CELLULAR RECEPTORS: BINDING OF RADIOACTIVELY LABELED ANTI-ALLOANTISERUM

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It has been shown previously that antisera raised in (A X B) F1 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 X B) F1 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 alloantisera 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. F1 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 X Lewis) F1 animals. On day 0, each animal to be immunized received 10 intradermal injections of 0.1 ml of plain alloantisera 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 X 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 1% 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).

| Anti-Alloantisera Used in the Present Experiments |
|--------------------------------------------------|
| Anti-Alloantisera injected as antigen | Animals immunized | Anti-alloantisera obtained |
| DA anti-BN | (DA X BN) F1 | Anti-(DA anti-BN) |
| BN anti-DA | (DA X BN) F1 | Anti-(BN anti-DA) |
| DA anti-Lewis | (DA X Lewis) F1 | Anti-(DA anti-Lewis) |
| Lewis anti-DA | (DA X Lewis) F1 | Anti-(Lewis anti-DA) |

Upon receipt of the iodinated preparations from Würenlingen, they were immediately passed through a 2.5 X 90 cm column of Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Upsala, 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 F1 Cells.—Before being used in any of the tests to be described, the radioactive anti-alloantibodies were absorbed with spleen cells from F1 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 X 10^6 (BN X DA) F1 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 X 10^6 fresh, washed (5 X) 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 × 10⁶/ml, or normal lymphoid cells, usually 30 × 10⁶/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 × 10⁶ cpm of ¹²⁵I. The reaction between particulate antigen and radioactive anti-alloanfibody 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 re-suspended 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 1.34 × 2 inches sodium iodide crystal was used. The counter was calibrated with the aid of a ¹³⁷Cs 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-alloanfibody. 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-alloanfibody 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 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

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### Table II

| 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.
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

| 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
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 × 10⁶ BN cells | Anti-(BN anti-DA)          | 4373 ± 222                              |
| 50 × 10⁶ BN cells |                           | 9573 ± 764                              |
| 75 × 10⁶ BN cells |                           | 15,903 ± 194                            |
| 100 × 10⁶ BN cells |                           | 20,785 ± 373                            |
| 25 × 10⁶ BN cells | Anti-(DA anti-BN)          | 1443 ± 226                              |
| 50 × 10⁶ BN cells |                           | 961 ± 77                                |
| 75 × 10⁶ BN cells |                           | 1038 ± 196                              |
| 100 × 10⁶ BN cells |                           | 1154 ± 147                              |
| 25 × 10⁶ DA cells | Anti-(BN anti-DA)          | 1166 ± 93                               |
| 50 × 10⁶ DA cells |                           | 1410 ± 111                              |
| 75 × 10⁶ DA cells |                           | 1454 ± 156                              |
| 100 × 10⁶ DA cells |                           | 1419 ± 162                              |
| 25 × 10⁶ DA cells | Anti-(DA anti-BN)          | 3593 ± 227                              |
| 50 × 10⁶ DA cells |                           | 7533 ± 203                              |
| 75 × 10⁶ DA cells |                           | 11,199 ± 574                            |
| 100 × 10⁶ 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 F₁ cells was particularly revealing. Thus, (Lewis × DA)F₁
cells should not be able to bind radioactive anti-(BN anti-DA), since the F₁ cells carry the DA antigen and should therefore lack RS(DA). On the other hand, these same (Lewis × DA)F₁ 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 | Anti-(Lewis anti-DA)            | 12,289 ± 796                          |
| DA cells  |                                 | 1601 ± 50                             |
| BN cells  |                                 | 8166 ± 443                            |
| Lewis cells | Anti-(DA anti-Lewis)           | 1632 ± 107                            |
| DA cells  |                                 | 13,436 ± 856                          |
| BN cells  |                                 | 10,036 ± 1544                         |

* 50 × 10⁶ normal lymphoid cells from lymph nodes and spleen.
‡ Mean ± standard error.

### TABLE VI

**Specificity of Cellular Receptors on F₁ Hybrid Cells**

| Antigen*  | Radioactive reagent added       | Radioactivity (cpm) fixed by antigen |
|-----------|---------------------------------|--------------------------------------|
| BN cells  | Anti-(BN anti-DA)               | 6111 ± 897                           |
| DA cells  | (Lewis × DA)F₁ cells            | 1345 ± 131                           |
|           | Anti-(BN anti-DA)               | 1245 ± 116                           |
| BN cells  | Anti-(DA anti-BN)               | 787 ± 69                             |
| DA cells  | (Lewis × DA)F₁ cells            | 3345 ± 279                           |
|           | Anti-(DA anti-BN)               | 2970 ± 359                           |

* 50 × 10⁶ 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 × 10⁶ normal lymphoid cells; the supernatant was again absorbed with 50 × 10⁶ 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 re-
moved, 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 X 10⁶ 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 X 10⁶ normal lymph node and spleen cells per batch.
† Mean ± standard error.
§ 250 X 10⁶ 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 X 10⁶ 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

| Antigen  | Reaction mixture                      | 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 × 10⁶ 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 F₁ origin before using them in tests. F₁ 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 F₁ 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 allo-
antibody used to immunize F1 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 F1 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 F1 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 F1 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 F1 animal should not be capable of producing antibodies against
parental allotypes, since these are inherited codominantly (9–11). That allo-
types were not involved could also be shown by experiments such as those sum-
marized 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 immuno-
lobulins share the same allotype (10, 11).

To simplify further discussions, the actual names of inbred rat strains con-
cerned 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 × B)F1 animals. The activity obtained
was an anti-alloantiserum called anti-(A anti-B) and anti-(B anti-A), respect-
atively (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 F₁ cells: (A × C)F₁ cells fixed anti-(A anti-B), whereas (B × C)F₁ cells did not (Table VI). This can be easily understood: (A × C)F₁ cells possess RS(B), but (B × C)F₁ 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 alleotype (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 alleotypic 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 foray 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 lymph-
oid 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\textsubscript{1} hosts and the last two alloantisera into (DA × Lewis)F\textsubscript{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\textsuperscript{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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