SHARED IDIOTYPIC DETERMINANTS ON
B AND T LYMPHOCYTES REACTIVE AGAINST THE SAME
ANTIGENIC DETERMINANTS

I. Demonstration of Similar or Identical Idiotypes on IgG Molecules and
T-Cell Receptors with Specificity for the Same Alloantigens*

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The immunocompetent cells that can recognize foreign structures by their own
actively synthesized antigen-binding receptors are the lymphocytes (1). Two
major groups of lymphocytes exist, with separate functions and without ability to
transform into each other (2, 3). One group is called T lymphocytes, due to their
dependence on the presence of the thymus for development (2). The second group
of cells are called B lymphocytes, after the bursa of Fabricius in birds, where
maturation of these B cells do occur (3). T lymphocytes are responsible for the
major part of so called cell-bound immune reactions, whereas B lymphocytes pro-
duce conventional immunoglobulin antibodies.

The chemical structure of the antigen-binding receptors on the B lymphocytes is
generally considered to be that of a classical immunoglobulin, with minor modifications as
to make the molecule surface-attached (4). With regard to the antigen-binding receptors
on T lymphocytes opinions do vary considerably as to the nature of the receptor, and
classical immunoglobulins (5, 6) as well as molecules, determined by genes associated with
the loci determining the major histocompatibility antigens of the species (7, 8), have been
implied. Most workers have failed, by classical means, to detect significant amounts of
immunoglobulin on the surface of T lymphocytes (9–11).

One approach to the study of the molecular basis of the receptors on T lymphocytes
would be to use anti-idiotypic antibodies directed against the products of B lymphocytes,
that is against antibodies specific for a particular antigen determinant. If such
antiantibodies would have a detectable significant impact on T lymphocytes capable of
reacting with the same antigen, this would strongly suggest that B and T lymphocytes use
the same variable set of genes for the creation of antigen-binding sites on their receptors.
Indications that such anti-idiotypic antibodies will have the expected impact on the
relevant T lymphocytes have come from work in systems using an F, hybrid to parent
combination taking advantage of the immunogenetical filter in the production of such
specific antiantibodies (12, 13). Using this system substantial amounts of data have been
accumulated to suggest that B and T lymphocytes directed against the same alloantigenic
determinants may share idiotypic determinants (14–17). Final proof for such an identity is
still lacking, however, as in most tests either the lymphocyte populations or the antiserum

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Cancer Society, and NIH contract NO1-CB-33859.
used for immunization might have been contaminated with products from the other lymphocyte group.

In a previous set of articles we accumulated sizeable amount of results, indicating that anti-idiotypic antibodies produced in an F₁ hybrid rat against antibodies produced in one parental strain against the alloantigens of the other strain, will inhibit the T lymphocytes of the genotype of the antisera donor to function in the relevant, cell-bound immune reactions (16-17). In radioimmunoassays, however, we were only able to show that such F₁ antibodies would react in a detectable manner with B lymphocytes of the relevant strain (16, 17). As we considered this to be a matter of sensitivity of the test or quality of antibodies induced we have now tried and found it possible to obtain extremely high-titered antibodies produced in F₁ hybrid rats against T lymphocytes of one of the parental strains. Such antisera were found to react with highly purified IgG alloantibodies, produced in the T donor strain against alloantigens of the other parental strain. They were also found capable of reaction with the relevant immunocompetent normal parental T lymphocytes. Absorption experiments were then performed that showed that highly purified IgG alloantibodies could remove the reactivity of the F₁ anti-idiotypic antibodies against the relevant “pure” parental T lymphocytes and vice versa.

Thus the results obtained all directly demonstrate that B and T lymphocytes directed against the same antigenic determinants have antigen-binding receptors, which are similar or identical with regard to their antigen-binding sites as judged by their shared idiotypes.

Material and Methods

Animals. Rats of the inbred strains Lewis (L) (Ag-b¹), DA (Ag-B¹), and BN (Ag-B²) as well as F₁ hybrids between these strains were domestically raised and maintained. Either adult male or female rats were used within an experiment.

Rat Lymphocyte Preparations

SINGLE LYMPHOCYTE SUSPENSION. These were done from spleens and lymph nodes according to a standard procedure (18). Red cells were lysed by incubation in 0.84% NH₄Cl for 8 min at room temperature. Lymphoid cells were washed twice with Eagle's basal medium (BME)¹ containing 5% heat-inactivated fetal calf serum (FCS). The cell viability was judged by trypan-blue exclusion.

PREPARATION OF RAT T LYMPHOCYTES. This was done as already described by using Degalan V-26 beads coated with rat gamma globulin and rabbit antirat gamma globulin (16). Such T-cell preparations showed normally less than 2% B-cell contamination as judged by autoradiography using ¹²⁵iodine-labeled rabbit antirat gamma globulin or by immunofluorescence technique using rabbit antirat gamma globulin and FITC-coupled goat IgG antirabbit IgG.

PURIFICATION OF RAT B LYMPHOCYTES. For this a rabbit antirat T-lymphocyte serum was used in a cytolytic assay. A rabbit was inoculated with 5 × 10⁸ peripheral purified rat T lymphocytes twice at 2-wk intervals and bled 1 wk after last injection. The serum was heat-inactivated and then absorbed extensively with rat liver cells until a serum was produced that killed 30-40% of rat spleen cells and 60-70% of rat lymph node cells in the presence of guinea pig complement. The killing was at the same plateau level over several twofold dilutions up to 1:500 serum dilution. For purification of spleen B cells, cells were incubated with the antisera at 4°C for 1 h whereafter guinea pig complement was added and the suspension was incubated at 37°C for 40 min (using dilutions of antisera and complement as to achieve the above indicated percentual lysis of spleen cells). Cells were then filtered through sterile gauze to remove debris and dead cells and washed three times. Such cell suspensions were enriched for Ig surface-positive cells to more than 90% positive cells.

¹Abbreviations used in this paper: BME, Eagle's basal medium; FCS, fetal calf serum; MLC, mixed leukocyte culture; PBS, phosphate-buffered saline.
Production of Rat Antisera

Production of Alloantisera. Rat alloantisera of specificity Lewis anti-DA and DA anti-Lewis were raised by full thickness skin grafts from one strain to another (18). 3 wk after a single skin graft animals were bled and the sera heat-inactivated at 56°C for 30 min, Millipore filtered, and stored at –20°C. Such alloantisera showed titers between 1:2,048 and 1:8,192 as measured in a hemagglutination assay described elsewhere (19).

Production of anti-idiotypic antisera. See Results.

Purification of Rat IgG. This was done as described previously (13). Briefly, the gamma globulin fraction was precipitated with saturated (NH₄)₂SO₄ or by dialysis against 18% and 15% Na₂SO₄. The IgG fraction was then purified on DEAE-cellulose. Such preparations showed a single IgG peak on a calibrated G-200 column. In immunoelectrophoresis the preparations showed a single IgG precipitation line against a rabbit antiserum against whole rat serum.

Graft-vs.-Host Reactions. These were done in 5- to 8-wk old F₁ rats as described previously (15). 4 x 10⁸ viable parental lymphocytes in 0.1 ml BME were injected into each foot pad. Animals were killed 7 days later and the lymph nodes were excised and weighed (20).

Mixed Leukocyte Culture (MLC). Rats were anesthesized with ether and then killed by cervical dislocation and their spleens and lymph nodes aseptically teased with forceps in petri dishes containing RPMI 1640 (Flow Laboratories, Inc., Rockville, Md.) complemented with glutamin (2.5 mg/ml), 100 IU of penicillin, 100 μg of streptomycin, and 5% FCS (Flow Laboratories). The undissociated cells were allowed to settle for 5 min at 4°C. The single cell suspension was washed twice with the same medium. Stimulator cells were adjusted to 1 x 10⁸ cells per ml and were irradiated with 2,000 rad from an X-ray machine. The cells were washed again and adjusted to 7.5 x 10⁶ per ml. Untreated responder cells were adjusted to 1.5 x 10⁶ cells per ml. 5 x 10⁷ pelleted responder cells were incubated with 100 μl of 1:2 diluted anti-idiotypic or normal F₁ serum for 60 min at 4°C after which guinea pig complement (absorbed with agarose, 80 mg/ml serum) was added to a final dilution of 1:3. After a further incubation for 40 min at room temperature, cells were washed twice and finally adjusted to 1.5 x 10⁸ viable cells per ml. Both the F₁ anti-idiotypic and the normal F₁ serum were previously absorbed with 1 x 10⁷ (Lewis × DA)F₁ lymphoid cells per ml serum.

The one-way mixed leucocyte culture (MLC) was performed in Cooke round bottom microtiter plates (Cooke Laboratory Products, Div. Dynatech Laboratories, Alexandria Va.) using 1.5 x 10⁸ responder cells and 0.75 x 10⁶ stimulator cells in a total vol of 0.2 ml. The culture medium was RPMI 1640 (Flow Laboratories) complemented with glutamin, penicillin and streptomycin (see above) and 5% (Lewis × DA)F₁ normal rat serum previously absorbed with 1 x 10⁷ (Lewis × DA)F₁ lymphoid cells per ml serum. Control cultures contained responder cells or stimulator cells alone in concentrations as in the one-way MLC. All cultures were pulsed for 16 h on day 3 with 2 μCi of [³H]-thymidine (Radiochemical Centre, Amersham) with a spec act of 5 Ci per mmol in 20 μl culture medium without normal rat serum. On day 4 the cells were harvested using a Skatron collector and washed on glassfiber filters. The filters were dried and placed into plastic scintillation vials. All samples were counted in 3 ml scintillation fluid in an Intertechnique SL 30 liquid scintillation spectrometer.

Immunabsorbsens Technique. IgG preparations from Lewis anti-DA alloantibodies, Lewis, and (Lewis × DA)F₁ normal sera were coupled to CnBr-activated Sepharose 4 B (Pharmacia Fine Chemicals, Uppsala, Sweden) using carbonate buffer pH 9.0. After incubation for 4 h at room temperature the beads were washed once with coupling buffer followed by an incubation with 1 M ethanola- min pH 8.0 for 2 h at room temperature. Beads were then washed with isotonic glycine-HCl buffer pH 2.8 containing 2 M NaCl followed by 0.1 M carbonate buffer pH 8.0 containing 1 M NaCl. This was done three times in a cyclic way. The beads were finally washed with phosphate-buffered saline (PBS) and filled into plastic syringes and washed again with excess PBS. Serum to be analyzed was passed over the column and washed out with PBS. The eluate was collected and passed again through the column and finally sterile filtered. Columns were washed with excess PBS until the washing fluid contained less than 10 μg per ml of protein. Absorbed material was then eluted with isotonic glycine-HCl buffer pH 2.8 containing 2 M NaCl. The eluate was neutralized immediately, dialyzed against PBS and concentrated by vacuum dialysis and finally sterile filtered.

Indirect Radioimmunoassay

Using ¹²⁵I-radiolabeled rabbit anti-rat Ig. Normally, 1 x 10⁸ purified rat T lymphocytes were distributed into two or three parallel 2-ml plastic tubes. To the cell pellet 60 μl of 1:10 diluted (or as
indicated in the tables) serum was added. The mixture was incubated for 1 h at 4°C. Cells were then washed three times with BME containing 5% FCS. 0.1 ml of 111I-radiolabeled rabbit IgG antirat Ig (spec act 1 mCi per mg protein) corresponding to about 1-2 x 10^{6} cpm of 111I were added to the cell suspension and incubated for 1 h at 4°C after which the cells were washed five times. After the final wash the cells were transferred into new plastic tubes and counted for 1 min in an Intertechnique CG 30 automatic gamma spectrometer.

For quantitation of many samples the test was modified in the following manner (22). 1 x 10^{7} lymphoid cells in 25 μl were distributed in round bottom microtiter plates (Cooke Laboratory), 25 μl of 1:10 diluted (or as indicated) serum was added into each well and the plates gently shaken and incubated for 1 h at 4°C. The plates were then centrifuged for 10 min at 1,500 rpm in an X-3 Wifug (Winkelcentrifug, Stockholm) centrifuge in special holders fitting the microtiter plates. The supernates were smashed out into a sink and the cells resuspended with BME; cells were washed three times. 25 μl of 111I-labeled rabbit IgG antirat Ig (corresponding to about 2.5-5 x 10^{5} cpm) were then added into each well. After incubation for 1 h at 4°C the cells were washed again three times as described above. Finally the cells were transferred into plastic tubes for gamma counting.

Using 125I-labeled Protein A from Staphylococcus aureus. Protein A from Staphylococcus aureus is a molecule with specificity for Fc of most mammalian IgG classes (23). It can be iodinated and used as a sensitive probe for cell-bound IgG molecules (21). The procedure was identical to the second one described previously for radiolabeled rabbit antirat Ig, and has been described previously in detail (21).

Passive Hemagglutination Assay. This was done as described elsewhere (24). 300 μg IgG from relevant rat serum was dissolved in 200 μl saline, 10 μl of 0.07% CrCl_{3} solution and 100 μl of 50%, five times washed sheep red blood cells (SRBC) were added and the suspension well mixed. The CrCl_{3} was dissolved in saline and was kept half an hour at room temperature before used. After incubation of the elegant technical assistance of Berit Olsson, Ann Sjölund, and Lisbeth Ostberg is gratefully heat-inactivated FCS. After the final wash the coated SRBC were adjusted to a final concentration of 0.2% with saline containing 5% FCS. 25 μl of 0.2% coated SRBC were incubated with 25 μl of anti-idiotypic or normal sera dilutions for 1 h at room temperature followed by 2 h at 4°C or overnight at 4°C in V-bottom micro titer trays (BIO-cult, Linbor, IS MVC 96). Before used in this assay the anti-idiotypic sera were absorbed once with one tenth (vol/vol) of packed SRBC. As controls, uncoated SRBC or SRBC-treated with CrCl_{3} only were used.

Results

(DA x Lewis)F_{1} Hybrid Rats Inoculated with Parental T Lymphocytes Produce Antibodies Against Idiotypic Determinants on Relevant Parental Alloantibodies. Adult F_{1} hybrid rats were inoculated intraperitoneally with graded doses of normal parental T plus B lymphocytes (spleen and lymph node cells) or normal T lymphocytes (spleen and lymph node cells that had been filtered through an anti-Ig column). They would then be expected to produce antibodies against cell-bound receptors, present on the inoculated cells and with specificity for alloantigens of the other parental strain (12). At various times thereafter the animals were bled and their sera analyzed for possible content of antialloantibodies in the indirect hemagglutination assay. Representative results of such experiments are shown in Table I and indicate that the F_{1} hybrid rats will make such antibodies [as shown before (13, 16)]. The best way to obtain high-titered antialloantibodies was to inoculate column-purified parental T lymphocytes, whereas the presence of parental B lymphocytes, if anything, seemed to have an inhibitory effect. The present results would seem to indicate that either T and B lymphocytes directed against the same alloantigens do share idiotypic determinants or, alternatively a few contaminating B cells amongst the inoculated parental T cells do carry the antigen-binding receptors responsible for the induction of these anti-idiotypic determinants.
Table I

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Immunizing cells</th>
<th>Target cells</th>
<th>Days postimmunization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>DA, T + B, 5 × 10^7</td>
<td>SRBC, DA anti-Lewis IgG</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>DA, T + B, 2.5 × 10^7</td>
<td>SRBC, DA anti-Lewis IgG</td>
<td>2.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>DA, T 2.5 × 10^6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Lewis, T + B, 5 × 10^7</td>
<td>SRBC, Lewis Anti-DA IgG</td>
<td>3.0 ± 1.0</td>
</tr>
<tr>
<td>4</td>
<td>Lewis, T + B, 2.5 × 10^7</td>
<td>SRBC, Lewis Anti-DA IgG</td>
<td>2.4 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Lewis, T 2.5 × 10^6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All sera tested against SRBC coated with IgG from DA, Lewis or (DA × Lewis)F, normal sera as well as against SRBC coated with IgG from DA anti-Lewis or Lewis anti-DA immune sera. All sera were preadsorbed with SRBC before testing. Only groups where agglutination occurred are indicated in the table. Starting size of experimental groups normally five animals per group.

The rest of the present article we will use to try to prove that in fact T and B lymphocytes directed against the same alloantigens do have shared (or identical) antigen-binding receptors as judged by the use of anti-idiotypic antisera. For this purpose we inoculated a large number of F, hybrid rats intraperitoneally with 2 × 10^7 DA or Lewis column-purified T lymphocytes once every week and tested the antisera obtained at various time intervals for anti-idiotypic antibodies in the agglutination assay. A pool of unusually high-titered anti-idiotypic antibodies produced against Lewis T lymphocytes was obtained by such a procedure. Most of the present results to be described were carried out with this single, large pool of well-defined antibodies. This serum pool will be called 1003, and was obtained from a group of five (DA × Lewis)F, rats bled after inoculation 4–7 times with 25 × 10^6 Lewis T lymphocytes intraperitoneally. The pool was heat-inactivated at 56°C for 30 min, and was then adsorbed with normal (DA × Lewis)F, hybrid spleen and lymph node cells to remove possible autoimmune antibodies (25).

Characteristics of the (DA × Lewis)F, Serum 1003 Obtained by Immunization with Lewis T Lymphocytes

**Binding to Lewis-anti-DA IgG alloantibodies.** As stated before the 1003 serum was selected for its unusually high titer of anti-(Lewis anti-DA) antibodies. The titers and demonstration of the anti-idiotypic nature of the 1003 serum is shown in Table II. As seen, not only had the 1003 serum an extremely high titer against sheep erythrocytes coated with IgG from Lewis anti-DA alloantiserum (2148 in titer), but it was also precipitating in gel against such alloantiserum. A similar precipitating capacity of antialloantibodies has been reported before (26). As the 1003 serum in the hemagglutination or gel-diffusion tests with IgG did not react with any other normal or immune serum than the Lewis anti-DA serum (e.g., no reaction with Lewis anti-BN alloantiserum) we conclude that this serum must contain a high concentration of true anti-idiotypic antibodies with specificity for Lewis antibodies directed against DA alloantigens.

**Binding to normal Lewis T and B lymphocytes.** The 1003 serum was now tested for its capacity to react with normal lymphocytes from various rat strains.
or F₁-hybrids using the indirect radioimmunoassays as described in the methods section. Of the tested strains, only Lewis normal lymphocytes could be shown to bind 1003 antibodies as seen in Table III. When the distribution of binding Lewis lymphocytes was analyzed as to T or B type or to organ distribution sizeable,

**Table II**

**Presence of Anti-Idiotype Antibodies with Specificity for Lewis Anti-DA Alloantibodies in 1003 Serum**

<table>
<thead>
<tr>
<th>Indirect hemagglutination</th>
<th>Gel diffusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target</td>
<td>Titer</td>
</tr>
<tr>
<td>SRBC-Lewis normal IgG</td>
<td>Neg</td>
</tr>
<tr>
<td>SRBC-Lewis anti-DA IgG</td>
<td>2⁺⁻⁻⁻⁻²⁺⁺⁺⁺</td>
</tr>
<tr>
<td>SRBC-Lewis anti-BN IgG</td>
<td>Neg</td>
</tr>
<tr>
<td>SRBC-DA normal IgG</td>
<td>Neg</td>
</tr>
<tr>
<td>SRBC-DA anti-Lewis IgG</td>
<td>Neg</td>
</tr>
<tr>
<td>SRBC-(DA x L)F₁ normal IgG</td>
<td>Neg</td>
</tr>
</tbody>
</table>

**Table III**

**Uptake of Serum 1003 by Lewis Spleen Cells and Lewis Purified T Cells as Measured by ¹²⁵I-labeled Rabbit Antirat Ig**

<table>
<thead>
<tr>
<th>Lymphoid cells*</th>
<th>Incubated with:</th>
<th>Uptake of [¹²⁵I]rabbit antirat Ig† (Mean cpm of quadruplicates ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS</td>
<td>Serum 1003</td>
<td>16,878 ± 1,274</td>
</tr>
<tr>
<td>LS</td>
<td>N rat S</td>
<td>7,712 ± 1,018</td>
</tr>
<tr>
<td>LT</td>
<td>Serum 1003</td>
<td>12,756 ± 700</td>
</tr>
<tr>
<td>LT</td>
<td>N rat S</td>
<td>6,869 ± 534</td>
</tr>
<tr>
<td>L Thp</td>
<td>Serum 1003</td>
<td>1,269 ± 106</td>
</tr>
<tr>
<td>L Thp</td>
<td>N rat S</td>
<td>1,105 ± 44</td>
</tr>
<tr>
<td>DA S</td>
<td>Serum 1003</td>
<td>9,119 ± 1,513</td>
</tr>
<tr>
<td>DA S</td>
<td>N rat S</td>
<td>8,578 ± 489</td>
</tr>
<tr>
<td>DA T</td>
<td>Serum 1003</td>
<td>6,350 ± 457</td>
</tr>
<tr>
<td>DA T</td>
<td>N rat S</td>
<td>5,850 ± 552</td>
</tr>
<tr>
<td>DA Thp</td>
<td>Serum 1003</td>
<td>566 ± 53</td>
</tr>
<tr>
<td>DA Thp</td>
<td>N rat S</td>
<td>498 ± 55</td>
</tr>
<tr>
<td>F₁, S</td>
<td>Serum 1003</td>
<td>4,622 ± 306</td>
</tr>
<tr>
<td>F₁, S</td>
<td>N rat S</td>
<td>4,818 ± 112</td>
</tr>
<tr>
<td>F₁, T</td>
<td>Serum 1003</td>
<td>3,346 ± 490</td>
</tr>
<tr>
<td>F₁, T</td>
<td>N rat S</td>
<td>3,927 ± 523</td>
</tr>
<tr>
<td>F₁, Thp</td>
<td>Serum 1003</td>
<td>528 ± 41</td>
</tr>
<tr>
<td>F₁, Thp</td>
<td>N rat S</td>
<td>415 ± 33</td>
</tr>
<tr>
<td>BN p T</td>
<td>Serum 1003</td>
<td>4,196 ± 58</td>
</tr>
<tr>
<td>BN T</td>
<td>N rat S</td>
<td>5,393 ± 78</td>
</tr>
<tr>
<td>BN Thp</td>
<td>Serum 1003</td>
<td>480 ± 42</td>
</tr>
<tr>
<td>BN Thp</td>
<td>N rat S</td>
<td>342 ± 28</td>
</tr>
</tbody>
</table>

* S denotes a mixture of spleen and lymph node cells; T denotes T lymphocytes prepared by fractionation of spleen and lymph node cells over anti-Ig bead columns; Thp denotes thymocytes which have been passed over anti-Ig columns.

† Input per tube: 5 x 10⁴ cpm of [¹²⁵I]rabbit antirat Ig.
specific binding to both T and B lymphocytes was observed as shown in Tables III and IV. The binding to normal Lewis thymocytes, on the other hand, was insignificant. It should be noted that some of the tests in Table IV were performed using radiolabeled protein A from Staphylococcus, demonstrating that the 1003 serum contained IgG anti-idiotypic T-cell-reactive antibodies. The fact that the indirect radioimmunoassay even did function when using a mixture of T and B lymphocytes as targets (thus having a highly significant background noise due to the binding to surface Ig on normal B lymphocytes) would indicate a

![Table IV](image)

**Table IV**

<table>
<thead>
<tr>
<th>Cells*</th>
<th>Antiserum†</th>
<th>[125I]pA§</th>
<th>Mean ± SE11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lewis T</td>
<td>[125I]F, anti-Lewis T</td>
<td>–</td>
<td>6.399 ± 256</td>
</tr>
<tr>
<td>Lewis T</td>
<td>F, anti-Lewis T</td>
<td>+</td>
<td>1.074 ± 78</td>
</tr>
<tr>
<td>Lewis T</td>
<td>F, normal</td>
<td>+</td>
<td>222 ± 15</td>
</tr>
<tr>
<td>Lewis B</td>
<td>F, anti-Lewis T</td>
<td>+</td>
<td>26.335 ± 1.161</td>
</tr>
<tr>
<td>Lewis B</td>
<td>F, normal</td>
<td>+</td>
<td>12.445 ± 981</td>
</tr>
<tr>
<td>DA T</td>
<td>[125I]F, anti-Lewis T</td>
<td>–</td>
<td>2.098 ± 283</td>
</tr>
<tr>
<td>DA T</td>
<td>F, anti-Lewis T</td>
<td>+</td>
<td>250 ± 25</td>
</tr>
<tr>
<td>DA T</td>
<td>F, normal</td>
<td>+</td>
<td>342 ± 22</td>
</tr>
<tr>
<td>DA B</td>
<td>[125I]F, anti-Lewis T</td>
<td>–</td>
<td>1.870 ± 261</td>
</tr>
<tr>
<td>DA B</td>
<td>F, anti-Lewis T</td>
<td>+</td>
<td>12.075 ± 639</td>
</tr>
<tr>
<td>DA B</td>
<td>F, normal</td>
<td>+</td>
<td>14.349 ± 137</td>
</tr>
</tbody>
</table>

* Lewis T cells were prepared by fractionation of spleen and lymph node cells over anti-Ig bead columns. B cells were produced by cytolysis of T cells by a rabbit antirat T-cell serum in presence of complement. Purity with regard to T and B respectively exceeded 95%.
† Directly 125I-labeled or unlabeled F, anti-Lewis T serum was used. When 125I-labeled, the IgG fraction of the antiserum was used. Input per well: 3 × 10⁴ cpm.
§ + denotes secondary addition of [125I]protein A to the test. Input per well: 5 × 10⁴ cpm.
11 Mean ± SEM. Triplicates. Italicized figures denote significantly increased specific binding compared to DA cell controls.


high number of idioype-positive cells in the present system as subsequently found to be true.²

**Proof that the Binding of the 1003 Antibodies Occurs with Receptors Present on Normal Lewis T Lymphocytes and with Specificity for DA Alloantigens and Not with All Lewis T Cells.** We have previously found that F₁ hybrid antisera produced against parental alloantisera in rats, in the presence of complement, will inhibit the capacity of the relevant parental T lymphocytes to function in a given graft-vs.-host (GVH) reaction (17). We now first tested if the

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1003 serum could selectively inactivate Lewis T lymphocytes from participating in immune reactions against DA alloantigens, whilst sparing the reactivity against alloantigens of other strains intact. Two test systems were used: GVH reactions as measured by the local popliteal lymph node assay and the MLC system. Examples of the results obtained in the two systems are shown in Table V (see also Table VII) and demonstrate clearly that the 1003 serum contains antibodies specific for receptors on those Lewis T lymphocytes that can react with DA alloantigens. Thus, a close to complete inhibition of the reactivity against DA alloantigens was observed in both systems, whereas reactivity against BN was left largely untouched.

**Table V**

*Serum 1003 Specifically Eliminates Lewis Anti-DA GVH-Reactive T Cells*

<table>
<thead>
<tr>
<th>Cells injected*</th>
<th>Host</th>
<th>Injected cells treated with:</th>
<th>Mean of “Lewis” lymph node weights‡</th>
<th>Mean of “DA” lymph node weights‡</th>
<th>Mean of “BN” lymph node weights‡</th>
<th>Mean log ratio ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>L (L x DA)F₁</td>
<td>(L x DA)F₁, NS + C’</td>
<td>70.7 ± 8.6</td>
<td>70.2 ± 7.6</td>
<td>-0.01 ± 0.02§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA</td>
<td>(L x DA)F₁, NS + C’</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L (L x DA)F₁</td>
<td>Serum 1003 + C’</td>
<td>11.2 ± 0.9</td>
<td>73.2 ± 5.03</td>
<td>0.81 ± 0.02‖</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA</td>
<td>Serum 1003 + C’</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L (L x BN)F₁</td>
<td>(L x DA)F₁, NS + C’</td>
<td>33.7 ± 5.3</td>
<td>32.2 ± 2.5</td>
<td>-0.01 ± 0.05§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BN</td>
<td>(L x DA)F₁, NS + C’</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L (L x BN)F₁</td>
<td>Serum 1003 + C’</td>
<td>31.5 ± 3.7</td>
<td>31.9 ± 4.3</td>
<td>-0.00 ± 0.07§</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BN</td>
<td>Serum 1003 + C’</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*5 x 10⁶ spleen and lymph node cells were injected into each foot pad.
‡ Mean weights of four nodes (mg).
§ Not significantly different from 0.
‖ Significantly different from 0 (P < 0.01).

**Proof that the 1003 Antibodies Directed Against Idiotype Determinants on T Lymphocytes and Those Directed Against the Idiotype-Positive Lewis-Anti-DA IgG Alloantibodies Are the Very Same Antibody Molecules.** The antibodies in the 1003 that are capable of reaction with those Lewis T lymphocytes that are immunocompetent to react against DA alloantigens and those 1003 antibodies in the 1003 that are capable of reaction with those Lewis T lymphocytes that are immunocompetent to react against DA alloantigens and those 1003 antibodies that react with Lewis anti-DA IgG antibodies had now both been shown to be idiotypic. It now remained to finally demonstrate that the normal Lewis T lymphocytes with receptors for DA alloantigen did share idiotypic determinants with the Lewis anti-DA alloantibodies produced by B cells; that is, to prove identity or similarity between the antigen-binding sites of the two groups of reactive molecules. Two approaches were used. In the first, highly purified IgG was produced from Lewis anti-DA immunserum (see Methods) and covalently
linked to Sepharose beads. Such immunosorbant columns (and controls) were now analyzed for their capacity to remove from the 1003 serum the capacity to react with Lewis T lymphocytes as measured in radioimmunoassays or GVH or MLC reactions. The tests were performed testing both the immunosorbent column passed 1003 serum as well as the adsorbed and at low pH eluted 1003 material for inhibitory or binding capacity. Results of such experiments are shown in Tables VI, VII and VIII, and demonstrate the selective capacity of highly purified Lewis anti-DA IgG columns to remove in a recoverable form the 1003 antibodies with capacity to react with normal Lewis anti-DA-reactive T

TABLE VI
Capacity of Lewis Anti-DA Immunoabsorbens to Remove (Lewis × DA)F₁, Anti-Lewis T Anti-Idiotypic Antibodies

<table>
<thead>
<tr>
<th>Lymphoid cells</th>
<th>Uptake of [¹²⁵I] rabbit antirat Ig</th>
<th>Mean cpm of triplicates ± SE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT Serum 1003</td>
<td>70.074 ± 2.119</td>
<td></td>
</tr>
<tr>
<td>LT N rat S</td>
<td>22.367 ± 2.026</td>
<td></td>
</tr>
<tr>
<td>LT Serum 1003 absorbed on L anti-DA IgG</td>
<td>24.505 ± 787</td>
<td></td>
</tr>
<tr>
<td>LT Serum 1003 absorbed on L normal IgG</td>
<td>51.545 ± 6.648</td>
<td></td>
</tr>
<tr>
<td>LT Serum 1003 absorbed on (L × DA)F₁, normal IgG</td>
<td>56.551 ± 3.355</td>
<td></td>
</tr>
<tr>
<td>LT Serum 1003 bound and eluted from L anti-DA IgG</td>
<td>64.931 ± 9.626</td>
<td></td>
</tr>
<tr>
<td>LT Serum 1003 bound and eluted from L normal IgG</td>
<td>19.542 ± 4.69</td>
<td></td>
</tr>
<tr>
<td>LT Serum 1003 bound and eluted from (L × DA)F₁, normal IgG</td>
<td>21.618 ± 2.401</td>
<td></td>
</tr>
<tr>
<td>DA T Serum 1003</td>
<td>29.271 ± 2.819</td>
<td></td>
</tr>
<tr>
<td>DA T N rat S</td>
<td>25.444 ± 3.84</td>
<td></td>
</tr>
<tr>
<td>DA T Serum 1003 absorbed on L anti-DA IgG</td>
<td>32.788 ± 2.350</td>
<td></td>
</tr>
<tr>
<td>DA T Serum 1003 absorbed on L normal IgG</td>
<td>28.713 ± 1.164</td>
<td></td>
</tr>
<tr>
<td>DA T Serum 1003 absorbed on (L × DA)F₁, normal IgG</td>
<td>27.542 ± 1.037</td>
<td></td>
</tr>
<tr>
<td>DA T Serum 1003 bound and eluted from L anti-DA IgG</td>
<td>25.659 ± 3.04</td>
<td></td>
</tr>
<tr>
<td>DA T Serum 1003 bound and eluted from L normal IgG</td>
<td>28.245 ± 2.537</td>
<td></td>
</tr>
<tr>
<td>DA T Serum 1003 bound and eluted from (L × DA)F₁, normal IgG</td>
<td>25.786 ± 2.829</td>
<td></td>
</tr>
</tbody>
</table>

* Input per well: 2 × 10⁶ cpm of [¹²⁵I]rabbit antirat Ig.

The included controls demonstrated the Ig nature of the inhibitory capacity of the 1003 serum. Furthermore, the specificity of 1003 removing capacity of the IgG Lewis anti-DA column was further proven by the failure of such columns to remove DA antisheep red blood cell antibodies, thus also excluding the possibility that any anti-DA allotype-specific antibodies might have been on the column. Thus, these data strongly suggest the presence of identical idiotypes on IgG molecules produced in Lewis B cells against DA alloantigens and on the antigen-binding surface receptor on normal Lewis T cells with capacity to react against DA alloantigens.

To further prove this point normal Lewis T or T + B cells were used as specific immunosorbents to remove from the 1003 serum the antibodies, capable of agglutinating in the anti-idiotypic agglutination test, erythrocytes coated with
Table VII
Effect of Serum 1003 on the MLC Reaction. Selective Removal of Inhibitory Capacity with L anti-DA Alloantibody IgG Immunosorbant

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>L (L x DA)F₁</td>
<td>32.933 ± 245</td>
<td>1.017 ± 84</td>
<td>167 ± 11</td>
</tr>
<tr>
<td>L (L x DA)F₁, NS + C‘</td>
<td>32.112 ± 145</td>
<td>1.566 ± 302</td>
<td>187 ± 26</td>
</tr>
<tr>
<td>L Serum 1003 + C’</td>
<td>1.938 ± 29</td>
<td>1.133 ± 43</td>
<td>199 ± 2</td>
</tr>
<tr>
<td>L Serum 1003 abs L anti-DA IgG + C’</td>
<td>30.929 ± 292</td>
<td>1.006 ± 48</td>
<td>179 ± 23</td>
</tr>
<tr>
<td>L Serum 1003 abs LNS IgG + C’</td>
<td>1.660 ± 419</td>
<td>1.040 ± 62</td>
<td>223 ± 41</td>
</tr>
<tr>
<td>L Serum 1003 abs (L x DA)/F₁, IgG + C’</td>
<td>1.609 ± 113</td>
<td>1.015 ± 57</td>
<td>244 ± 49</td>
</tr>
<tr>
<td>L Serum 1003 bound and eluted from L anti-DA IgG + C’</td>
<td>3.013 ± 273</td>
<td>1.087 ± 72</td>
<td>214 ± 102</td>
</tr>
<tr>
<td>L Serum 1003 bound and eluted from LNS IgG + C’</td>
<td>31.525 ± 743</td>
<td>2.037 ± 741</td>
<td>249 ± 27</td>
</tr>
<tr>
<td>L Serum 1003 bound and eluted from (L x DA)F₁, IgG + C’</td>
<td>30.987 ± 1.155</td>
<td>1.777 ± 373</td>
<td>296 ± 65</td>
</tr>
<tr>
<td>L (L x BN)F₁</td>
<td>22.857 ± 801</td>
<td>1.814 ± 799</td>
<td>252 ± 111</td>
</tr>
<tr>
<td>L (L x BN)F₁, Serum 1003 + C’</td>
<td>21.844 ± 1.091</td>
<td>1.007 ± 154</td>
<td>366 ± 220</td>
</tr>
<tr>
<td>DA (L x DA)F₁</td>
<td>30.294 ± 528</td>
<td>1.134 ± 61</td>
<td>195 ± 72</td>
</tr>
<tr>
<td>DA (L x DA)F₁, Serum 1003 + C’</td>
<td>29.915 ± 257</td>
<td>1.227 ± 81</td>
<td>214 ± 35</td>
</tr>
</tbody>
</table>

Table VIII
Capacity of L Anti-DA Alloantibody IgG to Remove Anti-Idiotypic Antibodies from 1003 Serum as Measured by Inhibition of GVH-Reaction

<table>
<thead>
<tr>
<th>Cells injected*</th>
<th>Host</th>
<th>Injected cells treated with:</th>
<th>Mean of “Lewis” lymph node weights*</th>
<th>Mean of “DA” lymph node weights*</th>
<th>Mean log Ratio ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>L, DA (L x DA)F₁</td>
<td>Serum 1003</td>
<td>28.5 ± 3.3</td>
<td>26.4 ± 2.9</td>
<td>-0.02 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>L, DA Serum 1003 + C’</td>
<td>6.1 ± 1</td>
<td>32.6 ± 5.6</td>
<td>0.73 ± 0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L, DA Serum 1003 abs L anti-DA IgG + C’</td>
<td>27.7 ± 1.6</td>
<td>25.0 ± 0.3</td>
<td>-0.04 ± 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L, DA Serum 1003 abs LNS IgG + C’</td>
<td>6.2 ± 0.6</td>
<td>33.6 ± 3.5</td>
<td>0.70 ± 0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L, DA Serum 1003 (L x DA)F₁, N S IgG + C’</td>
<td>4.1 ± 0.4</td>
<td>33.0 ± 2.2</td>
<td>0.89 ± 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L, DA Serum 1003 bound and eluted from L anti-DA IgG + C’</td>
<td>5.1 ± 1.0</td>
<td>29.7 ± 2.7</td>
<td>0.78 ± 0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L, DA Serum 1003 bound and eluted from LNS IgG + C’</td>
<td>21.8 ± 1.0</td>
<td>22.9 ± 0.7</td>
<td>0.02 ± 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L, DA Serum 1003 bound and eluted from F₁, N S IgG + C’</td>
<td>26.2 ± 1.2</td>
<td>23.5 ± 1.4</td>
<td>0.05 ± 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 3 × 10⁶ spleen cells and lymph node cells were injected into each foot pad.

IgG Lewis anti-DA alloantibodies. As seen in Table IX purified Lewis T lymphocytes were, if anything, better than T + B cell mixtures in removing such specific anti-idiotypic antibodies. We could thus conclude that, by all measurements, Lewis normal T lymphocytes, with the capacity to react with the DA
TABLE IX
Capacity of Normal Lewis T Lymphocytes to Adsorb away Serum 1003 Antibodies as Subsequently Measured in the Indirect Hemagglutination Assay

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Inhibitory cells*</th>
<th>Agglutination titers$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lewis T</td>
<td>$2^4$</td>
</tr>
<tr>
<td></td>
<td>(Lewis x DA)F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>$2^{13}$</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>$2^{14}$</td>
</tr>
<tr>
<td>2</td>
<td>Lewis, T + B</td>
<td>$2^7$</td>
</tr>
<tr>
<td></td>
<td>Lewis, T</td>
<td>$2^9$</td>
</tr>
<tr>
<td></td>
<td>(Lewis x DA)F&lt;sub&gt;1&lt;/sub&gt;, T + B</td>
<td>$2^{11}$</td>
</tr>
<tr>
<td></td>
<td>(Lewis x DA)F&lt;sub&gt;1&lt;/sub&gt;, T</td>
<td>$2^{12}$</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>$2^{13}$</td>
</tr>
</tbody>
</table>

* $10^7$ cells of indicated type was added to each hole in the microplate, whereafter twofold dilutions of 1003 serum was added. Purity of T cells with regard to B-cell contamination = 98% or more. B-cell contamination in T + B cell populations = 50-60%. T + B = spleen and lymph node cells mixture. T = T cells purified out of the T + B population via passage through anti-Ig columns.

† Agglutination as assessed against SRBC coated with Lewis-anti-DA IgG antibodies. Controls including SRBC coated with IgG from normal Lewis, DA or F<sub>1</sub> sera as well as IgG from DA-anti-Lewis immune sera were negative.

strain alloantigens, have receptors with identical idiotypes to the IgG antibodies that Lewis B cells will produce against the same alloantigens.

Discussion
The aim of the present article is to demonstrate that the antigen-binding receptors on B and T lymphocytes reactive against the same antigenic determinants do share idiotypic determinants. F<sub>1</sub> hybrid animals can be immunized against parental lymphocytes or alloantibodies produced in one parental strain against alloantigens of the other parental strain. Such antireceptor antisera made in F<sub>1</sub> hybrid mice or rats have been found capable of selective interference with relevant parental lymphocytes as measured by in vitro (12-14, 16, 27) or in vivo (15, 17, 26-28) tests.

Using radiolabeled F<sub>1</sub> antialloantibody IgG molecules it was previously found possible to label parental lymphocytes of the relevant strain (13, 16). Here, the presence of idioantigens on normal parental lymphocytes was paralleled by the selective ability of such lymphocytes to bind in vitro to the relevant allogeneic monolayer of cells (16, 17). Although such antialloantibody serum was found able to selectively interfere with the specific binding to the monolayer of both B and T lymphocytes (16, 17), in the previous assays only B lymphocytes could be shown to bind enough radiolabeled anti-idiotypic alloantibody IgG molecules to allow detection. However, we then favored the interpretation that T cells as well as B cells should bind in such anti-idiotypic assays, provided the
radioimmunoassay became sensitive enough or the anti-idiotypic antibodies had the relevant specificity. The present results have indeed shown that when F₁ hybrids make high-titered antisera against parental T-cell receptors with specificity for alloantigen of the other parental strain, such sera will now also be capable of showing directly demonstrable binding to parental immunocompetent T lymphocytes carrying such idiotypic, antigen-binding receptors.

A great help in the analysis of anti-idiotypic antibodies produced in F₁ hybrids against inoculated alloantisera, produced in one parental strain against the alloantigens of the other parental, came with the development of a sensitive indirect hemagglutination assay to detect such antibodies (24, 26). Taking advantage of these findings we have been able to confirm and extend the results previously obtained in the radioimmunoassays and the cellular immune reactions (15-17), and as we believe, to finally prove the existence of shared idiotypic determinants on antigen-binding receptors on T or B lymphocytes reactive against the same antigenic determinants. The crucial issue to consider in such an approach is the purity of the reagents or cells used, as “pure” T lymphocytes could always be contaminated by a certain low but still decisive number of B cells (or cytophilic B-cell products) whereas on the other hand, alloantisera used in the previous assays might have contained soluble T-cell receptors (12, 14, 16, 17). Our approach in the present article was to immunize F₁ hybrids with “pure” parental T lymphocytes, trying to make a pool of high-titered antisera, reactive with idiotypic receptors or antibodies with the relevant antigen-binding specificity. T lymphocytes were used as “antigen” as we had previously had problems to get high enough titered antisera by the use of injection of alloantibodies only (16, 17). There are reasons to believe that receptors on immunocompetent T cells would be most immunogeneic in this system. We succeeded in producing such high-titered F₁ antisera using as a screening assay sheep erythrocytes coated with IgG from relevant immune parental alloantisera; that is, looking for antibodies directed against idiotypic determinants present on B-cell products, the IgG molecules. Subsequently we analyzed these high-titered F₁ hybrid antisera for their capacity to react in a radioimmunoassay with normal, parental “pure” T lymphocytes. We could find a nice positive correlation between anti-idiotypic titer as measured by agglutination of the alloantibody IgG-coated erythrocytes and the capacity to react with the relevant, parental T lymphocytes.²

We then demonstrated that the binding of F₁ antibodies as measured in the radioimmunoassay onto the normal parental T lymphocytes was indeed directed against idiotypic antigen-binding receptors on a minority of these cells. This was demonstrated by the capacity of such anti-idiotypic antisera to, in the presence of complement, selectively abolish the capacity of those parental T lymphocytes to react against the relevant allogeneic cells as measured in local GvH-reaction or in vitro MLC, whilst sparing the reactivity of the cell population towards other, third party allogeneic cells. Furthermore, the strength of the reaction allowed a direct enumeration of the idiotype-positive T and B cells.²

The final proof demonstrating identity between the anti-idiotypic antibodies reactive with the B-cell product, the alloantibodies of IgG type, and those that react with the idiotypic receptors on the parental T cells came from a series of
experiments, where reciprocal adsorptions using highly purified T cells or IgG-immunosorbents were used to selectively remove the anti-idiotypic antibodies reactive with the "other" idiootype group. Highly purified IgG from the relevant parental alloantiserum coupled to Sepharose was shown capable of selective removal of the F₁ anti-idiotypic antibodies reactive with the relevant T-cell receptors. As measurements were taken to ensure highest possible purity of the IgG alloantibodies before coupling to Sepharose, and contamination with soluble T-cell receptors for antigen would seem unlikely, unless such receptors are indeed physically unseparable from conventional IgG molecules. Reciprocally, purified parental T cells were capable of selective removal of F₁ antialloantibody antibodies as subsequently tested in the hemagglutination assay. Here the fact that normal purified parental T lymphocytes were seemingly more efficient than a mixture of T and B cells in removing specific anti-idiotypic antibodies as subsequently measured in the hemagglutination assay would strongly argue against possible B-cell contamination as a cause for the capacity of T cells to adsorb away these antibodies.

In conclusion, our results demonstrate that T and B lymphocytes directed against the same alloantigens are using antigen-binding receptors or antibodies with shared idiotypic determinants. The simplest interpretation of these findings would be that T and B lymphocytes are indeed using the same set (or subset) of variable genes to create the antigen-binding area of their respective receptors for antigen.

Summary

Antigen-binding receptors on T lymphocytes and IgG antibodies with the same antigen-binding specificity as the T-cell receptors display shared or identical idiootypes. This was shown using a system where adult F₁ hybrid rats between two inbred strains were inoculated with T lymphocytes from one parental strain. Such F₁ hybrid rats produce antibodies directed against idiotypic determinants present on IgG alloantibodies, produced in the T donor genotype strain and with specificity for the alloantigens of the other parental strain. The idiotypic nature of the F₁ antialloantibody serum against the parental alloantibodies was demonstrated both by indirect hemagglutination tests or by gel diffusion using alloantisera with different specificity as targets. Furthermore, the F₁ anti-T-lymphocyte sera could be shown to contain antibodies against idiotypic parental T lymphocytes as well. This was shown by the capacity of the antisera, in the presence of complement, to wipe out the relevant parental T-cell reactivity against the other parental strain (as measured in MLC or GVH) whilst leaving the T-lymphocyte reactivity against a third, unrelated allogeneic strain intact.

These findings demonstrate that F₁ hybrid rats inoculated with parental T lymphocytes make anti-idiotypic antibodies directed against both the T cell receptors and IgG alloantibodies of that parental strain with specificity for alloantigens of the other parental strain. In order to prove identity between the anti-idiotypic antibodies against the B and T-cell antigen-binding molecules the following experiments were carried out: highly purified IgG from relevant alloantibody-containing serum in immunosorbent form could be shown to selectively remove both anti-idiotypic activities from the F₁ antiserum. Further-
more, parental normal T lymphocytes could be shown capable of removing from
the anti-idiotypic antisera all those antibodies that would cause agglutination
of the relevant alloantibody-coated erythrocytes in the indirect agglutination assay.
We would thus conclude that T and B lymphocytes reactive against a given
antigenic determinant use receptors with antigen-binding areas coded for by the
same variable gene subset(s).

The elegant technical assistance of Berit Olsson, Ann Sjölund, and Lisbeth Östberg is gratefully
acknowledged.

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Note added in proof. Anti-idiotypic antibodies directed against a mouse-antistrepto-
coccal determinant can, if passively administered to normal mice, prime these animals for
helper activity against the streptococcal antigens (K. Eichmann, and K. Rajewsky, per-
sonal communication). It is possible that these findings have the same meaning as those
results in the present article, that is, that T and B lymphocytes reactive against the same
antigenic determinant may share idiotypic determinants.

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