CELLULAR RECEPTORS: BINDING OF RADIOACTIVELY LABELED ANTI-ALLOANTISERUM

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It has been shown previously that antisera raised in (A × B) F_1 animals (whereby A and B designate two strains of inbred rats) against alloantibodies A anti-B are capable of specifically blocking the recognition of B antigens by A lymphoid cells (1). The test system used to measure antigenic recognition in vitro was the PAR (product of antigenic recognition) assay (2), basically a short-term mixed lymphocyte culture. The anti-receptor activity of such anti-alloantibodies was indistinguishable, by this test, from that of sera induced in (A × B)F_1 rats by the injection of receptor-bearing parental strain lymphoid cells. This led to the conclusion that receptors present on lymphoid cells and those presumed to exist in the antigen-combining region of alloantiserum might be antigenically similar (1).

The existence of antibodies specifically directed against recognition structures (RS)[1] on lymphoid cells bears on fundamental aspects of immunology; it therefore seemed useful to seek confirmation by experiments entirely independent of the PAR test. The most straightforward approach appeared to be a demonstration of the uptake of radioactively labeled anti-alloantibodies by lymphoid cells. This uptake should occur preferentially by those cells presumed to carry receptors of similar combining specificity to the alloantibody against which the labeled anti-alloantibody was directed. The present communication shows that this premise was sustained.

Materials and Methods

Animals.—Rats of the following inbred strains were domestically maintained: Lewis, DA, and BN. Adults of either sex were used in the experiments. F_1 hybrids were raised as required.

Alloantisera.—Full-thickness skin grafts were performed from one rat strain to another (3). These grafts were rejected within 9–11 days. 3 wk after a single skin graft, animals were bled, and the serum was heated at 56°C for 30 min, Millipore filtered (Millipore Corp., Bedford, Mass.), and stored at −20°C.

Anti-Alloantisera.—Alloantiserum, for instance of specificity DA anti-Lewis, was injected into (DA × Lewis)F_1 animals. On day 0, each animal to be immunized received 10 intradermal injections of 0.1 ml of plain alloantiserum distributed over the skin of the back (1). On day 14, animals were bled and the serum pool was inactivated and Millipore filtered as described for alloantisera. The alloantisera serving as antigens, the hybrids immunized, and the specificity of the anti-alloantisera obtained are shown in Table I.

[1] *Abbreviation used in this paper:* RS, recognition structure.

Preparation and Iodination of IgG from Anti-Alloantisera.—Crude gamma globulin was precipitated from whole serum by dialysis against sodium sulfate (4). The final precipitate was dissolved in 0.015 M potassium phosphate buffer, pH 8.0, and dialyzed overnight against the same buffer. After removal of a small precipitate by centrifugation, the protein solution (10–20 mg/ml) was applied to a 2.5 × 40 cm diethylaminoethyl cellulose column (Serva, Heidelberg, Germany) equilibrated with the above buffer. Elution was carried out with an ionic strength gradient (0.015–0.25 M) of the same buffer (5). The first major fraction to emerge was concentrated by vacuum dialysis, sterilized by Millipore filtration, and stored at −20° C. Immunoelectrophoretic analysis showed this fraction to consist mainly of IgG.

Four IgG preparations (one from each of the anti-alloantisera listed in Table I) were iodinated with ^{125}I by the chloramine T method (6) at the Schweizerisches Institut für Reaktorforschung, Würenlingen, Switzerland. Free iodine was eliminated by passage through a Dowex 1-X8 Cl^- form column. The specific activities were: 0.93 mCi/mg for anti-(DA anti-BN), 0.83 mCi/mg for anti-(BN anti-DA), 2.0 mCi/mg for anti-(DA anti-Lewis), and 2.0 mCi/mg for anti-(Lewis anti-DA).

TABLE I

Anti-Alloantisera Used in the Present Experiments

Alloantiserum injected as antigen	Animals immunized	Anti-alloantiserum obtained
DA anti-BN	(DA × BN)F_1	Anti-(DA anti-BN)
BN anti-DA	(DA × BN)F_1	Anti-(BN anti-DA)
DA anti-Lewis	(DA × Lewis)F_1	Anti-(DA anti-Lewis)
Lewis anti-DA	(DA × Lewis)F_1	Anti-(Lewis anti-DA)

Upon receipt of the iodinated preparations from Würenlingen, they were immediately passed through a 2.5 × 90 cm column of Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden). A small amount of aggregated material which came off with the void volume and some radioactivity appearing in the small molecular region were discarded. The main radioactive peak behaved like a 7S globulin molecule. Gel filtration was repeated after 3 wk, again permitting separation of some radioactivity in the small molecular region. The four preparations gave satisfactory results during 3–5 wk, after which time they seemed to lose their specificity in a rather abrupt manner.

The anti-alloantibodies were monitored through the various steps of preparation with the PAR test (1, 2). For instance, serum anti-(BN anti-DA) had a receptor-blocking titer as measured by the PAR test of 1:200,000. The IgG fraction after ion exchange chromatography (10 mg/ml of protein) displayed a titer of 1:128,000. The iodinated fraction (2 mg/ml) revealed a titer of 1:4000.

Absorption of Iodinated Anti-Alloantibodies with F_1 Cells.—Before being used in any of the tests to be described, the radioactive anti-alloantibodies were absorbed with spleen cells from F_1 hybrids of the same cross which had produced the anti-alloantibody. For instance, the iodinated anti-(BN anti-DA) was absorbed three times with 2×10^9 (BN × DA)F_1 spleen cells/mg of iodinated protein at 37°C for 30 min. This procedure was intended to remove remnants of the alloantiserum used for immunization (in this case BN anti-DA) and to eliminate those iodinated molecules having the highest nonspecific affinity for lymphoid cells.

Alloantibody-Coated Red Blood Cells.—Equal volumes of a suspension containing 250×10^6 fresh, washed (5×) rat red blood cells/ml of the proper type and of undiluted, inactivated alloantibody of the corresponding specificity were mixed and incubated for 30 min at 37°C. The coated cells were washed three times with saline (phosphate-buffered saline, pH 7.2) at 800 *g* for 8 min and resuspended in saline to the original volume.

Normal Lymphoid Cell Suspensions.—These were prepared from lymph nodes and spleens according to a standard procedure (3). Lymphoid cells were washed three times at 130 *g* for 8 min in Basal Medium Eagle (Laboratoires Eurobio, Paris, France) and were counted in a hemacytometer under phase contrast. Cell viability, as judged by the ability to exclude trypan blue, was at least 95%.

General Procedure for Determining the Reaction between Iodinated Anti-Alloantibody and Cells.—1-ml aliquots of antigen (either coated red blood cells, usually 250×10^6/ml, or normal lymphoid cells, usually 50×10^6/ml) were distributed into 5–10 parallel 85 × 10 mm tubes. Radioactive reagent was added in a volume of 0.1 ml usually containing between 3 and 4×10^5 cpm of ^{125}I. The reaction between particulate antigen and radioactive anti-alloantibody was allowed to proceed at room temperature for 60 min. The tubes were then centrifuged at 130 *g* for 8 min and the supernatant was sucked off, leaving approximately 0.05 ml and the cell sediment behind. The cells were resuspended in 1 ml of Basal Medium Eagle by touching the tubes to a Vortex mixer (Scientific Instruments, Inc., Springfield, Mass.), and 3 ml of Basal Medium were added to the suspension. The cells were again sedimented at 130 *g* for 8 min. This washing procedure was repeated for a total of six times. The last cell sediment was resuspended in 2 ml of saline and the cell suspension was poured into plastic tubes (Packard No. 6001098) fitting the gamma counter.

Radioactivity Measurements.—A Packard Auto-Gamma spectrometer model 3380 (Packard Instrument Co., Downers Grove, Ill.) equipped with a $1\frac{3}{4} \times 2$ inches sodium iodide crystal was used. The counter was calibrated with the aid of a ^{137}Cs standard. Lower and upper discriminator settings were 10 and 100 kev, respectively. Background levels (usually between 80 and 200 cpm) were subtracted from the sample counts. Results are indicated as means ± standard error.

RESULTS

(*a*) *Interaction between Iodinated Anti-Alloantibody and Alloantibody-Coated Red Cells.*—To test whether the immunization procedure had, in fact, induced the formation of immunoglobulin molecules capable of reacting with the antigen employed, an assay had to be arranged in which alloantibody (considered as an antigen) was allowed to interact with iodinated anti-alloantibody. Since both partners in this reaction were immunoglobulins, the Farr technique or its derivatives could not be employed. Rather, use was made of the unique antibody character of the antigen by allowing it to coat rat red cells of appropriate histocompatibility phenotype. For instance, Lewis red cells were incubated with BN anti-Lewis serum (as indicated in Materials and Methods); the coated red cells were then brought into contact with radioactive anti-(BN anti-Lewis) or with radioactive anti-(Lewis anti-BN). The amount of radioactivity fixed by the coated red cells was determined after thorough washing. The outcome of an experiment in which the concentration of alloantiserum used to coat the red cells was varied is shown in Table II.

Experiments using the other two labeled anti-antisera (see Table I) yielded similar results. The radioactive label was preferentially fixed by red cells which had been coated with the alloantibody used to immunize the anti-alloantibody donors. Coating with an alloantiserum dilution of 1:2 or 1:10 did not make much difference; alloantiserum at 1:100 dilution was apparently insufficient. Normal serum of the corresponding rat strains was ineffective.

(*b*) *Specificity of Interaction between Alloantibody and Iodinated Anti-Alloantibody.*—The three rat strains used differ at major histocompatibility loci (7). Although the exact specificities involved have been less extensively studied than in the mouse, several specificities are known to exist, some of which are unique for some strains, whereas others are shared by two or more strains (8). Therefore, it was interesting to see to what extent an anti-alloantiserum reacting with its "proper" antigen would cross-react with a "wrong" antigen of

TABLE II

Fixation of Iodinated Anti-Alloantibody by Alloantibody-Coated Red Cells

Antigen*	Radioactive reagent added	Radioactivity (cpm) fixed by antigen‡
Lewis anti-DA 1:2		7108 ± 565
Lewis anti-DA 1:10	Anti-(Lewis anti-DA)	5804 ± 577
Lewis anti-DA 1:100		1332 ± 95
Normal Lewis serum 1:2		1173 ± 137
Lewis anti-DA 1:2		1766 ± 331
Lewis anti-DA 1:10	Anti-(DA anti-Lewis)	734 ± 76
Lewis anti-DA 1:100		611 ± 123
Normal Lewis serum 1:2		822 ± 92
DA anti-Lewis 1:2		1592 ± 200
DA anti-Lewis 1:10	Anti-(Lewis anti-DA)	2030 ± 467
DA anti-Lewis 1:100		1230 ± 55
Normal DA Serum 1:2		1641 ± 110
DA anti-Lewis 1:2		8447 ± 920
DA anti-Lewis 1:10	Anti-(DA anti-Lewis)	8452 ± 799
DA anti-Lewis 1:100		1622 ± 282
Normal DA serum 1:2		1906 ± 304

* Rat red cells coated with alloantibody. DA cells were coated with Lewis anti-DA and Lewis cells were coated with DA anti-Lewis at the indicated concentrations.

‡ Mean ± standard error.

similar alloantibody specificity. Table III gives an example of such an experiment. The two radioactively labeled anti-alloantibodies, anti-(DA anti-Lewis) and anti-(Lewis anti-DA), were reacted with four alloantibodies (considered as antigens), namely: DA anti-Lewis and BN anti-Lewis fixed on Lewis red cells, and Lewis anti-DA and BN anti-DA fixed on DA red cells.

The highest amount of radioactivity was always bound by the "correct" alloantibody, i.e., that used for immunizing the anti-alloantibody donors. However, substantial amounts of radioactivity were also fixed by other alloantibodies of like specificity. Thus, whereas red cells coated with DA anti-Lewis absorbed approximately 11,000 counts of anti-(DA anti-Lewis), red

cells coated with BN anti-Lewis still fixed close to 3500 counts of the same reagent. These differences were statistically significant.

It appears clearly from Table III that the important point was similarity of combining specificity of two alloantibodies, not similarity of origin. For instance, BN anti-DA fixed substantial amounts of radioactivity from anti-(Lewis anti-DA), but BN anti-Lewis did not.

(*c*) *Interaction between Iodinated Anti-Alloantibody and Normal Rat Lymphoid Cells.*—Most immunological theories demand that recognition of foreign histocompatibility antigens be a function of "receptors" or "recognition structures" (RS) located on lymphoid cells. It has frequently been postulated that such

TABLE III

Specificity of Iodinated Anti-Alloantibody

Antigen*	Radioactive reagent added	Radioactivity (cpm) fixed by antigen‡
Lewis anti-DA	Anti-(Lewis anti-DA)	7517 ± 750
DA anti-Lewis		1010 ± 132
BN anti-DA		6613 ± 501
BN anti-Lewis		1474 ± 212
Lewis anti-DA	Anti-(DA anti-Lewis)	1310 ± 120
DA anti-Lewis		11,042 ± 1305
BN anti-DA		1557 ± 174
BN anti-Lewis		3429 ± 753

* Rat red cells coated with alloantibody. Lewis anti-DA and BN anti-DA were used at 1:10 dilution to coat DA red cells, and DA anti-Lewis and BN anti-Lewis were used at 1:10 dilution to coat Lewis red cells.

‡ Mean ± standard error.

recognition structures should bear some resemblance to circulating alloantibodies. Since, as shown above, our iodinated reagents did react with alloantibody of the proper specificity, a direct test for the presence of RS on normal lymphoid cells seemed feasible.

Normal lymphoid cells (from lymph nodes and spleens) were left in contact with radioactive anti-alloantibody and washed thoroughly, and the radioactivity which they had taken up was determined (see Materials and Methods). Table IV shows an example in which the numbers of lymphoid cells was varied, the input radioactivity being kept constant.

The amount of radioactivity fixed by the correct lymphoid cells was much higher than the amount absorbed by the wrong cells, and it increased with increasing cell dose. Thus, the radioactive anti-(BN anti-DA), presumed to have activity against receptors for DA antigens, was fixed by BN cells, which are supposed to carry receptors for DA transplantation antigens [RS(DA)], but

not by DA cells, which cannot carry receptors for their own histocompatibility phenotype.

(*d*) *Specificity of Interaction between Normal Lymphoid Cells and Iodinated Anti-Alloantibody.*—The same considerations made in section *b* with respect to alloantibodies of like specificity but of different origin apply for lymphoid cells. Thus, DA cells carry RS(Lewis) and are therefore capable of fixing anti-(DA anti-Lewis) [= anti-RS(Lewis)]. However, BN cells also carry RS(Lewis),

TABLE IV

Fixation of Iodinated Anti-Alloantibody by Normal Rat Lymphoid Cells

Antigen*	Radioactive reagent added	Radioactivity (cpm) fixed by antigen‡
25×10^6 BN cells	Anti-(BN anti-DA)	4373 ± 222
50×10^6 BN cells		9573 ± 764
75×10^6 BN cells		15,903 ± 194
100×10^6 BN cells		20,785 ± 373
25×10^6 BN cells	Anti-(DA anti-BN)	1443 ± 226
50×10^6 BN cells		961 ± 77
75×10^6 BN cells		1038 ± 196
100×10^6 BN cells		1154 ± 147
25×10^6 DA cells	Anti-(BN anti-DA)	1166 ± 93
50×10^6 DA cells		1410 ± 111
75×10^6 DA cells		1454 ± 156
100×10^6 DA cells		1419 ± 162
25×10^6 DA cells	Anti-(DA anti-BN)	3593 ± 227
50×10^6 DA cells		7533 ± 203
75×10^6 DA cells		11,199 ± 574
100×10^6 DA cells		15,143 ± 600

* Normal lymphoid cells from lymph nodes and spleen.

‡ Mean ± standard error.

although RS(Lewis) on BN cells is probably not identical to RS(Lewis) on DA cells. A partial identity between these two recognition structures of like specificity should reveal itself by a certain amount of cross-reaction with the radioactive anti-(DA anti-Lewis). Table V contains an example of such an experiment.

Whereas Lewis cells fixed about 12,000 counts of anti-(Lewis anti-DA), BN cells absorbed approximately 8000 counts, a quantity well in excess of the 1600 counts taken up by DA cells, which must be entirely devoid of any RS for DA antigens.

The behavior of F_1 cells was particularly revealing. Thus, (Lewis × DA)F_1

cells should not be able to bind radioactive anti-(BN anti-DA), since the F_1 cells carry the DA antigen and should therefore lack RS(DA). On the other hand, these same (Lewis × DA)F_1 cells should be capable of fixing some radioactive anti-(DA anti-BN), since they must have RS(BN). As shown in Table VI, this expectation was fulfilled.

TABLE V

Specificity of Cellular Receptors for Iodinated Anti-Alloantibody

Antigen*	Radioactive reagent added	Radioactivity (cpm) fixed by antigen‡
Lewis cells		12,289 ± 796
DA cells	Anti-(Lewis anti-DA)	1601 ± 50
BN cells		8168 ± 443
Lewis cells		1632 ± 107
DA cells	Anti-(DA anti-Lewis)	13,436 ± 856
BN cells		10,036 ± 1544

* 50×10^6 normal lymphoid cells from lymph nodes and spleen.
‡ Mean ± standard error.

TABLE VI

Specificity of Cellular Receptors on F_1 Hybrid Cells

Antigen*	Radioactive reagent added	Radioactivity (cpm) fixed by antigen‡
BN cells		6111 ± 897
DA cells	Anti-(BN anti-DA)	1345 ± 131
(Lewis × DA)F_1 cells		1245 ± 116
BN cells		787 ± 69
DA cells	Anti-(DA anti-BN)	3345 ± 279
(Lewis × DA)F_1 cells		2970 ± 359

* 50×10^6 normal lymphoid cells from lymph nodes and spleen.
‡ Mean ± standard error.

(*e*) *Absorption of a Specific Subpopulation from Iodinated Anti-Alloantibody.*— Since the radioactive input was always very large when compared with the radioactivity fixed by cells, it seemed important to show that the uptake of this small fraction was actually specific. This was done by serial absorption experiments: A radioactive input of approximately 400,000 cpm of anti-alloantibody was absorbed first with 50×10^6 normal lymphoid cells; the supernatant was again absorbed with 50×10^6 fresh cells, and this procedure was repeated two more times. The radioactivities fixed by each lot of cells as well as that of the last supernatant were determined. Table VII shows the results of two experiments. After four absorption cycles the supernatant still contained more than

90% of the initial counts. However, the specific counts had been largely removed, as shown by the fact that the fourth batch of lymphoid cells was no longer able to fix significant amounts of specific radioactivity.

TABLE VII

Absorption of Iodinated Anti-Alloantibody by Lymphoid Cells

Antigen used for absorption*	Radioactive reagent added	Radioactivity (cpm) fixed by antigen‡
First batch of Lewis cells	Anti-(Lewis anti-DA)	9036 ± 1319
Second batch of Lewis cells	Supernatant of first absorption	2564 ± 695
Third batch of Lewis cells	Supernatant of second absorption	1432 ± 370
Fourth batch of Lewis cells	Supernatant of third absorption	2696 ± 541
First batch of DA cells	Anti-(DA anti-Lewis)	22,891 ± 6015
Second batch of DA cells	Supernatant of first absorption	13,654 ± 2107
Third batch of DA cells	Supernatant of second absorption	9893 ± 1986
Fourth batch of DA cells	Supernatant of third absorption	6668 ± 687

* 50×10^6 normal lymph node and spleen cells per batch.

‡ Mean ± standard error.

TABLE VIII

Absorption of Iodinated Anti-Alloantibody by Lymphoid Cells. Parallel Decrease of Binding by Lymphoid Cells and by Alloantibody-Coated Red Cells

Antigen used for absorption*	Radioactive reagent added	Radioactivity (cpm)‡ fixed by	
		Lymphoid cells	Coated cells§
First batch of DA cells	Anti-(DA anti-Lewis)	10,914 ± 1156	—
Second batch of DA cells	Supernatant of first absorption	6634 ± 411	12,252 ± 91
Third batch of DA cells	Supernatant of second absorption	2413 ± 390	2915 ± 180
Fourth batch of DA cells	Supernatant of third absorption	1240 ± 78	1109 ± 143
	Supernatant of fourth absorption	—	1303 ± 18

* 50×10^6 normal lymph node and spleen cells per batch.

‡ Mean ± standard error.

§ 250×10^6 Lewis red cells coated with DA anti-Lewis.

(f) Identity of the Radioactive Subpopulation Fixed by Lymphoid Cells with that Absorbed by Alloantibody.—An experiment similar to the one shown on Table VII was used to answer the following question: Are the labeled molecules taken up by lymphoid cells the same as those which can be fixed by red cells coated with alloantibody? Iodinated anti-(DA anti-Lewis) was absorbed with four batches of 50×10^6 DA cells each. An aliquot of each supernatant was reacted

with Lewis red cells coated with DA anti-Lewis. The results are shown in Table VIII.

The radioactivity which could still be fixed by alloantibody-coated red cells diminished in parallel to that which had affinity for lymphoid cells. It thus appeared that the same molecules were involved in both reactions.

The same phenomenon could be shown in a slightly different experiment. Here lymphoid cells of type DA were reacted with radioactive anti-(DA anti-Lewis) in the presence of a 1:10 dilution (presumably a large excess) of DA anti-Lewis. The radioactivity fixed by the DA cells was substantially reduced in comparison with the control where a 1:10 dilution of normal DA serum had been added (Table IX). Tables VIII and IX indicate that lymphoid cells of the

TABLE IX

Inhibition by Free Alloantibody of the Reaction between Iodinated Anti-Alloantibody and Lymphoid Cells

Antigen*	Reaction mixture: Radioactive reagent + Serum added‡	Radioactivity (cpm) fixed by cells§
DA cells	Anti-(DA anti-Lewis) + Normal DA	10,073 ± 1462
DA cells	Anti-(DA anti-Lewis) + DA anti-Lewis	1474 ± 84
Lewis cells	Anti-(Lewis anti-DA) + Normal Lewis	4852 ± 1292
Lewis cells	Anti-(Lewis anti-DA) + Lewis anti-DA	1216 ± 115

* 50×10^6 normal lymph node and spleen cells.

‡ A 1:10 dilution of heat-inactivated serum was added to the reaction mixture.

§ Mean ± standard error.

proper type and circulating alloantibody of the proper specificity compete for the same radioactive anti-alloantibody.

DISCUSSION

Two technical factors may have contributed to the success of our experiments. The first was the use of rats as experimental animals. The iodination of antibodies of mouse origin reportedly yields erratic results (Dr. G. M. Iverson, personal communication). Preliminary experiments with iodinated rat alloantibodies had convinced us that they were easy to prepare without undue loss of activity. We had therefore reasons to hope that the iodination of anti-alloantibodies from rats would not present intractable difficulties. In fact, this aspect of our work proved surprisingly unproblematic.

The second factor was the possibility of exhaustively absorbing our radioactive reagents with lymphoid cells of F_1 origin before using them in tests. F_1 cells must be chemically very similar to the parental lymphoid cells used in the actual experiments, and yet they lacked the specific recognition structure in which we were interested. At first sight, absorption with F_1 cells might appear

unnecessary, since the serum must have already been absorbed in vivo by the cells of the very animal which produced it. However, remnants of the alloantibody used to immunize F_1 animals might still have been present at the time of bleeding. Furthermore, some iodinated molecules could conceivably have a propensity for nonspecific adsorption onto lymphoid cells of any kind, and these were conveniently removed by F_1 cells.

One may ask why alloantibodies should be such good antigens in the first place. A possible answer is that their antibody nature endows them with a "homing device" directing them at strategically important points of the immune system of the inoculated F_1 host.

Could our findings be explained by assuming that our radioactive reagents were directed at histocompatibility antigens? This is most unlikely. Rat histocompatibility antigens are inherited codominantly (8), hence F_1 animals should be incapable of forming anti-histocompatibility antibodies against parental antigens. One would also have to postulate that, for instance, Lewis red cells coated with DA anti-Lewis exhibited the same histocompatibility antigen as normal DA lymphoid cells, a rather elaborate assumption.

Could the existence of allotypes account for our findings? The answer again is no. An F_1 animal should not be capable of producing antibodies against parental allotypes, since these are inherited codominantly (9–11). That allotypes were not involved could also be shown by experiments such as those summarized in Table III: red cells coated with Lewis anti-DA absorbed a large amount of radioactive anti-(Lewis anti-DA), whereas red cells coated with BN anti-Lewis absorbed little of the same reagent; yet Lewis and BN immunoglobulins share the same allotype (10, 11).

To simplify further discussions, the actual names of inbred rat strains concerned will be reduced to A, B, and C. RS stands for recognition structure, A RS(B) represents that recognition structure which enables A cells to recognize the foreignness of the histocompatibility complex B. Similarly, A anti-B is an alloantiserum made by an animal A in response to a skin transplant from B. The antisera which were radioactively labeled were induced by injecting A anti-B serum or B anti-A serum into $(A \times B)F_1$ animals. The activity obtained was an anti-alloantiserum called anti-(A anti-B) and anti-(B anti-A), respectively (Table I).

As expected, anti-(A anti-B) was preferentially fixed by rat red cells of type B coated with A anti-B antibody (Table II). Absorption of A anti-B onto B target cells apparently did not impede its capacity to react with anti-(A anti-B). This perhaps offers an explanation for the recently reported failure of anti-receptor sera to inhibit cytotoxicity of alloantibodies (12). It had been shown previously, with the aid of the PAR test, that A anti-B must be antigenically similar to A RS(B) (1). This was shown again by the present experiments: Anti-(A anti-B) was preferentially fixed by lymphoid cells of type A, and anti-(B anti-A) was preferentially taken up by cells B, because the former

antibody represents anti-RS(B) and the latter anti-RS(A), respectively (Table IV). Cells of type C fixed a certain amount of both anti-(A anti-B) and anti-(B anti-A) (Table V). This was to be expected, since cells of type C contain both RS(A) and RS(B). However, it should be noted that RS(A) on cells C is different from RS(A) on cells B. Cells of type C can recognize on A cells only that subset of histocompatibility specificities as foreign by which the two strains, A and C, differ. This is not, in general, the same subset by which strains A and B differ. Therefore only partial cross-reactions can be expected between recognition structures of like specificity located on cells of different histocompatibility type.

A beautiful illustration of the principles involved is offered by the behavior of F_1 cells: (A $\times$ C)F_1 cells fixed anti-(A anti-B), whereas (B $\times$ C)F_1 cells did not (Table VI). This can be easily understood: (A $\times$ C)F_1 cells possess RS(B), but (B $\times$ C)F_1 cells, having themselves the antigen B, cannot carry RS(B).

From our absorption and inhibition experiments it appears that those radioactive molecules which were fixed by appropriate lymphoid cells were the same as those fixed by alloantibody-coated red cells (Tables VIII and IX). We interpret this to mean that anti-(A anti-B) specifically binds to both A anti-B and to A RS(B). Symbolically, one could therefore write: anti-(A anti-B) = anti-A RS(B).

Hence there must exist an antigenic similarity between circulating alloantibody A anti-B and the corresponding cellular receptor, A RS(B). This idea has been expressed previously by stating that alloantibody and the corresponding recognition structure are of the same aliotype (13).

The existence of antibodies directed against individual-specific determinants of other antibodies is well recognized (14–17). These specificities are usually called idiotypes. The difference between idiotypes as observed by others and our present findings is that all alloantibodies of a given specificity, e.g. A anti-B, induce the same anti-(A anti-B) antibody, whereas ordinary antibodies can have different idiotypes even if they are of the same specificity (15). The similarity between recognition structure and circulating alloantibody probably means that the antibodies appearing in the course of skin graft rejections are "true" copies of the receptors occurring in nonstimulated lymphoid cells. The aliotypic specificity therefore can be defined as an antigen characterizing all alloantibodies of a given specificity and the corresponding recognition structures (13).

It has been suggested by some that anti-(A anti-B) might consist of a complex of anti-B + B. While this interpretation seems farfetched and is unlikely to be true on several counts (18), the present experiments were not designed with a view of proving or disproving it. However, the ease with which our anti-RS activity could be iodinated and its behavior in gel filtration and ion exchange chromatography strongly suggest that the activity resides in a bona fide immunoglobulin molecule.

The present paper is a first forage into an area which promises vast oppor-

tunities for research. It will be necessary to delineate clearly the extent of cross-reactions between recognition structures of like specificities, and to correlate these with the histocompatibility types involved. It might be possible, by saturating the receptors on lymphoid cells, to estimate their numbers and distribution. One of the questions which are of particular actuality concerns the nature of the lymphoid cells that fix the labeled anti-alloantibody. Are they B cells or T cells? The alloantibodies used as immunogens are supposedly of B cell origin. However, the recognition of histocompatibility antigens is usually attributed to T cells. Preliminary experiments suggest that "educated" rat thymocytes bind much more of our radioactive reagents than do normal lymphoid cells (H. Binz and J. Lindenmann, unpublished data). A definite proof that the lymphoid cells involved are T cells would be important, since it might shed light on the hitherto elusive nature of the T cell receptor.

SUMMARY

Rat alloantisera of the following specificities were obtained after single skin graft rejection: DA anti-BN, BN anti-DA, DA anti-Lewis, and Lewis anti-DA. Anti-alloantibodies were raised by injecting the first two alloantisera into (DA × BN)F_1 hosts and the last two alloantisera into (DA × Lewis)F_1 hosts. In this manner, four anti-alloantisera were raised: anti-(DA anti-BN), anti-(BN anti-DA), anti-(DA anti-Lewis), and anti-(Lewis anti-DA). From each anti-alloantiserum a gamma globulin fraction was prepared and trace labeled with ^{125}I.

The labeled anti-alloantibodies could be shown to be fixed preferentially by red cells coated with the alloantibody used to induce them. They were also preferentially fixed by normal rat lymphoid cells presumed to carry recognition structures similar to alloantibody. For instance, anti-(DA anti-BN) was fixed by BN red cells coated with DA anti-BN and by normal DA lymphoid cells presumed to carry RS(BN). Conversely, anti-(BN anti-DA) was fixed by DA red cells coated with BN anti-DA and by normal BN lymphoid cells, carrying RS(DA).

Absorption and inhibition experiments showed that alloantibodies and normal lymphoid cells compete for the same labeled anti-alloantibodies. We conclude that cellular receptors and circulating alloantibody are antigenically similar: They have the same aliotype.

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REFERENCES

1. Ramseier, H., and J. Lindenmann. 1971. Cellular receptors. Effect of anti-alloantiserum on the recognition of transplantation antigens. *J. Exp. Med.* **134:**1083.

2. Ramseier, H. 1969. Quantitative studies on antigenic recognition. I. Immunological and nonimmunological parameters of the response. *J. Exp. Med.* **130:** 1279.
3. Billingham, R. E., and W. K. Silvers, editors. 1961. Transplantation of Tissues and Cells. The Wistar Institute Press, Philadelphia.
4. Marrack, J. R., H. Hoch, and R. G. S. Johns. 1951. The valency of antibodies. *Br. J. Exp. Pathol.* **32:**212.
5. Fahey, J. L.9 167. Chromatographic separation of immunoglobulins. *In* Methods in Immunology and Immunochemistry. C. A. Williams and M. W. Chase, editors. Academic Press, Inc., New York. **1:**322.
6. Hunter, W. M., and F. C. Greenwood. 1962. Preparation of iodine–131 labelled human growth hormone of high specific activity. *Nature (Lond.).* **194:**495.
7. Palm, J., and G. Black. 1971. Interrelationships of inbred rat strains with respect to Ag-B and non-Ag-B antigens. *Transplantation.* **11:**184.
8. Palm, J. 1971. Immunogenetic analysis of Ag-B histocompatibility antigens in rats. *Transplantation.* **11:**175.
9. Barabas, A. Z., and A. S. Kelus. 1967. Allotypic specificity of serum protein in inbred strains of rats. *Nature (Lond.).* **215:**155.
10. Armerding, D. 1971. Two allotypic specificities of rat immunoglobulin. *Eur. J. Immunol.* **1:**39.
11. Gutman, G. A., and I. L. Weissman. 1971. Inheritance and strain distribution of a rat immunoglobulin allotype. *J. Immunol.* **107:**1390.
12. Levinson, A. I., and D. B. Wilson. 1972. Failure to inhibit cytotoxic alloantibody with F_1 anti-parental receptor site substance. *Fed. Proc.* **31:**3023.
13. Ramseier, H., and J. Lindenmann. 1972. Similarity of cellular recognition structures for histocompatibility antigens and of combining sites of corresponding alloantibodies. *Eur. J. Immunol.* **2:**109.
14. Kunkel, H. G., M. Mannik, and R. C. Williams. 1963. Individual antigenic specificity of isolated antibodies. *Science (Wash. D C.).* **140:**1218.
15. Oudin, J., and M. Michel. 1969. Idiotypy of rabbit antibodies. I. Comparison of idiotypy of antibodies against *Salmonella typhi* with that of antibodies against other bacteria in the same rabbits, or of antibodies against *Salmonella typhi* in various rabbits. *J. Exp. Med.* **130:**595.
16. Gell, P. G. H., and A. S. Kelus. 1967. Anti-antibodies. *Adv. Immunol.* **6:**461.
17. Daugharty, H., J. E. Hopper, A. B. MacDonald, and A. Nisonoff. 1969. Quantitative investigations of idiotypic antibodies. I. Analysis of precipitating antibody populations. *J. Exp. Med.* **130:**1047.
18. Ramseier, H., and J. Lindenmann. 1972. Aliotypic antibodies. *Transplant. Rev.* **10:**57.