulus) A None	B Donor WBC‡	C Unrelated WBC‡	Cells cultured in recipient serum (antigenic stimulus) D None	E Donor WBC‡	F Unrelated WBC‡	Ratio: Response in normal serum / Response in recipient serum Donor WBC (B − A/E − D)	Unrelated WBC (C − A/F − D)
1	66	188	3,060	4,326	208	110	1,162	∞	4.3
2	791	277	2,014	3,890	2,353	1,249	6,488	∞	0.9
	991	245	6,353	18,341	139	590	12,267	13.5	1.5
3	414	2,272	12,140	28,750	174	286	10,092	88.1	2.7
	614	913	8,171	12,444	125	217	5,271	78.9	2.2
4	1,034	262	972	8,988	244	366	6,998	5.8	1.3
5	650	150	986	4,616	182	586	4,334	2.1	1.1
	834	190	598	6,955	400	275	10,671	∞	0.7
6	1,274	3,394	174	5,486	2,498	242	11,766	0.0	0.2
7	214	71	223	3,852	82	473	6,429	0.4	0.6
	559	578	376	16,051	102	310	10,884	0.0	1.4
8	47	128	413	429	1,420	1,906	15,302	0.6	0.0
	175	294	265	309	620	1,949	14,971	0.0	0.0
	177	496	428	5,040	530	2,000	20,118	0.0	0.2
9	18	217	5,459	2,066	218	6,375	5,356	0.9	0.4
	33	204	2,379	6,687	240	2,436	5,343	1.0	1.3
	284	186	1,523	6,936	131	305	204	7.7	92.5

* All data expressed as counts per minute per 10^6 cultured leukocytes.
‡ WBC = peripheral blood leukocytes irradiated with 4000 R.

Mittal et al. (24) was used, as well as two modifications of this technique which increased its sensitivity: (*a*) prolongation of the incubation time with antiserum to 60 min and the incubation time with complement to 120 min, and (*b*) washing the lymphocytes three times with 5 μl of 0.15 M NaCl buffered with 0.01 M sodium phosphate, pH 7.4 (PBS), before the addition of complement and at the same time, using long incubation times mentioned above. Normal serum from a type AB, Rh-positive person was likewise included as a negative control.

Membrane immunofluorescence. Membrane immunofluorescence was performed following a modification (20) of the technique described by Möller (19). Donor lymphocytes (0.4×10^6) were incubated with 100 μl of test serum at 22° for 1 h in Beckman microtubes (Beckman Instruments, Inc., Fullerton, Calif.). The lymphocytes were then sedimented through 0.25 ml 5% bovine serum albumin (BSA) in PBS in Beckman microtubes by centrifuging 1½ min in a Beckman microfuge (model 152) at 2,000 *g*, resuspended in 30 μl of the appropriate fluorescein-conjugated antihuman immunoglobulin heavy-chain serum, and incubated an additional 30 min at 22°C. The cells were then resedimented through 5% BSA in PBS two times, resuspended in 5–10 μl PBS, and mounted under buffered glycerine (PBS: glycerine ratio 1:1). The number of cells showing membrane fluorescence per 100 cells was counted and the appropriate correction made for the number of cells which intrinsically carry surface immunoglobulin as revealed by examination of the same donor cells incubated in autologous serum before exposure to the fluorescein-conjugated antisera. All slides were coded and read without knowledge of the particulars of each experiment with a Leitz Ortholux microscope (E. Leitz, Inc., Rockleigh, N. J.) fitted with a Corning 2 mm No. 7-60 excitation filter (Corning Glass Works, Corning, N. Y.) and a Kodak No. 430 barrier filter (Eastman Kodak Co., Rochester, N. Y.) using the 54 × oil objective.

Anti-immunoglobulin reagents. Commercial preparations of antisera specific for human γ-, α-, or μ-immunoglobulin heavy chains [2] were used in the antiglobulin microcytotoxicity test and the membrane immunofluorescence technique. In addition, a rabbit antihuman IgG prepared in our own laboratory was used in the antiglobulin test. The specificity of each reagent was confirmed by immunoelectrophoresis and double diffusion in agar and appropriate cross adsorptions with insoluble immunoadsorbents (25) were done to assure that each antiglobulin reagent was specific for the heavy chains of a single immunoglobulin class.

RESULTS

Inhibition of MLC by recipient's serum

Serum from six of the nine renal allograft recipients suppressed the one-way MLC to some degree when the recipient's lymphocytes were used as responder cells and irradiated donor lymphocytes were used as stimulator cells (Tables I and II). In the first three patients listed in Tables I and II, the suppression was relatively specific and profound. In patients 4 and 5, the response to donor cells was not as vigorous as in patients 1, 2, and 3; still the recipient's serum consistently suppressed the small response which their cells made to the donor al-

[2] The following rabbit antihuman immunoglobulin antisera were used: lot 1245 (γ-chain) and lot 1482 (μ-chain), Behring Diagnostics Inc., Subsidiary of American Hoechst Corporation, Cincinnati, Ohio. The following goat antihuman fluorescein-conjugated antisera were used: lot FGHG C 101 (γ-chain), lot FGHA C 102 (α-chain), and lot FGHM C 103 (μ-chain), Meloy Laboratories, Mel Labs, Inc., Biological Div., Springfield, Va.

TABLE II

Specificity of Serum Inhibitory Effect; Ratio of Blastogenic Responses in Normal and Recipient Serum

Patient No.	Days post-transplant	Donor WBC	Unrelated WBC	Phytohem-agglutinin	Strepto-lysin O	Varidase
1	66	∞	4.3	2.0	8.7	2.7
2	791	∞	0.9	0.5	3.2	∞
	991	13.5	1.5	1.3	1.3	ND*
3	414	88.1	2.7	1.5	0.5	0.9
	614	78.9	2.2	0.9	0.8	ND*
4	1,034	5.8	1.3	1.5	3.2	0.5
5	650	2.1	1.1	1.1	1.8	2.6
	834	∞	0.7	1.0	1.3	ND*
6	1,274	0.0	0.2	0.5	1.2	ND*
7	214	0.4	0.6	1.9	NS‡	NS‡
	559	0.0	1.4	1.0	1.8	0.0
8	47	0.6	0.0	0.3	0.2	0.4
	175	0.0	0.0	0.3	0.8	1.0
	177	0.0	0.2	0.0	3.3	0.2
9	18	0.9	0.4	3.2	0.5	0.6
	33	1.0	1.3	0.8	0.6	0.8
	284	7.7	92.5	1.9	3.6	5.4

* Not done.

‡ No stimulation.

loantigens (Fig. 1). In the last patient (9), the suppressor effect was only seen 284 days after transplantation and was not limited to donor target cells. Cells from one donor-recipient pair (patient 6), showed no blastogenic response in either direction in the MLC reaction. HL-A phenotyping suggested that the two may be histo-identical siblings although a family was not available to study for confirmation. Two recipients (Nos. 7 and 8) showed essentially no blastogenic response to specific donor lymphocytes although neither was histo-identical to his respective donor. Both responded to lymphocytes from unrelated individuals in culture. MLC studies were repeated several months later on patients 2, 3, 5, 7, 8, and 9. In all instances but one (patient 9), results obtained in the subsequent studies were similar to those seen during the first.

Dose response curves for sera from the five patients who demonstrated some degree of specific MLC suppression are shown in Fig. 1. The responder and stimulator cells were cultured in decreasing amounts of recipient serum diluted to a constant volume of 1 ml with normal donor serum. Not only were the undiluted serums suppressive in MLC (Fig. 1), but in some cases, the effect was still evident when the serum was diluted as much as 1:6. Although the magnitude of the [^{3}H]thymidine incorporation in normal donor serum by lymphocytes from patients 4 and 5 was not as great as in patients 1, 2, and 3, a dose-related suppressive effect was seen in the MLC tests which contained sera from these two patients.

Specificity of the serum inhibitory effect on blastogenesis

The suppression of lymphocyte blastogenesis by recipient serum was restricted for the most part to tests set up using donor leukocytes as antigen, except in the case of patient 9 (Table II). Although the ratio of blastogenic responses to cells from an unrelated donor in normal vs. recipient serum for patients 1 and 3 was between 2 and 5, this ratio was considerably lower than occurred when cells from the kidney donor were used to provide stimulator cells. Only in the study performed 284 days after transplantation in patient 9 was the suppressive effect of recipient serum stronger against an unrelated random leukocyte donor than against his kidney donor. Patient 9 was atypical in other respects since his day 284 serum also suppressed reactivity to SLO and varidase and possibly to PHA as well. In certain other patients, the ratio of the blastogenic responses to SLO or varidase in normal versus recipient serum was also

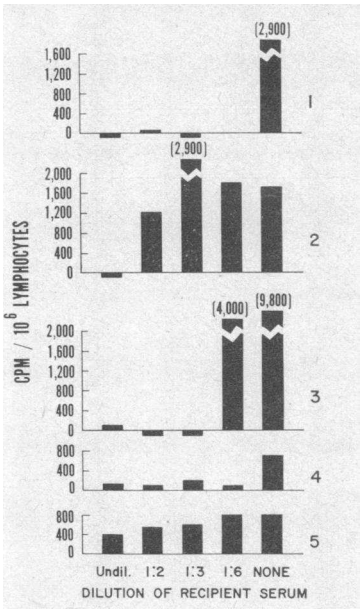

FIGURE 1 Dose response effect of recipient serum in suppressing one-way MLC responses to irradiated donor lymphocytes in five renal allograft recipients. The abscissa indicates the dilution of recipient serum with serum from a normal donor used in the leukocyte cultures. Each vertical bar represents the mean of duplicate or triplicate cultures. Because the quantity of lymphocytes was not sufficient, an MLC test containing a 1:6 dilution of recipient serum was not set up for patient 1.

greater than 2, suggesting that the recipient serum may sometimes also exert a suppressive effect on the leukocyte culture response to these agents.

Tests for alloantibody reactivity

Cytotoxicity. Serum samples from eight of the nine recipients had no detectable reactivity against specific donor cells by either of the three direct or by the sensitive antiglobulin microcytotoxicity tests (Table III). Antibody against donor lymphocytes was detected in sera from patient 7 by the direct microcytotoxicity test and also by the antiglobulin microcytotoxicity test using antihuman IgG. No antibodies against autologous cells were demonstrable in sera from any of the nine patients.

Membrane immunofluorescence. Sera from one of the nine recipients showed significant binding of IgG to lymphocytes (Table III). This was the same patient (no. 7) in whom cytotoxic antibodies were also detected. None of the recipients had antibodies detectable against donor lymphocytes using conjugated antisera specific for α- or μ-heavy chains.

DISCUSSION

Adaptation to histo-incompatible allografts may occur as a result of several mechanisms: actively acquired immunological tolerance (26, 27), the action of nonspecific humoral immunosuppressive factors (28), and immunological enhancement (6, 10–12) have all been suggested as possibilities. Consistent with the notion of immunological tolerance, recipients have been identified who seem to have become specifically unresponsive to donor leukocytes in MLC, regardless of the source of serum used to support the leukocytes in culture (27). Although patients 4 through 8 did not respond strongly in MLC to their respective donor leukocytes, we doubt that any represent examples of acquired immunological tolerance.

For example, patients 4 and 5's donor-specific MLC reactions, though rather weak, were both inhibited by the presence of autologous serum (Fig. 1), suggesting that both patients had responded to the donor antigens and had developed a circulating inhibitor of cellular reactivity. Patient 7 was also not tolerant since he was the only patient whose serum contained antibodies reactive with donor leukocytes. Although Patient 8 was poorly responsive in MLC to leukocytes from his first allograft donor, he lost that kidney either because of recurrence of rapidly progressive nephritis, which destroyed his original kidneys, or because of rejection (see Table III). Since he has adapted well to a second cadaveric kidney transplant, it seems likely that rejection, rather than recurrent disease, was responsible for the loss of his first kidney.

A weak response in MLC to donor cells after transplantation is not by itself evidence for successful adaptation to the graft. It may provide additional evidence as in the case of Patient 6 that the donor and recipient are HL-A identical siblings. Or in cases which do not yield a clinically successful result it may only indicate that despite a poor blastogenic response in vitro the recipient generated sufficient killer lymphocytes to reject the graft. A strong correlation exists between the lymphocyte blastogenic response and the numbers of effector lymphocytes generated during a cell-mediated immune response (29–31). Nevertheless, recent evidence suggests that the production of effector lymphocytes is not always dependent upon a previous blastogenic response to the same antigens (32, 33).

More positive evidence that an active regulatory process is involved in adaptation to the allograft is provided by those models in which one can demonstrate that one component of the immune response depresses another component of the same response. This type of suppression can be nonspecific as in the case of patient 9 or restricted to certain antigens as in patients 1, 2, and 3, and possibly in patients 4 and 5. Exogenous factors such as accumulated renal excretory products or the residue of immunosuppressive drugs carried over in the serum may suppress in vitro cellular immune responses nonspecifically (34). However we do not believe that these

TABLE III

Alloantibody Activity Against Donor Leukocytes in Sera of Renal Transplant Recipients

Recipient No.	Days post-transplant	Donor of kidney and stimulator cells for MLC	Direct cytotoxicity	Anti-globulin cytotoxicity	Membrane immunofluorescence	Serum creatinine	Renal functional* impairment	Immunosuppressive drugs daily dose: Prednisone	Azathioprine	Cyclophosphamide
						mg/100 ml			*mg*	
1	66	Brother	−	−	−	2.4	C	40	50	
2	791	Mother	−	−	−	0.8	A	10		12.5
	991		−	−	−	0.9	A	10		12.5
3	414	Uncle	−	−	−	0.9	A	10	50	
	614		−	−	−	0.7	A	10	50	
4	1,034	Mother	−	−	−	1.1	A	2	200	
5	650	Father	−	−	−	1.1	A	20		50
	834		−	−	−	1.4	B	20		50
6	1,274	Sister	−	−	−	1.2	A	0	125	
7	214	Sister	+	+	+	1.8	B	10	100	
	559		+	+	ND	3.0	C	20	150	
8‡	47	Mother	−	−	−	5.6	F	25		
	175		−	−	ND	1.4	B	60	100	
9	18	Mother	−	−	−	2.1	B	60	100	
	33		−	−	−	1.6	B	50	125	
	284		−	−	ND	1.6	B	20	150	

‡ Rejection on day 6; functional cadaver allograft implanted 148 days after first transplant, all reported studies performed using lymphocytes from first allograft donor.

* Classification according to criteria established by Council on Kidney in Cardiovascular Disease (17). A = glomerular filtration rate (GFR) within normal predicted limits; B = GFR reduced less than 50% of predicted normal; C = GFR reduced 20% to 50% of predicted normal; F = GFR less than 5% of predicted normal; ND = not done.

were the immunosuppressor substances present on day 284 in the serum of patient 9. At the time this serum effect appeared, he was not uremic and was receiving only slightly more azathioprine and much less prednisone than at the time of earlier studies which did not show this effect. We cannot exclude the possibility that he may have developed a type of nonspecific immunosuppressive factor associated with the plasma alpha globulins in certain human sera, since these factors have been shown to be able to suppress lymphocyte blastogenic responses in vitro (28).

In the remaining patients, the factors which depressed in vitro lymphocyte blastogenic activity were relatively specific for donor cells. Because of this specificity, it has seemed reasonable to postulate that these suppressor factors may be enhancing antibodies (3–7, 10–12). Considerable evidence indicates that immunological enhancement is an important mechanism in facilitating host adaptation to histoincompatible tumor grafts (1, 35, 36). Immunological enhancement of nonmalignant tissue has been more difficult to produce, especially by passive transfer of the appropriate alloantibodies (37–40, 3–5, 7). But there is good evidence that the presence of donor-specific alloantibody is essential to enhancement of normal tissue grafts as well (5, 37–40). The evidence that immunological enhancement can and does occur in man is more tenuous and is based on one possibly successful attempt to cause passive enhancement of a kidney transplant (6) and on the two observations previously cited in the introduction: (*a*) transplant recipients and cancer patients have been identified whose cell-mediated immune responses to donor tissue or autocthonous tumor are specifically suppressed in vitro by factors in their own serum (10–12, 41, 42); and (*b*) allogeneic antisera containing antibodies of the type sometimes found in transplant recipients will specifically depress MLC reactions in vitro when the stimulator cells carry the alloantigens with which the antibodies react (13, 14).

Our attempt to relate these two observations by showing that well-adapted renal transplant recipients whose serum contains factors which specifically suppress in vitro lymphocyte reactivity have circulating antibodies reactive with donor alloantigens has not been successful. Our results do not exclude the possibility that immunological enhancement is responsible for graft adaptation in these cases. But they suggest that if these sera contain enhancing antibodies, they must either be present in extremely low concentrations, do not belong to the IgA, IgG, or IgM classes of immunoglobulin, or are com-

plexed with soluble donor antigen and therefore are not free to react with donor cells in vitro.

Previous experience with passive immunological enhancement of tumors in mice has emphasized that very low concentrations are optimal in facilitating tumor growth (35); less antibody than is needed to coat all cells in the graft will cause vigorous immunological enhancement (1, 8). In certain experimental models, the presence of enhancing antibody may be so difficult to detect that the enhanced animal may appear to be immunologically tolerant (43). Haughton and Adams, while studying mechanisms by which humoral immune responses are regulated, similarly observed that miniscule amounts of antibody are needed to suppress the generation of new antibody forming cells (44).

It is conceivable that an extremely low concentration of alloantibody will effect MLC suppression and not be detected by the serologic methods employed. Alternatively the antibody could be reactive with a subpopulation of lymphocytes which is so small as not to be identified in the serologic tests.

The majority of investigators who have studied the physical properties of enhancing antibodies in animals have concluded that these reside among the IgG_2 immunoglobulins which are generally considered to fix complement rather well (9). However, Voisin's work has suggested that antibodies of the IgG_1 or IgA classes may be the agents of immunological enhancement in certain species (8, 43). In either case, the techniques we employed, if they were sufficiently sensitive, should have been able to demonstrate IgA or IgG_1 alloantibodies and their subclasses. The possibility that the MLC suppression is mediated by an antibody not of the IgG, IgA, or IgM heavy-chain classes cannot be ruled out.

Regarding the role of antigen-antibody complexes in enhancement, Amos and Klein have recently shown, using sera which can passively enhance the growth of histo-incompatible tumors, that the antibodies in these sera react with the tumor cell antigens to form complexes which depress the generation of specific antigen-reactive cells (45, 46). It has been suggested that antigen-antibody complexes rather than free antibody alone may actually be the effector substance which suppresses a variety of cell-mediated immunological reactions (46–50). Complexes of homologous cell surface antigen and specific antibody, for example, can suppress the killer cell response to allogeneic tumors (46–48). It is possible that complexes of antigen and specific alloantibody may have played a role in prolonging the survival of rat renal allografts in the experiments of Stuart, Saitoh, and Fitch (7) since treatment of the recipients with antigen or antibody alone did not produce as striking graft prolongation as the combination of antibody and antigen administered. Antigen-antibody complexes acting at the level of the antigen-reactive cell may also be responsible for suppressing the generation of antibody-forming cells in vitro (49). Oppenheim's studies of the lymphocyte blastogenic response in vitro to complexes of diphtheria or tetanus toxoids with their respective antitoxins have shown that free antibody or soluble complexes in antibody excess specifically inhibited the response of sensitized cells to free antigen, but antigen-antibody complexes precipitated at equivalence or soluble complexes prepared in antigen-excess provoked a greater response than that produced by optimal concentrations of antigen alone (50). The mechanism by which antigen-antibody complexes suppress or facilitate specific responsiveness in various in vitro models has not been resolved, although in each, they appear to act at the level of the antigen-reactive lymphoid cell (46, 49, 50).

If antigen-antibody complexes were responsible for the specific suppression of MLC reactivity which we observed, the antibodies involved must have been highly avid. Otherwise one would expect that the excess of donor antigen, produced by incubating the sera with large numbers of donor lymphocytes, would transiently dissociate some antibody molecules from the complex and permit their reaction with sufficient numbers of donor cells to be detected by one of the methods used in this study.

Using techniques already applied to the study of suppressor factors in sera of tumor-bearing animals (45–48), it should be possible to evaluate whether soluble antigen-antibody complexes are responsible for the suppressive effect of recipient sera on the MLC response of kidney transplant patients to their donor's cells. The larger question, whether these MLC suppressor factors influence the process of immunologic adaptation to the transplant will need to be answered in long-term clinical studies. If it can be shown that complexes of alloantibody and antigen are the effector substances which promote both specific MLC suppression and renal allograft adaptation, then one can entertain the possibility of using such complexes therapeutically to facilitate allograft survival.

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