

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

II. Determination of Frequency and  
Characteristics of Idiotypic T and B Lymphocytes in Normal  
Rats using Direct Visualization\*

BY HANS BINZ AND HANS WIGZELL

*(From the Department of Immunology, Uppsala University, Uppsala, Sweden)*

The specific antigen-binding molecules on B lymphocytes would seem to be conventional immunoglobulins, maybe somewhat different from serum immunoglobulins as to allow a more stable membrane attachment but otherwise seemingly identical (1). The molecules with the same function on T lymphocytes, however, are still a matter of dispute (2–10). Whereas most researchers fail to detect conventional immunoglobulins on or in T lymphocytes (4,7–9) others report contrariwise (2,3). The finding that immune response genes linked to the major histocompatibility locus systems are of major importance for T-lymphocyte function, has also led to the hypothesis that T lymphocytes may use the products of such response genes as their specific antigen-binding receptors instead of using classical immunoglobulin genes (5,6). In support of this have been reports that antigen-binding specific factors from T lymphocytes may carry antigenic markers indicating them to be products of such response genes (10,11).

Our approach in the analysis of the relationship between antigen-binding receptors on T vs. B lymphocytes has been to use anti-idiotypic antibodies, that is, antibodies against the variable region (frequently involving the actual antigen-binding area) of the antibody or antigen-binding receptor. This was initiated under the assumption that it was comparatively easier to perceive the creation of a T-cell receptor for antigen that contained constant regions of a new "type" but would still be using for its variable region one of, or the same, subset of variable genes as would the B lymphocytes. By the use of such anti-antibodies produced against alloantisera or T-lymphocyte receptors using parent to F<sub>1</sub> hybrid combinations (12) sizeable data have accumulated indicating the feasibility of this kind of approach in the analysis of the antigen-binding receptors on T lymphocytes (13–18). In our latest experiments we have thus been successful via the use of highly purified B-cell products (antibodies of IgG class) and purified T cells (with receptors for a given antigen) to obtain results showing the presence

---

\* This work has been supported by a grant from the Swiss Foundation for Scientific Research to Hans Binz, by the Swedish Cancer Society, and by NIH contract N01-CB-33859 to Hans Wigzell.

of shared idiotypes on normal T and B lymphocytes with antigen-binding receptors for the same antigenic determinants (9). This could be shown using a combination of techniques involving radioimmunoassays, indirect hemagglutinations, and specific inhibition of T-lymphocyte activity against the relevant antigen. We would thus conclude that T and B lymphocytes in their creation of receptors for antigen most likely are able to use at least one common pool of variable subsets of genes.

In the present article we have set as our task to determine the frequency of normal idiotypic T and B lymphocytes reactive against an antigenic determinant(s), using as our probe anti-idiotypic antibodies of the kind previously described (9,16,18). The conditions allowing such studies, the frequencies of the cells carrying the idiotypic receptors, and the density of idiotypic markers as an indication of receptor density on T vs. B lymphocytes have been studied using autoradiography and/or quantitative single cell fluorospectrophotometry. In an accompanying article the actual direct linkage between specific immune capacity of a given cell in relation to its capacity to display a given idotype will be described.

## Materials and Methods

*Animals.* Adult male or female rats of the inbred strains Lewis (L)<sup>1</sup> (Ag-B<sup>1</sup>) and DA (Ag-B<sup>4</sup>) as well as F<sub>1</sub> hybrids between these two strains were domestically raised and maintained and used in the present experiments.

### *Preparation of Rat Lymphoid Cell Suspensions*

**SINGLE LYMPHOID CELL SUSPENSION.** Splens and lymph nodes were teased with forceps in Petri dishes containing RPMI 1640 (Flow Laboratories, Inc., Rockville, Md.) complemented with 2.5 mg/ml of glutamin, 100 IU/ml of penicillin, 100  $\mu$ /ml of streptomycin, and 10% heat-inactivated (30 min, 56°C) fetal calf serum (FCS). The suspension was filtered through a net and the single cell suspension was washed once with the same medium. Red cells were lysed by incubation with 0.84% (wt/vol) NH<sub>4</sub>Cl for 8 min (including 6 min centrifugation at 800 g). Cells were washed again twice with medium. Cell viability was judged by trypan blue exclusion.

**PREPARATION OF RAT T LYMPHOCYTES.** Single lymphoid cell suspensions from spleen and lymph nodes were passed through Degalan beads previously coated with rat Ig and rabbit antirat Ig (16). Cells were collected on ice and washed again with the above medium complemented further with 10 mM HEPES and 10 mM NaN<sub>3</sub> and kept at 0°C until used for the experiments. Purity of these T-cell preparations is indicated under results.

**PURIFICATION OF RAT B LYMPHOCYTES.** A rabbit was injected with  $5 \times 10^8$  peripheral purified rat T lymphocytes (purified as described above) twice at 2-wk intervals and bled 1 wk after the last injection. The serum was heat inactivated (30 min, 56°C) and then absorbed extensively with rat liver cells until a serum was obtained that killed 30–40% of rat spleen cells and 60–70% of rat lymph node cells in the presence of absorbed (agarose 80 mg/ml serum) guinea pig complement (C). For the present report  $5 \times 10^8$  spleen and lymph node cells were incubated with 10 ml 1:50 diluted rabbit anti-T-cell serum for 1 h at 0°C. Cells were washed five times with medium and absorbed guinea pig C was added to a final dilution of 1:5. Cells were washed again with medium and then trypsinized for 10 min at room temperature with 0.25% trypsin, filtered through a net, and then allowed to recover overnight at 37°C in medium containing 20% FCS. Cells were filtered again through a net and finally handled as described for rat T cells. For the purity of such B-cell preparations see the Results.

### *Production of Anti-Idiotypic Antisera*

**SERUM POOL 1003.** This was obtained from five (Lewis  $\times$  DA)F<sub>1</sub> rats which have been inocu-

<sup>1</sup> Abbreviations used in this paper: FCS, fetal calf serum; FITC, fluorescein isothiocyanate.

lated four to seven times with  $2.5 \times 10^7$  Lewis T lymphocytes in 3-wk intervals. Animals were bled 1 wk after each injection. The serum pool was heat inactivated (30 min, 56°C) and kept at  $-70^\circ\text{C}$ . (For details of the anti-idiotypic tests on this pool see reference 9.)

**SERUM POOL 3.** This was obtained from four (Lewis x DA)<sub>F</sub> rats inoculated eight times with  $2.5 \times 10^7$  Lewis peripheral T lymphocytes in 3-wk intervals. The pool was obtained 6 days after the last immunization, heat inactivated at 56°C for 30 min, and kept at  $-70^\circ\text{C}$  until use. Similar tests of anti-idiotypic specificity as for 1003 have been performed.

Both pools were absorbed extensively with (Lewis x DA)<sub>F</sub> lymphocytes to eliminate possible autoantibodies (19). Both serum pools showed specific binding to Lewis T lymphocytes as described in a radioimmunoassay described elsewhere (9). Most important, both pools showed specific suppressor capacity of relevant graft-vs.-host reactions and mixed leukocyte culture responses as reported previously (9), indicating their true anti-idiotypic nature.

*Coupling of Ig from Serum Pool 3 with Fluorescein Isothiocyanate (FITC).* The Ig fraction from serum pool 3 was obtained from whole serum by ammonium sulfate precipitation (40% saturation). The precipitate was washed several times with 40% ammonium sulfate and finally dialyzed against phosphate-buffered saline. 20 mg of Ig at a concentration of 10 mg/ml were incubated with 10 mg FITC (Becton, Dickinson & Co., Cockeysville, Md.) at pH 9.0 for 2 h at room temperature. Unconjugated FITC was eliminated by passing the mixture through a Sephadex G-25 column. The FITC-conjugated protein was sterile filtered and kept at  $-70^\circ\text{C}$ . The FITC:protein ratio was 1.8:1. In the same way IgG of a rabbit antirat Ig was conjugated with FITC.

*Staining of Idiotypic-Positive Lymphocytes with FITC-Conjugated Anti-Idiotypic Antibodies.*  $5 \times 10^6$  lymphocytes to be stained were incubated with 1:2 or 1:5 diluted FITC-conjugated anti-idiotypic antibodies using RPMI 1640 complemented with glutamin, penicillin, streptomycin (see above), 10% FCS, 10 mM HEPES, and 10 mM NaN<sub>3</sub> as a medium for 1 h at  $0^\circ\text{C}$ . Before use the conjugated antibodies were centrifuged for 8 min in a Beckman 152 Microfuge (Beckman Instruments, Fullerton, Calif.).

Cells were washed six times with medium for 6 min at 800 g and kept on ice. Cells were either read with a Leitz Microscope (Leitz SM-Lux microscope with a Ploem fluorescence-illuminator for "naked eye" readings) or using a Zeiss microcytofluorometer equipped for peak readings (kindly lent by the Department of Pathology, Uppsala University, Uppsala, Sweden) for quantitative recordings of the fluorescence intensity of single cells.

*Iodination of Rabbit IgG against Rat Ig.* The IgG fraction from rabbit antirat Ig serum was obtained as described earlier (20). The IgG was iodinated with <sup>125</sup>Iodine by the method of Hunter and Greenwood using a sp act of 1 mCi/mg protein.

*Autoradiographs.* Cells to be analyzed were suspended in RPMI 1640 complemented as described for staining with FITC-conjugated antibodies (see above).  $5 \times 10^6$  cells were incubated with sterile filtered 1:2 diluted anti-idiotypic antiserum or normal F<sub>1</sub> serum for 1 h at  $0^\circ\text{C}$ . Cells were washed five times with medium and then incubated with <sup>125</sup>Iodine-radiolabeled rabbit antirat Ig ( $5 \times 10^6$  cpm/sample) for 1 h at  $0^\circ\text{C}$ . Cells were washed again five times with medium and then smeared on precleaned glass slides and dried under a fan for 24 h at  $37^\circ\text{C}$ . Cells were fixed for 24 h in 100% methanol. Slides were dipped into Ilford G-5 emulsion (Ilford Ltd., Essex, England), kept at  $46^\circ\text{C}$ , and dried in a slanted position. The autoradiographs were exposed at  $4^\circ\text{C}$  for 5 days and then developed with Kodak ID-19 developer and fixed with 5% Na-thiosulfate. Cells were stained with Giemsa at pH 5.75.

*Radioimmunoassays on Large Populations of Cells.* In some experiments populations of cells rather than single cells were analyzed as to their capacity to bind anti-idiotypic antibodies. The principle design was identical as depicted under Autoradiograph section, and details were as previously described (9).

## Results

*Failure to Demonstrate any Significant Impact of Temperature on the Binding of Anti-Idiotypic Antibodies to Specific T-Lymphocyte Receptors.* It has previously been reported that T lymphocytes may fail to express significant antigen-binding capacity at  $4^\circ\text{C}$  in comparison to  $37^\circ\text{C}$  (21,22). Also, the failure by several "conventional" immunosorbant techniques to remove specific T lym-

phocytes could, among other things, be due to a possible easy detachment of the antigen-binding receptors from the T-cell surface (23). As both our present single cell assays involve frequent incubation and washing steps, we required knowledge as to whether variations in temperature during the experimental procedures would be of any major importance. We thus carried out radioimmunoassays on Lewis T lymphocytes in the batch assay using incubation temperatures of 37°C or 4°C. As shown in Table I very similar binding values were obtained at the two temperatures. We decided anyhow to carry out our further experiments at 0–4°C whenever possible, although incubations at higher temperatures would seem to carry no measurable disadvantage, at least when measured at the population level.

*Frequency of Lewis Normal T and B Lymphocytes Displaying Idiotypic Markers of Lewis-Anti-DA Specificity.* Having established that T lymphocytes express their idiotypic markers to the same degree at 4°C compared to 37°C (see Table I) we now felt justified to compare the frequencies of Lewis normal T or B lymphocytes carrying idiotypic markers signifying anti-DA activity using 4°C as our working temperature. B lymphocytes have previously been found to be roughly equal in their immunoglobulin presentation or antigen-binding capacity at 4°C compared to 37°C (22). We now tested using two different batches of anti-Lewis-anti-DA idiotypic antisera for the frequency of normal Lewis T and B lymphocytes carrying such markers using the direct fluorescent antibody technique. The results of one of these antisera (the other gave comparative figures) are shown in Table II. Several facts emerge from these studies: The first and basic finding was that it is indeed possible to visualize idio-type-positive cells by such fluorescent anti-idiotypic antibodies. The fluorescence as judged by the naked eye on the idiotypic T as well as B cells was a typical membrane reaction, with the stain distributed in a somewhat patchy formation as shown in Fig. 1. It should be recalled that these fluorescent antibody tests were carried out using conditions reducing the formation of caps (see Materials and Methods). The frequency of T lymphocytes with idiotypic markers of anti-DA specificity is higher than on B cells (6.2 vs. 1.1%); both figures being quite high in comparison to most (24) but not all (K. Eichmann, personal communication) reports on frequencies of idiotypic cells. The purity of T population with regard to B cells was more than 99% whereas the B-cell population could at most have been contaminated with 2% T lymphocytes (now assuming surface Ig positivity as a "sure" B-cell marker). The figures mentioned above could accordingly not be influenced to any significant degree by contamination from the other lymphocyte population. Finally, the results obtained using the anti-idiotypic antiserum at two different dilutions were identical, demonstrating that the test was carried out under conditions of antibody excess.

Maybe the most striking findings in Table II are the high frequencies of normal T lymphocytes carrying idiotypic markers of a given specificity. However, it should be recalled that very similar figures of immunocompetent T lymphocytes from normal individuals and with reactivity against the major histocompatibility antigens of the species have been reported from functional assays (25,26). To further confirm the present findings we also applied another technique, namely autoradiography. Results of such experiments [now reporting on

TABLE I  
*Equal Binding of 1003 Serum Antibodies to Lewis Cells at 4°C and 37°C*

T cells*	Incubated with:	Incubated at:	Mean‡ cpm of duplicates ± SE
		°C	
L	1003	4	9.348 ± 1.145
L	1003	37	9.494 ± 3.311
L	F <sub>1</sub> NS§	4	3.375 ± 147
L	F <sub>1</sub> NS	37	2.663 ± 66
DA	1003	4	2.240 ± 301
DA	1003	37	1.950 ± 402
DA	F <sub>1</sub> NS	4	1.984 ± 62
DA	F <sub>1</sub> NS	37	1.691 ± 9

\*  $5 \times 10^6$  T lymphocytes were added to each well.

‡  $5 \times 10^4$  cpm of  $^{125}\text{I}$ -labeled protein A was added to each sample.

§ NS, normal serum.

TABLE II  
*Percentage of Idiotypic-Positive T and B Lewis Lymphocytes with "Anti-DA" Idiotypes*

Cells	Incubated with:	Total cells counted	Positive cells	Positive cells
				%
Lewis T	Pool 3-FITC 1:2	2,361	147	6.23
Lewis T	Pool 3-FITC 1:5	2,148	134	6.24
DA T	Pool 3-FITC 1:2	516	0	0
DA T	Pool 3-FITC 1:5	433	0	0
Lewis B	Pool 3-FITC 1:2	3,210	33	1.03
Lewis B	Pool 3-FITC 1:5	2,624	28	1.07
DA B	Pool 3-FITC 1:2	614	0	0
DA B	Pool 3-FITC 1:5	439	0	0
L T	R antirat-FITC 1:20	213	1	0.47
DA T	R antirat-FITC 1:20	133	1	0.75
L B	R antirat-FITC 1:20	168	165	98.21
DA B	R antirat-FITC 1:20	211	207	98.10

experiments obtained using another anti-(Lewis-anti-DA) antiserum] are shown in Table III. After subtracting for the minor B-cell contamination in the population, similar figures as compared for those obtained using fluorescent antibody techniques were found. Using an arbitrary cutoff level for highly labeled cells at 40 grains per cell as the limit (above that number actual grain counts became rapidly inaccurate) we could thus show the presence of 6.3% T lymphocytes from normal adult Lewis rats with idiotypic markers of anti-DA specificity. The actual intensity of reaction of anti-idiotypic antibodies with single T lympho-

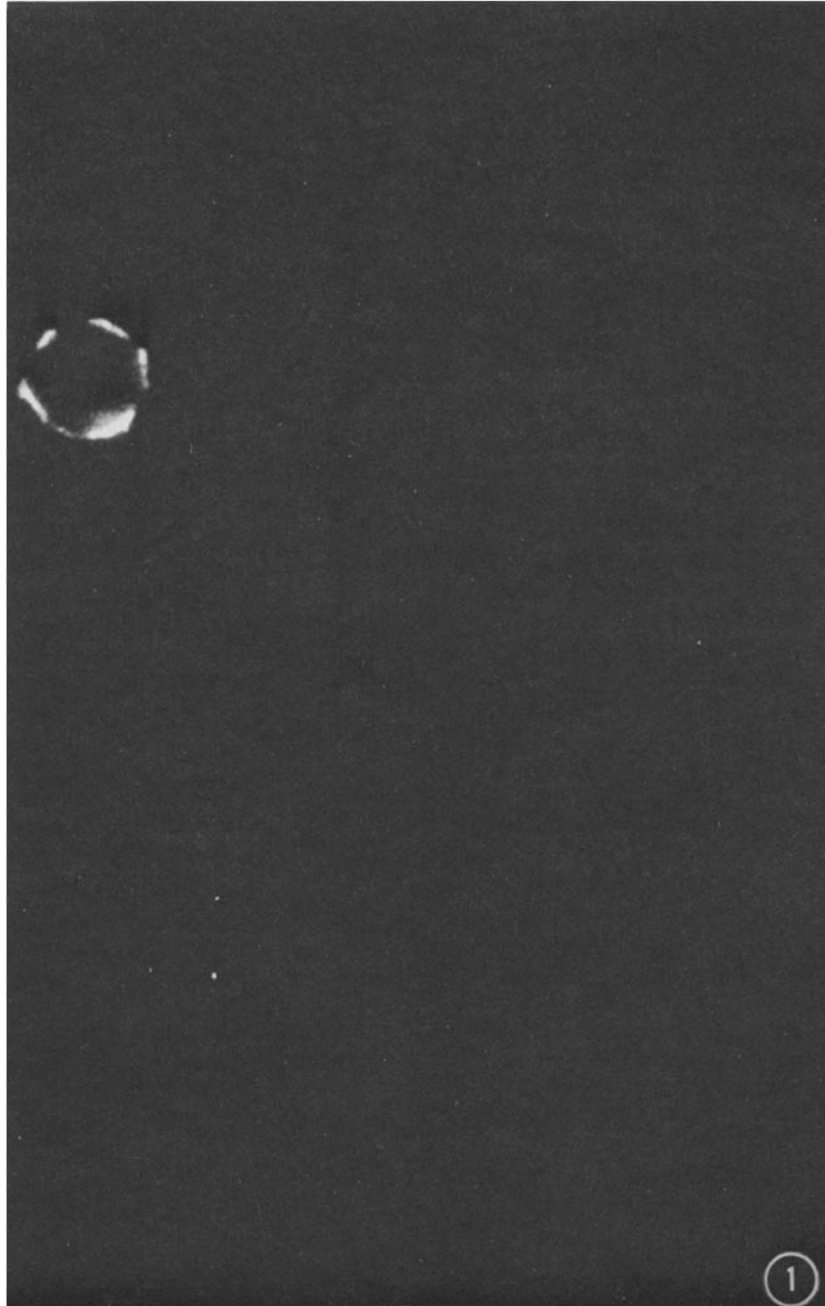


FIG. 1. Picture of "anti-DA" idiotype-positive normal Lewis T-lymphocyte incubated with FITC-labeled antibodies from serum pool 3. Test carried out under "non-capping" conditions.

TABLE III  
*Frequency Distribution of Grains on Lewis or (Lewis × DA)F<sub>1</sub>T Lymphocytes Incubated with (Lewis × DA)F<sub>1</sub> Anti-Lewis T Serum or (Lewis × DA)F<sub>1</sub> Normal Serum*

Cells*	Serum†	Grain count distribution (in percent of total population)									
		0-4	5-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80 or more
Lewis T	F <sub>1</sub> , anti-Lewis T	88	3	0.7	0	0.3	0	0	0.3	0	7.0
Lewis T	F <sub>1</sub> , normal serum	93	6	0.2	0.5	0.2	0	0	0	0	0.5
(Lewis × DA)F <sub>1</sub> T	F <sub>1</sub> , anti-Lewis T	96	3	0	0.4	0	0	0	0	0	0.7
(Lewis × DA)F <sub>1</sub> T	F <sub>1</sub> , normal serum	90	8	1.1	0	0	0	0	0	0	1.4

\* Prepared from normal spleen and lymph node cells filtered through anti-Ig columns. 300-400 cells counted per group.  
 † (Lewis × DA)F<sub>1</sub> normal or anti-Lewis T serum, followed by radiolabeled rabbit-antirat Ig antibodies.

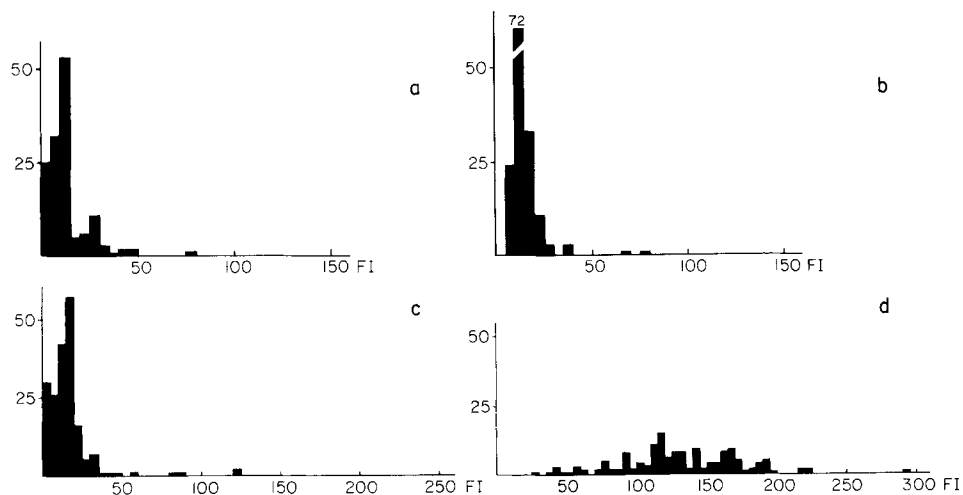
cytes as compared to the anti-Ig reaction with B lymphocytes (see control results in Table III) suggested similar orders of magnitude.

*Quantitative Cytofluoroscrophotometry on Idiotype-Positive T Lymphocytes using Anti-Idiotypic Antisera.* As the results using both naked eye determinations and autoradiography suggested that T lymphocytes do carry a comparatively high number of idiotypic sites per cell we carried out some preliminary experiments using single cell measurement in quantitative cytofluoroscrophotometry. Here, comparisons were made between the reactivity for surface Ig of B lymphocytes using fluorescein-labeled antipolyvalent Ig antibodies in relation to the staining observed on single idiotypic T lymphocytes using FITC-labeled anti-idiotypic antibodies. As the experiments are highly time consuming, such high numbers of cells as analyzed in Table II could not be reached. Consequently we would not observe any significantly increased number of stained B cells in these experiments due to the fact that the background "noise" in relation to the low frequency of idiotypic B cells became too disturbing.

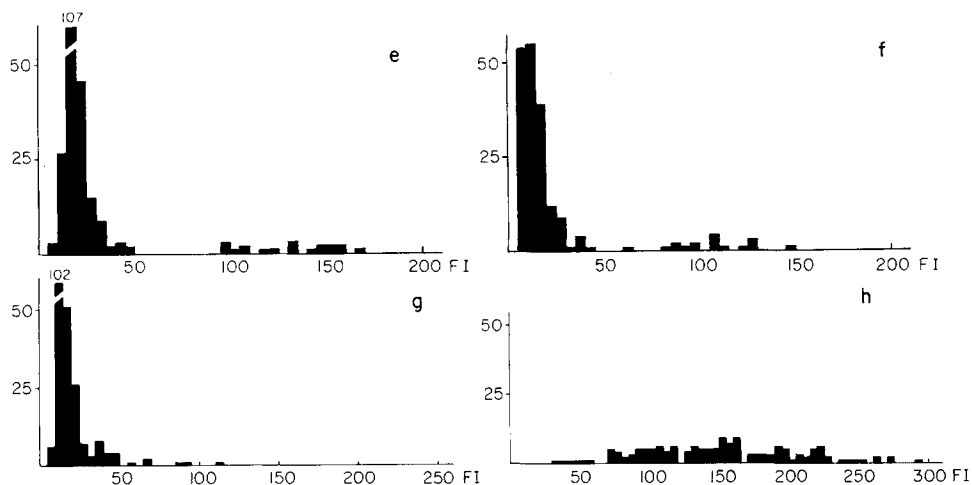
However, the frequency of T lymphocytes with the same idiotypic markers would (as expected from Tables II and III) be expected to be higher. Indeed, such cells could be studied under quantitative fluorometric conditions as seen in Fig. 2. Two facts emerge from these measurements: The first being that the anti-idiotypic antibodies were indeed in excess as already suggested by the data in Table II. Thus, the average intensity of the Lewis T lymphocytes with "anti-DA" idiotypes was the same at two different serum dilutions (Figs. 2 *e* and *f*). From previous data we know that our rabbit-antirat Ig serum was also used in excess. Thus, although direct comparisons between the two FITC-labeled antisera should be interpreted with caution a comparison of the intensities of fluorescence obtained on the B lymphocytes using the rabbit-antirat IgG serum with the anti-idiotypic reaction on the T lymphocytes suggests similar density of the antigenic sites on the two types of cells.

### Discussion

In the study of the antigen-binding structures of T lymphocytes attempts have been made to visualize these molecules via antigen-binding experiments (22,27,28). Such approaches have met with varying success and the controversial



FIGS. 2 *a-d*. (*a-h*) Fluorescence intensity (FI) of single cells on the abscissa with number of cells on the ordinate. Bg, average background in the fluorescence of the preparation. Background not subtracted. n, total number of indicated cells analyzed. (*a*) DA T cells; pool 3, 1:2; n, 141; and Bg, 4. (*b*) DA T cells; pool 3, 1:5; n, 148; and Bg, 7. (*c*) DA T cells; rabbit-antirat Ig; n, 189; and Bg, 4. (*d*) DA B cells; rabbit-antirat Ig; n, 148; and Bg, 10.



FIGS. 2 *e-h*. (*e*) Lewis T cells; pool 3, 1:2; n, 233; and Bg, 13. (*f*) Lewis T cells; pool 3, 1:5; n, 192; and Bg, 7. (*g*) Lewis T cells; rabbit-antirat Ig; n, 216; and Bg, 7. (*h*) Lewis B cells; rabbit-antirat Ig; n, 151; and Bg, 13.

results obtained have indicated a density of receptors varying from comparatively few to as many receptors as are found present on B lymphocytes (27,28). The failure to demonstrate antigen-binding structures on T cells in relation to function in several immunosorbant systems (23) has been ascribed to unique features of the T-cell antigen-recognizing system. Thus, several reports exist suggesting a striking requirement for higher temperatures for T lymphocytes to express prolonged antigen-binding capacity (21,22,29). In order to circumvent factors that could seriously influence the outcome of such binding experiments



(such as affinity of T-cell receptors) we chose to investigate the receptors for antigen on T lymphocytes using anti-idiotypic antibodies directed against the supposed variable regions of the specific T-cell receptors for a given antigen. We have in the present article been using anti-idiotypic antibodies directed against T-cell receptors present in one strain of inbred rats and with specificity for major histocompatibility antigens of another strain of rats. Previously, such anti-idiotypic antisera could be shown to react both with T receptors for a given antigen as well as with IgG antibodies with the same specificity as the T-cell structures (9,16,18). Thus, we concluded that as the T and B receptors for antigen share idiotypic determinants it is most likely that they also share at least one of the two variable regions comprising the antigen-binding site of a conventional Ig molecule (9).

Using anti-idiotypic antisera we have previously been able to visualize, in an analogous system in the mouse (30), the actual idiotypic molecules on the outer membranes of normal T lymphocytes. In the present experiments in the rat system we have extended these studies using highly defined anti-idiotypic antisera to analyze the frequency of normal T and B lymphocytes carrying the same idiotypic markers as well as to study the density of idiotypic markers on such cells. We first satisfied ourselves that when using anti-idiotypic antibodies there was no detectable impact of temperature with regard to expression of idiotypic markers on T lymphocytes (see Table I), thus justifying a comparison of T and B lymphocytes using 4°C conditions. Our estimates on frequency of idio-type-positive peripheral T and B lymphocytes from normal adult Lewis rats studying idiotypic markers signifying a reactivity against DA major histocompatibility antigens (9) yielded figures of a few percent of idio-type-positive T lymphocytes, whereas the frequency of B lymphocytes was in the order of 1%. These figures would seem to express the upper level as the tests could be shown to be carried out in antibody excess. Still, the matter of finding a few percent of idiotypic T lymphocytes might at first seem an extraordinary high figure. First, it should be remembered, however, that the present figures obtained by serology using anti-idiotypic antibodies are very similar to figures obtained when using specific functional activation of immunocompetent T lymphocytes in analogous systems (26,27). From other data we also know that the idiotypic T lymphocytes are in fact the very T lymphocytes that carry the expected immunocompetence against the relevant alloantigens (31). We could thus conclude that the present figures on frequencies of immunocompetent T lymphocytes do match very well with earlier findings using entirely different approaches.

Still, such a high figure deserves further discussion as to how it can be created. Here, it should first be stated that the present idiotypic system is known to frequently involve more than one idiotypic determinant (antigen-binding specificity?) (32). The multitude of idiotypes would in a corresponding manner increase the frequency of idio-type-positive cells. In the rat the clonality of antibodies directed against the major histocompatibility locus antigens within the species frequently is of restricted heterogeneity with a few dominating clones (K. Welsh, personal communication).

We would then be left with the problem of the relatively higher frequency of idiotypic T in comparison to B lymphocytes. Here it might be relevant to

consider an important factor in the yield of frequency of idiotypic cells. Idiotypic determinants on conventional immunoglobulins can be created by a combination of variable regions of two chains (33) but they can also be found exclusively on one of the two chains (34). Idiotypes created by a combination of two variable regions would produce very rare idiotypes (frequency of a given possibility of one variable region multiplied by the frequency of the other one). The "single chain" idio type would be significantly more frequent as it would only represent the variation provided for by the V genes coding for the variable region of that particular chain type. From the present frequencies we would predict that the idiotypes analyzed are of "single chain" type. Experiments are in progress to determine if this is true as such an approach would yield further information as to the nature of the V genes coding for the T-cell receptors for antigen. A speculative explanation why we find more idiotypic T than B lymphocytes would read as follows: Our present idiotypic marker(s) is of "single chain" type. Our anti-idiotypic antisera have been made against T-cell receptors (T-cell idiotypes) and react with both T and B receptors specific for the same antigens. Earlier experiments using anti-idiotypic antibodies against B-cell receptors (humoral antibodies of a given specificity) could be shown to yield antibodies that react preferentially with B- and to a lesser extent with T-cell receptors for the same antigen (16,18). We would now say that our present antisera might react with a "single chain"-type idio type, that is present in both B and T receptors specific for the same alloantigen. It is possible, however, that the T receptor is a single chain molecule and, if so, the additional chain in the B receptor might, according to its variable sequence, block or not block the relevant idiotypic markers. That single chains express antigenic markers that can be masked when the chain combines with another chain is well documented by the work on antigenic determinants expressed by free light chains in comparison to when light chains are bound to the heavy chain (35). The corresponding result would then, when using sera of the present type, result in a relatively lower frequency of idiotypic B v. T cells.

The intensity measurements on the density of idiotypic markers on the surface of T vs. B lymphocytes should be considered as preliminary. However, they readily document that T lymphocytes express on their outer surface a high number of idiotypic markers as visualized by intensity of reaction measured both in autoradiography and quantitative immunofluorescence. We would thus consider that the present data as well as earlier ones in the mouse (30) indicate a density of antigen-binding receptors on T lymphocytes in the same order of magnitude as on the B cells. That these antigen-binding receptors are actually produced by the T lymphocytes and not acquired in a passive manner has been proven by a variety of experiments including trypsin removal and recovery (H. Binz, unpublished results), and failure to demonstrate any constant region markers of immunoglobulin type on T lymphocytes (9). The discontinuous distribution pattern of the idiotypic marker in the lymphocyte population and the fact that T lymphocytes with reactivity against different antigens express different idiotypes (31) does also strongly argue against cytophilic products.

In conclusion, we have been able to use anti-idiotypic antibodies to visualize T and B lymphocytes in normal individuals as to allow a frequency analysis as

well as a study on the density of these idiotypic markers on the single lymphocyte of the two types. In an accompanying article the use of such idiotypic markers in the selective enrichment or depletion of immunocompetent T lymphocytes potentially reactive against a given antigen will be described (31).

### Summary

Anti-idiotypic antibodies made against the antigen-binding receptors of T lymphocytes for a given antigen (*Ag-B* locus antigens in rats) can be shown to react with IgG antibodies of the same antigen-binding reactivity. Using such anti-idiotypic antibodies, normal Lewis T lymphocytes of B and T type can be visualized by the use of anti-(Lewis-anti-DA) antibodies. Visualization was made possible by the use of direct fluorescent antibody tests or by autoradiography. Using the first technique and naked eye observations 6.2% of normal Lewis T lymphocytes expressed idiotypic markers signifying anti-DA reactivity, whereas anti-DA-reactive B lymphocytes as measured by this approach was in the order of 1.1%. Autoradiography on purified normal Lewis T lymphocytes gave similar figures. When comparing the intensity of fluorescence at the single cell level using quantitative cytofluorometry anti-idiotypic antibodies reactive with T lymphocytes gave a similar degree of intensity as was obtained using anti-Ig antibodies against B lymphocytes.

Dr. K. Pachmann made some preliminary, helpful measurements in the quantitative cytofluorometry assay. The elegant technical assistance of Berit Olsson, Ann Sjölund, and Lisbeth Östberg is gratefully acknowledged.

*Received for publication 8 July 1975.*

### References

1. Wigzell, H. 1973. On the relationship between cellular and humoral antibodies. *Contemp. Top. Immunobiol.* 3:77.
2. Marchalonis, J. J., R. E. Cone, and J. L. Atwell. 1972. Isolation and partial characterization of lymphocyte surface immunoglobulins. *J. Exp. Med.* 135:956.
3. Feldmann, M. 1974. T cell suppression in vitro. II. Nature of the suppressive specific factor. *Eur. J. Immunol.* 4:667.
4. Vitetta, E. S., J. W. Uhr, and E. A. Boyse. 1973. Immunoglobulin synthesis and secretion by cells in the mouse thymus that do not bear theta antigen. *Proc. Natl. Acad. Sci. U. S. A.* 70:834.
5. Benacerraf, B., and H. O. McDevitt. 1972. Histocompatibility-linked immune response genes. *Science (Wash. D. C.)* 175:273.
6. Shevach, E. M., I. Green, and W. E. Paul. 1974. Alloantiserum-induced inhibition of immune response gene product function. II. Genetic analysis of target antigens. *J. Exp. Med.* 139:679.
7. Crone, M., C. Koch, and M. Simonsen. 1972. The elusive T cell receptor. *Transplant. Rev.* 10:36.
8. Mond, J. J., A. L. Luzzati, and G. J. Thorbecke. 1972. Surface antigens of immunocompetent cells. IV. Effect of anti-b5 allotype on immunologic responses of homozygous b5 rabbit cells in vitro. *J. Immunol.* 108:566.
9. Binz, H. and H. Wigzell. 1975. 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. *J. Exp. Med.* 142:197.
10. Tada, T., K. Okumura, and M. Taniguchi. 1973. Regulation of homocytotrophic antibody formation in the rat. VIII. An antigen-specific T cell factor that regulates anti-hapten homocytotrophic antibody response. *J. Immunol.* 111:952.
  11. Munro, A. J., M. J. Taussig, R. Campbell, H. Williams, and Y. Lawson. 1974. Antigen-specific T-cell factor in cell cooperation: physical properties and mapping in the left-hand (K) half of *H-2*. *J. Exp. Med.* 140:1579.
  12. Ramseier, H., and J. Lindenmann. 1971. Cellular receptors. Effect of anti-alloantiserum on the recognition of transplantation antigens. *J. Exp. Med.* 134:1083.
  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. Binz, H., J. Lindenmann, and H. Wigzell. 1973. Inhibition of local graft-versus-host reaction by anti-alloantibodies. *Nature (Lond.)*. 246:146.
  15. Ramseier, H. 1973. T cells in recognition of histocompatibility antigens. *Nature (Lond.)*. 246:351.
  16. Binz, H., J. Lindenmann, and H. Wigzell. 1974. Cell-bound receptors for alloantigens on normal lymphocytes. I. Characterization of receptor-carrying cells by the use of antibodies to alloantibodies. *J. Exp. Med.* 139:877.
  17. Ramseier, H. 1974. Antibodies to T cell receptors and to histocompatibility antigens. *Cell. Immunol.* 8:177.
  18. Binz, H., J. Lindenmann, and H. Wigzell. 1974. Cell-bound receptors for alloantigen on normal lymphocytes. II. Antialloantibody serum contains specific factors reacting with relevant immunocompetent T lymphocytes. *J. Exp. Med.* 140:731.
  19. Lindholm, L., L. Rydberg, and Ö. Strannegård. 1973. Development of host plasma cells during graft-versus-host reactions in mice. *Eur. J. Immunol.* 3:511.
  20. Binz, H., and J. Lindenmann. 1972. Cellular receptors: binding of radioactively labeled anti-alloantiserum. *J. Exp. Med.* 136:872.
  21. Golstein, P., E. A. J. Svedmyr, and H. Wigzell. 1971. Cells mediating specific in vitro cytotoxicity. I. Detection of receptor-bearing lymphocytes. *J. Exp. Med.* 134:1385.
  22. Hämmerling, G. J., and H. O. McDevitt. 1974. Antigen binding T and B lymphocytes. I. Differences in cellular specificity and influence of metabolic activity on interaction of antigen with T and B cells. *J. Immunol.* 112:1726.
  23. Wigzell, H. 1971. Cellular immunosorbants. *Prog. Immunol.* 1:1105.
  24. Natvig, J., F. Salsano, S. Fröland, and P. Stavhem. 1975. Same idiotype of membrane-bound IgD and IgM on single cells. In International Symposium on Membrane Receptors of Lymphocytes. M. Seligmann, editor. North-Holland Publishing Co., Amsterdam, The Netherlands.
  25. Wilson, D. B., J. L. Blyth, and P. C. Nowell. 1968. Quantitative studies on the mixed lymphocyte interaction in rats. III. Kinetics of the response. *J. Exp. Med.* 128:1157.
  26. Ford, W. L., S. J. Simmonds, and R. C. Atkins. 1975. Early events in a systemic graft-vs.-host reaction. II. Autoradiographic estimates of the frequency of donor lymphocytes which respond to each Ag-B-determined antigenic complex. *J. Exp. Med.* 141:681.
  27. Roelants, G. E., and A. Ryden. 1974. Dose dependence of antigen binding to B and T lymphocytes. *Nature (Lond.)*. 247:104.
  28. Jensenius, J. C., M. Crone and C. Koch. 1975. Antigen-binding by non-bursa-derived chicken leukocytes. *Scand. J. Immunol.* 4:151.
  29. Martz, E. 1975. Early steps in specific tumor cell lysis by sensitized mouse T lymphocytes. I. Resolution and characterization. *J. Immunol.* In press.

30. Binz, H., T. Bächli, H. Wigzell, H. Ramseier, and J. Lindenmann. 1975. Idiotypic positive T cells visualized by autoradiography and electron microscopy. *Proc. Natl. Acad. Sci. U. S. A.* 72:in press.
31. Binz, H., and H. Wigzell. 1975. Shared idiotypic determinants on B and T lymphocytes reactive against the same antigenic determinants. III. Physical fractionation of specific immunocompetent T lymphocytes by affinity chromatography using anti-idiotypic antibodies. *J. Exp. Med.* 142:1231.
32. McKearn, T. J. 1974. Antireceptor antiserum causes specific inhibition of reaction to rat histocompatibility antigens. *Science (Wash. D. C.)*. 183:94.
33. Hopper, J. E., and A. Nisonoff. 1971. Individual antigenic specificity of immunoglobulins. *Adv. Immunol.* 13:58.
34. Huser, H., J. Haimovich, and J. C. Jaton. 1975. Antigen-binding and idiotypic properties of reconstituted immunoglobulins G derived from homogeneous rabbit anti-pneumococcal antibodies. *Eur. J. Immunol.* 5:206.
35. Epstein, W. V. 1965. Specificity of macroglobulin antibody synthesized by the normal human fetus. *Science (Wash. D. C.)* 148:1591.