

## CELLULAR RECEPTORS

### EFFECT OF ANTI-ALLOANTISERUM ON THE RECOGNITION OF TRANSPLANTATION ANTIGENS\*

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The injection of parental strain lymphoid cells into F<sub>1</sub> hybrid animals is known to lead to manifestations of graft-*versus*-host (GVH)<sup>1</sup> reactions. It is generally agreed that the initiation of this process is set in motion by parental strain cells recognizing transplantation antigens the F<sub>1</sub> host has inherited from the other parent (1). This recognition process is assumed to be accomplished by recognition structures (RS) presumably located on the surface of parental strain lymphoid cells. If such a parent-to-F<sub>1</sub> GVH reaction is considered from a genetical point of view, it seems reasonable to assume that an F<sub>1</sub> animal fails to detect on injected parental strain lymphoid cells any foreignness, with the notable exception of particular recognition structures.

It was demonstrated earlier (2-4) that these structures were antigenic. The injection of moderate numbers of parental strain immunocompetent cells into F<sub>1</sub> hosts led to the production of anti-recognition structure (anti-RS) antisera. The activity of these sera could be demonstrated by their ability to block the corresponding recognition structures on parental strain cells and thus prevent antigenic recognition. Anti-RS sera have, furthermore, been shown to be of exquisite specificity. Since for genetic reasons an F<sub>1</sub> host can be expected to react only against RS which it does not possess, its serum will contain only antibody to the newly introduced parental RS. It could be shown that treatment of a suspension of parental strain lymphoid cells with such an antiserum blocked the RS in question but left RS for other transplantation antigens free to recognize corresponding antigens (3, 4).

While these anti-RS sera have all been provoked by injections of low numbers of parental strain lymphoid cells into adult F<sub>1</sub> hybrid hosts, the present communication intends to show that similar antibodies can be elicited by a seemingly remote immunization procedure. Instead of considering only immunocompetent cells as bearers of recognition structures for foreign trans-

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<sup>1</sup> Abbreviations used in this paper: BSS, balanced salt solution; GVH, graft-*versus*-host; PAR, product of antigenic recognition; PMN, polymorphonuclear; RS, recognition structure.

plantation antigens, it does not seem altogether impossible to view antigenic determinants on the combining region of alloantibodies as possibly similar structures. That this may be so is forcefully indicated by the fact that alloantibodies combine with antigens in immunologically specific ways giving rise to a variety of serological manifestations (5, 6). In following the consideration that alloantibodies might represent recognition structures for the immunizing antigen,  $F_1$  animals were immunized with an alloantiserum possessing an antigen-binding specificity directed against one of the antigens of the host. It was hoped that in this way an anti-alloantiserum could be provoked which would in effect be an anti-RS serum. The results of this paper show that such a serum behaved precisely like an anti-RS serum raised in similar  $F_1$  hosts by the injection of parental strain lymphoid cells.

#### *Materials and Methods*

*Animals and Provocation of Antisera.*—Adult male and female rats of inbred strains DA, Lewis, and BN, as well as  $F_1$  hybrids between these strains, were employed.

An alloantiserum DA anti-Lewis (serum 620) was prepared by grafting six female DA rats once with full-thickness skin taken from female Lewis rats according to a standard procedure (7). 3 wk postoperatively animals were exsanguinated and the serum pool was heat inactivated (30 min at 56°C) and sterilized by Millipore filtration (Millipore Corp., Bedford, Mass.).

An anti-RS serum of specificity (Lewis  $\times$  DA) $F_1$  anti-DA RS(Lewis) (serum 619) was provoked by a single injection of  $10^6$  female DA lymph node cells (suspended in 0.5 ml of Hanks' balanced salt solution [BSS]) by the intravenous route into each of four (Lewis  $\times$  DA) $F_1$  female rats. Animals were bled 2 wk later, individual sera pooled, heat inactivated, and sterilized by filtration. A control serum (serum 746) was obtained under identical conditions by injecting  $10^6$  spleen cells from (Lewis  $\times$  DA) $F_1$  male donors into five (Lewis  $\times$  DA) $F_1$  male hosts.

An anti-alloantiserum (serum 663) of specificity (Lewis  $\times$  DA) $F_1$  anti-(DA anti-Lewis) was elicited in five (Lewis  $\times$  DA) $F_1$  male rats by injecting equal volumes of DA anti-Lewis serum 620 and Freund's complete adjuvant (Difco Laboratories, Inc., Detroit, Mich.). Each host received in its belly skin 10 doses of 0.1 ml of this mixture. 2 wk later this procedure was repeated and after another 2 wk, the animals were exsanguinated, individual sera were pooled, heat inactivated, and sterilized.

A control serum (serum 675) was prepared in five (Lewis  $\times$  DA) $F_1$  female rats in exactly the same way, except that heat-inactivated and filtered serum from normal DA rats was injected.

*Product of Antigenic Recognition (PAR) Assay.*—This test was employed to estimate quantitatively recognition of transplantation antigens present on  $F_1$  hybrid cells by immunocompetent cells of parental origin. Procedures of co-cultivating histoincompatible lymphoid cells to obtain a product of antigenic recognition and of measuring this product have been described in full (8). Briefly, suspensions of dissociated spleen cells were prepared according to a standard procedure (7) using Hanks' BSS (General Biochemicals, Chagrin Falls, Ohio) to which penicillin (100 IU/ml) and streptomycin (50  $\mu$ g/ml) had been added. Suspensions of viable cells were adjusted to contain  $20 \times 10^6$  cells/ml. To 4 ml of Hanks' BSS (containing penicillin and streptomycin) in Falcon tissue culture dishes (Falcon Plastics, Los Angeles, Calif.) (60  $\times$  15 mm) were added 0.5 ml of  $20 \times 10^6$ /ml from each of the histoincompatible cell suspensions (mixed cultures) or 1 ml of  $20 \times 10^6$ /ml of histocompatible cell suspensions (unmixed controls). Cell cultures were incubated for 4 or 7 hr at 37°C in a humid atmosphere

of 95% air to 5% CO<sub>2</sub>, culture supernatants were harvested, centrifuged, and concentrated by lyophilization and reconstitution with 0.06 M phosphate buffer, pH 7.9, in one-fifth of the original volume. After 1 hr at 4°C supernatants from mixed and unmixed cultures formed precipitates spontaneously. These were washed once in the same buffer and were resuspended in buffer at one-tenth of the original volume (0.5 ml for 5 ml of culture fluid or multiples thereof). Well-mixed samples were injected into skin sites marked on the back of normal Syrian hamsters of agouti type (Tierzuchtinstitut, University of Zurich). PAR, but not control culture fluids, called forth accumulations of considerable numbers of polymorphonuclear (PMN) cells in ensuing skin lesions. To estimate quantitatively infiltrated cells, hamster hosts were killed 24 hr after intracutaneous injections and three of four replicate skin reactions from a given sample were excised, cut into small fragments, and were gently trypsinized. PMN cells released were counted conventionally and counts from supernatants of experimental cell mixtures were corrected for those determined in controls (8).

*Determinations of Activities of Antisera in the PAR Assay.*—In mixed spleen cell cultures two populations of cells with distinct properties are employed. Parental strain cells are the active partner endowed with the ability to recognize foreign antigens (8, 9). Transplantation antigens were offered to these aggressor cells in the form of appropriate F<sub>1</sub> hybrid spleen cells, called target cells.

Depending on whether a given antiserum addressed its activity to the recognizing properties of aggressors or to the transplantation antigens of targets, the PAR assay was set up accordingly. Either aggressors or targets were suspended in concentrations of  $20 \times 10^6$  cells/ml in various dilutions of a serum (using Hanks' BSS as diluent), incubated for 30 min at 37°C in a humid atmosphere of 95% air and 5% CO<sub>2</sub>, and washed three times in Hanks' BSS by centrifugation for 8 min at 130 g. After counting and readjustment to  $20 \times 10^6$ /ml, cells were employed in mixed or unmixed cultures. The other, untreated partner of cell mixtures was processed identically but in plain Hanks' BSS.

While titers of the various antisera were determined in the correct setup, i.e. treatment of F<sub>1</sub> target cells with alloantisera or treatment of aggressor cells with antisera directed against recognition structures, it was important to show the activities of these antisera in the reversed setup. Thus, alloantisera were also used to treat aggressor cells and anti-RS sera were employed in the treatment of target cells.

## RESULTS

*Activity and Specificity of Alloantiserum DA Anti-Lewis.*—A serum obtained after rejection of Lewis skin grafts by DA hosts was expected to be capable of blocking Lewis transplantation antigens on (Lewis × DA)F<sub>1</sub> target spleen cells. The results in Table I demonstrate that serum 620 inhibited recognition of this antigen up to a dilution of 1:512. The same serum had no effect on DA aggressor cells, since it failed to block recognition of Lewis alloantigens by DA aggressor spleen cells even at high concentrations (1:32).

*Activity and Specificity of a (Lewis × DA)F<sub>1</sub> Serum Obtained after Injection of DA Node Cells.*—As outlined previously (2-4), the injection of DA parental strain lymphoid cells into (Lewis × DA)F<sub>1</sub> hosts will lead to the formation of an antiserum directed against structures on DA cells by means of which Lewis transplantation antigens are recognized; we call these structures RS(Lewis). Employment of this serum (serum 619) for the treatment of DA aggressor cells blocked recognition of Lewis antigens in a dilution of 1:2048, as shown

in the upper part of Table II. The F<sub>1</sub> serum, therefore, possessed anti-DA RS(Lewis) activity. However, since immunocompetent and potentially antibody-forming cells were injected into F<sub>1</sub> hybrids, the serum also contained alloantibodies formed by the inoculated cells. For genetic reasons DA strain

TABLE I  
*Titer and Specificity of Alloantiserum DA Anti-Lewis 620 Provoked by Rejection of Lewis Skin Grafts by DA Rats*

Mixed spleen cell cultures*		PAR† (±SE)
Aggressors	Targets	
DA untreated	(Lewis × DA) F <sub>1</sub> untreated	5.79 ± 0.41
DA untreated	(Lewis × DA) F <sub>1</sub> + 620 1:32	0.55 ± 0.25
" "	" × " " + 620 1:64	0 ± 0
" "	" × " " + 620 1:128	0.50 ± 0.20
" "	" × " " + 620 1:256	0 ± 0
" "	" × " " + 620 1:512	0 ± 0
" "	" × " " + 620 1:1024	6.00 ± 0.87
" "	" × " " + 620 1:2048	6.17 ± 0.21
DA + 620 1:32	(Lewis × DA) F <sub>1</sub> untreated	6.37 ± 0.16

\* Cultivation was for 7 hr at 37°C.

† × 10<sup>6</sup> PMN cells/skin reaction. Average of six skin reactions.

TABLE II  
*Titer and Specificity of Serum 619 Provoked by Injection of DA Lymph Node Cells into (Lewis × DA) F<sub>1</sub> Hosts*

Activity	Mixed spleen cell cultures*		PAR† (±SE)
	Aggressors	Targets	
Anti-DA RS (Lewis)	DA untreated	(Lewis × DA)F <sub>1</sub> untreated	8.53 ± 0.60
	DA + 619 1:256	(Lewis × DA)F <sub>1</sub> untreated	0 ± 0
	" + 619 1:512	" × " " "	0.31 ± 0.18
	" + 619 1:1024	" × " " "	0 ± 0
	" + 619 1:2048	" × " " "	0 ± 0
	" + 619 1:4096	" × " " "	9.78 ± 0.12
DA anti-Lewis	DA untreated	(Lewis × DA)F <sub>1</sub> + 619 1:16	6.75 ± 1.04
	" "	" × " " + 619 1:32	0 ± 0
	" "	" × " " + 619 1:64	0 ± 0
	" "	" × " " + 619 1:128	0.35 ± 0.18
	" "	" × " " + 619 1:256	6.75 ± 0.53

\* Cultivation was for 7 hr at 37°C.

† × 10<sup>6</sup> PMN cells/skin reaction. Average of 6-18 skin reactions.

donor cells are accepted by (Lewis  $\times$  DA) $F_1$  hosts which also provide the antigenic stimulus (Lewis transplantation antigens); in response to these, donor cells produced DA anti-Lewis alloantibodies. As shown in the lower part of Table II, these prevented recognition of Lewis alloantigens by DA aggressor cells to a comparatively low titer of 1:128. Thus, while the anti-RS activity of this serum is because of a host-anti-inoculum reaction, that of alloantibody is the result of an inoculum-anti-host reaction.

If this is correct, a serum obtained by injecting lymphoid cells from (Lewis  $\times$  DA) $F_1$  rats into (Lewis  $\times$  DA) $F_1$  hosts should display neither anti-RS nor alloantibody activity. The results in Table III show that, even when tested in high concentration (1:32), such a serum (serum 746) failed to prevent recognition of Lewis antigens by treated DA aggressors. The same serum also lacked activity against Lewis alloantigens since recognition was normal after treatment of target cells.

TABLE III  
*Lack of Anti-RS and of Alloantibody Activity of Serum 746 Provoked by Injection of (Lewis  $\times$  DA) $F_1$  Spleen Cells into (Lewis  $\times$  DA) $F_1$  Hosts*

Activity	Mixed spleen cell cultures*		PAR $\ddagger$ ( $\pm$ SE)
	Aggressors	Targets	
	DA untreated	(Lewis $\times$ DA) $F_1$ untreated	9.06 $\pm$ 0.28
? Anti-DA RS (Lewis)	DA + 746 1:32	(Lewis $\times$ DA) $F_1$ untreated	8.87 $\pm$ 0.37
? DA anti-Lewis	DA untreated	(Lewis $\times$ DA) $F_1$ + 746 1:32	10.00 $\pm$ 0.33

\* Cultivation was for 7 hr at 37°C.

$\ddagger$   $\times 10^6$  PMN cells/skin reaction. Average of six skin reactions.

*Activity and Specificity of an Anti-(DA Anti-Lewis) Antiserum.*—A serum obtained by injecting alloantiserum DA anti-Lewis (serum 620) into (Lewis  $\times$  DA) $F_1$  hosts was tested for its anti-RS and alloantibody activities, as well as for the specificity of its anti-RS activity. The results obtained with this anti-antiserum (serum 663) are summarized in Table IV. Treatment of DA aggressor cells with serum 663 completely blocked their ability to recognize Lewis alloantigens on (Lewis  $\times$  DA) $F_1$  target cells up to a surprisingly high titer of 1:8000. In contrast to this clear-cut inhibition, treatment of Lewis aggressor cells with this serum failed to block recognition of DA alloantigens offered by the same target cells. Specificity of serum 663 was revealed by the observation that this serum blocked on DA aggressor cells recognition structures for Lewis alloantigens only, but not those for transplantation antigens of a third-party rat strain. Thus, when treated DA cells were confronted with (DA  $\times$  BN) $F_1$  target cells, BN alloantigens were recognized normally. Similarly,

TABLE IV  
*Titer and Specificity of Anti-Alloantiserum 663 Provoked by Injection of Alloantiserum  
 DA Anti-Lewis into (Lewis × DA)F<sub>1</sub> Hosts*

Activity	Mixed spleen cell cultures*		PAR‡ (±SE)
	Aggressors	Targets	
	DA untreated	(Lewis × DA)F <sub>1</sub> untreated	8.84 ± 0.36
Anti-RS (Lewis)	DA + 663 1:32-1:8192	(Lewis × DA)F <sub>1</sub> untreated	0 ± 0
	“ + 663 1:16384	“ × “ “ “	11.58 ± 0.91
	Lewis untreated	(Lewis × DA)F <sub>1</sub> untreated	8.12 ± 0.34
	“ + 663 1:32	“ × “ “ “	8.87 ± 0.10
	“ + 663 1:64	“ × “ “ “	7.20 ± 0.16
	DA untreated	(DA × BN)F <sub>1</sub> untreated	8.77 ± 0.27
	“ + 663 1:32	“ × “ “ “	7.28 ± 0.18
	“ + 663 1:64	“ × “ “ “	7.62 ± 0.45
	BN untreated	(DA × BN)F <sub>1</sub> untreated	7.75 ± 0.46
	“ + 663 1:32	“ × “ “ “	8.31 ± 0.64
	“ + 663 1:64	“ × “ “ “	9.38 ± 0.46
? DA anti-Lewis	DA untreated	(Lewis × DA)F <sub>1</sub> + 663 1:16	7.83 ± 0.08
	“ “	“ × “ “ + 663 1:32	8.30 ± 0.05
	“ “	“ × “ “ + 663 1:64	7.71 ± 0.12
	“ “	“ × “ “ + 663 1:128	7.30 ± 0.45
	“ “	“ × “ “ + 663 1:256	7.50 ± 0.25
? Lewis anti-DA	Lewis untreated	(Lewis × DA)F <sub>1</sub> + 663 1:16	8.08 ± 0.25
	“ “	“ × “ “ + 663 1:32	7.66 ± 0.08
	“ “	“ × “ “ + 663 1:64	7.75 ± 0.25
	“ “	“ × “ “ + 663 1:128	8.50 ± 0.50

\* Cultivation was for 4 hr at 37°C in recognition DA → BN and BN → DA or for 7 hr at 37°C for all others.  
 ‡ × 10<sup>6</sup> PMN cells/skin reaction. Average of 6-24 skin reactions.

TABLE V  
*Lack of Anti-RS(Lewis) Activity of Serum 675 Provoked by Injection of Normal DA Serum  
 into (Lewis × DA)F<sub>1</sub> Hosts*

Aggressors	Mixed spleen cell cultures*		PAR‡ (±SE)
	Untreated targets		
DA untreated	(Lewis × DA)F <sub>1</sub>		8.21 ± 0.18
DA + 675 1:32	(Lewis × DA)F <sub>1</sub>		7.10 ± 0.57
“ + 675 1:64	“ × “ “		6.94 ± 0.56
“ + 675 1:128	“ × “ “		6.58 ± 0.38
“ + 675 1:256	“ × “ “		7.31 ± 0.05
“ + 675 1:512	“ × “ “		6.89 ± 0.07

\* Cultivation was for 7 hr at 37°C.

‡ × 10<sup>6</sup> PMN cells/skin reaction. Average of 12 skin reactions.

treatment of BN aggressor cells with serum 663 failed to inhibit their ability to recognize DA alloantigens.

When the anti-alloantiserum was tested for either DA anti-Lewis or for Lewis anti-DA activity by treating (Lewis  $\times$  DA) $F_1$  target cells, normal responses were obtained. This indicated that serum 663 lacked both activities.

To ascertain that a serum provoked similarly as serum 663 but by injections of normal DA rat serum failed to display anti-RS(Lewis) activity, the experiments outlined in Table V were done. The results of this control serum 675 show that it was totally inactive.

#### DISCUSSION

The experiments reported demonstrate that the activities of antisera obtained after rejection of skin allografts, after injections of parental strain immunocompetent cells into  $F_1$  hosts, and after injections of an alloantiserum into similar hosts can be determined by the PAR test. Activities and titers revealed themselves according to whether a serum directed itself to the recognizing (aggressor cells) or to the recognized (target cells) partner of mixed cell cultures.

The unambiguous demonstration of the activity of a serum obtained after rejection of skin allografts is important. A posttransplantation serum appears to be directed exclusively against transplantation antigens. It failed to display activity against recognizing cells (Table I). From this and from other experiments with mouse and hamster alloantisera,<sup>2</sup> it may be concluded that such sera lack anti-recognition structure (anti-RS) activity. In contrast to this, antisera provoked in  $F_1$  hybrid animals by the injection of parental strain lymphoid cells manifest two activities (3). The high-titered activity is directed against recognition structures of donor-type cells for the antigen in question; the low-titered serum component addresses itself to transplantation antigens and thus appears to be an alloantiserum (Table II). The existence of this latter activity in an  $F_1$  serum must be traced to the antibody-forming capacity of the injected immunocompetent parental strain cells and to the fact that these cells encounter foreign transplantation antigens in a host that cannot reject them. While this is a plausible interpretation, it is surprising that the alloantibody activity produced by a small ( $10^6$  cells) inoculum and its descendants is not completely removed by host tissue. The activity that can be detected in the serum is perhaps low affinity antibody which escaped host absorption. That its activity could be demonstrated at all is witness to the sensitivity of the test system employed.

<sup>2</sup> Ramseier, H., and J. Lindenmann. Quantitative studies on antigenic recognition. IV. Specific inhibition of the recognition process by alloantisera. Paper submitted for publication.

The object of this study was to demonstrate that an antiserum directed against a particular set of recognition structures of lymphoid cells cannot only be produced by injecting cells bearing such structures, but also by injecting the corresponding alloantiserum into suitable  $F_1$  hosts. The latter procedure seemed feasible since immunoglobulins of alloantisera are known to combine with immunizing antigen. They might, therefore, have in their antigen-binding region the equivalent of what is considered to be the recognition site of immunocompetent cells. The results summarized in Table IV constitute strong evidence for the view that this indeed is so. Alloantiserum DA anti-Lewis, which we viewed as representing RS(Lewis), elicited, after injection into (Lewis  $\times$  DA) $F_1$  hosts, an anti-alloantiserum which could be shown to act like an anti-RS(Lewis) serum. Thus treatment of DA spleen cells, expected to possess RS(Lewis), with this anti-alloantiserum prevented recognition of Lewis antigens. The serum proved ineffective when employed in the treatment of Lewis spleen cells because these cells lack recognition structures directed against their own antigens. The anti-alloantiserum was of high specificity. Treatment of DA lymphoid cells resulted in blockage of RS(Lewis) only, whereas recognition of BN alloantigens remained perfectly normal. Likewise, BN spleen cells treated with this anti-RS(Lewis) serum were capable of recognizing DA alloantigens. Clearly then, specificity as revealed with this anti-alloantiserum was equal to that observed for anti-RS sera obtained by injections of immunocompetent cells (3, 4). In both cases serum-mediated inhibition was towards a particular set of RS only. Recognition structures not affected were free to interact with corresponding transplantation antigens.

Our interpretation of the activity of this anti-alloantiserum received support by the observation that a control serum, elicited in exactly the same way as the active serum except that normal DA rat serum rather than DA anti-Lewis serum was used to immunize  $F_1$  hosts, failed to show any anti-RS(Lewis) activity (Table V).

It appears from these results that there might be no fundamental difference between antisera to recognition structures prepared either by injecting whole cells bearing antigenic RS or by injecting alloantibodies carrying a specific antigen-binding site representing immunogenic RS. However, sera obtained by these two immunization procedures have important secondary qualities by which they differ. As the data in Table IV illustrate, an anti-RS(Lewis) serum in the form of an anti-alloantiserum failed to display activity when used to treat (Lewis  $\times$  DA) $F_1$  target cells. The serum showed neither anti-Lewis nor anti-DA activity. While the latter activity was not expected to be present in this serum, it was considered possible that remnants of the immunizing DA anti-Lewis serum, passaged through  $F_1$  hosts, might have been present in the  $F_1$  serum. Despite the fact that each  $F_1$  host received a total of 1 ml of this alloantiserum, no DA anti-Lewis activity could be found in the  $F_1$  serum pool.



This is sharply in contrast to the result obtained when RS-carrying cells were used as antigen (Table II). In this case a small but well-defined DA anti-Lewis titer was present in the F<sub>1</sub> serum pool. This then might indicate that when alloantisera are used as antigen pure anti-RS antisera are formed. The other quality by which F<sub>1</sub> sera produced by cells and those provoked by alloantisera differ is the titer. For the two anti-RS sera presented this difference is fourfold. At first sight it seems surprising that an inoculum known to proliferate in F<sub>1</sub> hosts (10-14) should yield a less active antiserum than a dead inoculum. Two observations might help to explain this. First, it has been observed that the activity of an anti-RS serum can be neutralized by a corresponding alloantiserum and, conversely, that the activity of an alloantiserum can be neutralized by a fitting anti-RS serum.<sup>3</sup> Since in an F<sub>1</sub> host that received parental strain lymphoid cells these two serum activities coexist, a neutralization process must continuously go on. This might result in a diminished anti-RS activity as well as in a diminished alloantibody activity. The second point is interconnected to the first. Although the subject of current investigations, observations made so far have revealed that the injection of 10<sup>6</sup>, 6 × 10<sup>6</sup>, and even 200 × 10<sup>6</sup> parental strain lymphoid cells into F<sub>1</sub> hosts all resulted in similar anti-RS titers. Even though there is little doubt that donor cells will multiply in F<sub>1</sub> animals in response to foreign transplantation antigens (10-14), this process appears to plateau-off quite soon. It seems that irrespective of the dose of donor cells used, mutual neutralization takes place, the only difference being the level at which this process works. The employment of alloantiserum instead of lymphoid cells appears to circumvent this neutralization. While the injected serum might serve as the neutralizer of the product it calls forth, only quantitative aspects seem to influence the outcome. The observation that with the presently used immunization regime F<sub>1</sub> sera lacked alloantibody activity would indicate that the serum might have been used up during immunization. Consequently, none or very little could act as an *in vivo* neutralizer of the anti-alloantiserum activity.

The significance of this study lies in the demonstration that the antigen-combining site of alloantibodies can be used as antigen in the preparation of antisera to cellular recognition structures. This observation strongly favors the view that antigen-combining sites of alloantibodies and cellular receptors for transplantation antigens might be very similar entities. The antigen-combining sites of alloantibodies are known to be located in the variable region of immunoglobulin molecules. Since antibody can be raised against this region and since the activity of this antibody and that prepared against cellular recognition structures is not distinguishable, both being directed against

<sup>3</sup> Ramseier, H., and J. Lindenmann. Similarity of cellular recognition structures for histocompatibility antigens and of combining sites of corresponding alloantibodies. Paper submitted for publication.

specific recognition structures, we conclude that the important part of cellular receptors for transplantation antigens might also consist of the variable region of an immunoglobulin.

This conclusion is strongly supported by another set of experiments<sup>3</sup> to be mentioned briefly. In these, it was shown that the activity of a mouse anti-RS serum provoked by the injection of immunocompetent parental strain cells into F<sub>1</sub> hosts could be neutralized by a mouse alloantiserum representing the corresponding RS. Thus a serum, (A × CBA)F<sub>1</sub> anti-A RS(CBA) capable of preventing recognition of CBA alloantigens by A aggressor cells, completely lost its inhibitory activity upon simple incubation with alloantiserum A anti-CBA, representing RS(CBA). Coincubation of this anti-RS(CBA) serum with a nonmatching alloantiserum CBA anti-C57BL/6, representing RS-(C57BL/6), failed to neutralize. Conversely, the activity of mouse alloantiserum A anti-CBA, representing RS(CBA), could be neutralized by the fitting anti-RS serum (A × CBA)F<sub>1</sub> anti-A RS(CBA) but not by the nonfitting (A × C57BL/6)F<sub>1</sub> anti-A RS(C57BL/6) antiserum.

These experiments and those reported in the present communication strongly favor an antigenic similarity between the antigen-binding region of immunoglobulin molecules and of cellular recognition structures. Antigenic determinants located on the variable region of immunoglobulin molecules are called idiotypes (15–17). For reasons given elsewhere<sup>3</sup> we suggest that the antigenic determinants characterizing alloantibodies be called “aliotypes”. Using this terminology, we can state that recognition structures on lymphoid cells and the corresponding alloantibodies are of the same aliotype.

The core of data presented means nothing else than direct experimental evidence for what has been expressed before by many researchers (18–26). To quote Mitchison (27) it has to be assumed “that nothing except antibody recognizes antigen, and we must therefore assume that the receptor for antigen is antibody already present at a site, in or on the cell, prior to exposure to antigen.” The results of this report add weight to the likelihood of this interpretation.

#### SUMMARY

The possibility that a rat alloantiserum DA anti-Lewis possesses similar recognition structures for Lewis transplantation antigens, as do DA immunocompetent cells, was investigated by raising an antiserum against this alloantiserum in (Lewis × DA)F<sub>1</sub> hosts. This antiserum, as well as one provoked by injecting DA lymphoid cells, was active against recognition structures for Lewis antigens of DA immunocompetent cells. The anti-(DA anti-Lewis) antiserum displayed the same degree of specificity as was found previously for anti-recognition structure sera prepared by injecting parental strain lymphoid cells into F<sub>1</sub> hosts. Since the activities of antisera raised against cell-bound

receptors or against the antigen-binding region of an immunoglobulin were indistinguishable, it was concluded that the functional part of cell-associated receptors might be structurally similar to the variable portion of an immunoglobulin.

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