

CELL-BOUND RECEPTORS FOR ALLOANTIGENS ON NORMAL LYMPHOCYTES

I. CHARACTERIZATION OF RECEPTOR-CARRYING CELLS BY THE USE OF ANTIBODIES TO ALLOANTIBODIES*

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It is possible to produce antibodies against the antigen-combining site of an antibody or its immediate vicinity. Such antibodies are called anti-idiotypic antibodies (1). Anti-idiotypic antibodies are regularly produced by F₁ hybrid animals inoculated with alloantibodies raised in one of the parental strains against the other parent (2, 3). The fact that these antialloantibodies are indeed made by the F₁ hybrid is indicated by their allotype (4) and the kinetics of their production (5).

Such antialloantibodies have been shown to inhibit the specific *in vitro* recognition of immunocompetent parental cells of the relevant alloantigen in some systems (2) but not in others (6). Recently, there has also been accumulating evidence that such antialloantisera can exert specific inhibitory activity *in vivo* as expressed by the selective inhibition of graft-v.-host (GvH)¹ reactions of parental cells in F₁ hybrid recipient (7). In view of the latter finding implicating a specific effect on T cells, we have studied the distribution on normal B and T lymphocytes of idiotypic receptors for alloantigens.

A combination of techniques involving radiolabeled antialloantibodies, separation procedures to produce "pure" T and B lymphocytes, and specific, allogeneic immunosorbents, have been used. The results obtained strongly suggest that only B cells have sufficient amounts of membrane-bound idiotypic receptors to accumulate radioactive antialloantibodies at significant levels.

Materials and Methods

Animals.—Rats of inbred strains Lewis (L), DA, and BN as well as F₁ hybrids between these strains were domestically raised and maintained. Adults of either sex were used in the experiments.

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¹ *Abbreviations used in this paper:* BME, Eagle's basal medium; BSS, balanced salt solution; FCS, fetal calf serum; GvH, graft-vs.-host; L, Lewis.

Alloantisera.—Alloantisera were raised by full thickness skin grafts from one strain to another (8). 3 wk after a single skin graft, animals were bled and the serum was heat-inactivated at 56°C for 30 min, Millipore-filtered and stored at -20°C.

Antialloantisera.—Antialloantibodies, for instance of specificity anti-(Lewis anti-DA), were elicited in (Lewis × DA)F₁ animals by injecting alloantiserum of specificity Lewis anti-DA. On day 0 each animal received five intradermal injections of 0.2 ml of plain alloantiserum distributed over the belly skin. Animals were immunized in the same way every 3 wk and received a minimum of three injections of alloantiserum. Animals were bled 14 days after the last injection by heart puncture and the serum pools were handled as described for alloantisera.

Isolation and Iodination of IgG Fraction of Antialloantisera.—IgG fractions from antialloantisera were purified by sodium sulfate precipitation followed by DEAE ion-exchange chromatography as previously described (3). Purified IgG was iodinated with ¹²⁵I by the Chloramine-T method (9) at the Eidgenössisches Institut für Reaktorforschung, Würenlingen (Switzerland), with a sp act of 1 mCi/mg protein. Upon receipt of the iodinated antialloantibodies they were passed through a calibrated Sephadex-G 200 column (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) to remove aggregated iodinated material and free iodine and for an additional check on the molecular weight.

Absorption of Iodinated Antialloantisera with F₁ Cells.—Iodinated antialloantisera were absorbed with spleen cells from F₁ animals of the same cross which had produced the antialloantiserum. Absorption was done three times with 2 × 10⁹ cells/mg iodinated protein at 37°C for 30 min (3).

Rabbit Antirat-Ig Antiserum.—Rat immunoglobulin of outbred rat serum was prepared by using 40% saturated ammonium sulphate precipitation and washing the precipitate twice at 40% saturation. The precipitate was then dissolved in phosphate-buffered solution and stored at -20°C. 1 mg/ml in Freund's complete adjuvant was used at a volume of 2 ml per rabbit distributed subcutaneously. Rabbits were bled 4 wk thereafter for antiserum, which was heat-inactivated at 56°C for 30 min before use.

Rabbit Anti-T Serum.—Rabbits received twice 10⁹ mouse thymocytes intravenously at 2-wk intervals and were bled 1 wk thereafter. Sera were heat-inactivated at 56°C for 30 min. Mouse brain suspension was washed twice with balanced salt solution (BSS) (1,500 rpm, 10 min) and fixed with three volumes of 0.5% glutaraldehyde for 15 min at 4°C. After two additional washes with BSS, the brain suspension was incubated for 10 min with isotonic glycine-HCl buffer pH 2.8, washed again twice with BSS, and stored at 4°C. One volume of fixed brain cells was incubated for 1 h at 4°C with three volumes of antiserum. After centrifugation, the supernate, still containing some anti-T antibodies, was saved and the sediment was washed twice with BSS. Specific rabbit anti-T antibodies were eluted from brain cells by adding isotonic glycine-HCl buffer pH 2.8 for 5 min at room temperature. After centrifugation, the supernate was immediately neutralized with 1.0 N NaOH and concentrated by vacuum dialysis. The brain cells were washed twice with BSS and kept at 4°C for further absorptions. The eluted antibody was specific for rat T cells as measured by a cytotoxic test with complement. It would kill 100% of thymocytes and close to 100% of spleen cells passed through an anti-Ig column at titers above 1:500 but only about 30% of normal spleen cells even at dilution 1:5.

Normal Lymphoid Cell Suspension.—There were prepared from spleens and lymph nodes according to a standard procedure (8). Cells were washed twice with Eagle's basal medium (BME) complemented with 10% fetal calf serum FCS and 100 IU/ml of penicillin and 50 µg/ml of streptomycin. Cell viability was judged by trypan blue exclusion.

Procedure for Determining the Reaction Between Iodinated Antialloantibodies and Lymphoid Cells.—The method has been described in full previously (3). Briefly, 50 × 10⁶ viable lymphoid cells were reacted with 3-4 × 10⁵ cpm of ¹²⁵I-labeled antialloantibodies, at room temperature for 30-60 min. Cells were then washed five times with ice cold BME, resuspended in 2 ml of saline and transferred into plastic tubes for gamma counting.

Cytotoxic Assay.—To 25 μ l of a serial dilution of either rabbit anti-T or rabbit anti-Ig, 25 μ l of a lymphoid cell suspension containing 5×10^6 viable cells per ml were added. After incubation for 30 min at 4°C, 25 μ l of fresh undiluted guinea pig serum (complement) were added. After further incubation for 40 min at 37°C, cells were put on ice and cell viability was read under a phase contrast microscope immediately after adding 25 μ l of trypan blue. Controls contained either anti-T or anti-Ig or complement alone.

Rabbit Antirat-Ig Column.—Degalan beads (Degussa Wolfgang AG, Hanau, German Federal Republic) were washed and decanted to remove fine granules using tap water, distilled water and BSS. Beads were poured into a 1.5 \times 90 cm column and crude rat Ig at a concentration of 10 mg/ml was allowed to fill the column and to remain in contact with it for 12 h at 4°C. Beads were then washed with three bed volumes of BSS and the column was filled with rabbit anti-rat Ig for 2 h at 4°C, after which it was washed again with BSS. Lymphoid cell suspensions, containing not more than 50×10^6 viable cells/ml, were passed through the column with a fluid rate of 30 drops/min. Passing cells were harvested in test tubes on ice (10).

Purification of B Cells.—Normal lymphoid cells from spleens and lymph nodes were incubated with an excess of rabbit anti-T (usual dilution 1:50) for 1 h at 4°C, after which fresh guinea pig complement was added to a final concentration of 1:3 or 1:5. After incubation for 40 min at 37°C, cells were washed twice with BME containing antibiotics. Washed cells were trypsinized with 0.25% trypsin for 10 min at 37°C to destroy dead cells. Trypsinization was stopped by adding 10% FCS. Cells were washed twice with BME and filtered through nylon mesh and then incubated overnight in tissue culture medium 199 complemented with 15% FCS and antibiotics. The next day, cells were washed twice with BME and then checked for T-cell contamination using rabbit anti-Ig and rabbit anti-T as described under cytotoxic assay.

Rat Fibroblast Monolayers.—Rat embryos from strains Lewis and DA were removed from the mothers as soon as pregnancy was detected. Heads, legs, and the intestine were removed and the rest was cut into small pieces and trypsinized for 15 min at room temperature in 0.25% trypsin containing 0.03% EDTA. Trypsinization was repeated once, the harvested cells were pooled and washed twice with BME containing 10% FCS and antibiotics and were adjusted to 40×10^6 viable cells per ml. 1 ml of this cell suspension was put together with 20 ml of BME into large Falcon plastic flasks (no. 3002, 60 \times 15 mm) (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). The next day the attached cells were washed with BME and then fed twice weekly. Normally one passage was carried out each week, the cells from one flask being divided into three new ones.

Pretreatment of Lymphoid Cells with Different Antisera.—Lymphoid cells were treated before adding the iodinated antialloantibodies with different antisera (see Table IV). The following antisera and concentrations were used: rabbit antirat Ig and rabbit anti-T in concentrations of 1:50. Antialloantisera and normal rat sera undiluted or diluted 1:3. In cytotoxic assays, cells were incubated first with antiserum 30 min at 4°C. Fresh guinea pig complement was added to a final concentration of 1:3 or 1:5. After incubation for 40 min at 37°C cells were washed twice with BME and then trypsinized and handled as described for purification of B cells.

RESULTS

Significant Uptake of [¹²⁵I]Antialloantibody Ig by B But Not by T Lymphocytes.—Normal lymphoid cells from DA or Lewis rats used were mixtures of lymph node and spleen cells. From these pure T lymphocytes were obtained by filtration through anti-Ig columns and pure B cells were obtained by treatment with a rabbit anti-T-cell serum and complement as indicated in Materials and

Methods. The starting population of cells contained approximately 40–45% B cells as judged by anti-T and complement cytotoxicity; the same test revealed between 0–3% of contaminating cells in purified suspensions of T and B cells. As seen in Table I, uptake of the proper ^{125}I -labeled antialloantibody occurred

TABLE I
Uptake of ^{125}I -Labeled Antialloantibody Ig by Purified B and T Lymphocytes

Exp.	Labeled antialloantibody	Cells*	Uptake† of ^{125}I by:	
			DA cells	L cells
			<i>cpm</i>	
1	Anti-(DA anti-L)	B	2,411 (1304)	922
	Anti-(DA anti-L)	B + T	1,742 (627)	1,069
	Anti-(DA anti-L)	T	656 (–13)	478
	Anti-(L anti-DA)	B	1,107	3,136 (2214)
	Anti-(L anti-DA)	B + T	1,115	1,722 (653)
	Anti-(L anti-DA)	T	669	670 (192)
2	Anti-(DA anti-L)	B	2,900 (1972)	946
	Anti-(DA anti-L)	B + T	1,470 (570)	738
	Anti-(DA anti-L)	T	578 (68)	538
	Anti-(L anti-DA)	B	928	3,176 (2230)
	Anti-(L anti-DA)	B + T	900	1,610 (872)
	Anti-(L anti-DA)	T	510	503 (–35)
3	Anti-(DA anti-L)	B + T	1,232 (356)	772
	Anti-(DA anti-L)	T	ND	410
	Anti-(L anti-DA)	B + T	876	2,064 (1298)
	Anti-(L anti-DA)	T	ND	411 (1)
4	Anti-(DA anti-L)	B + T	1,780 (968)	521
	Anti-(DA anti-L)	T	568 (–31)	ND
	Anti-(L anti-DA)	B + T	812	2,031 (1510)
	Anti-(L anti-DA)	T	599	ND

* B and T means more than 95% purity of each kind. B + T = approximately 40% B cells.

† Figures within brackets denote in cpm the actual specific cpm (uptake of relevant ^{125}I antialloantibody minus uptake of irrelevant ^{125}I Ig on the same cell population).

Figures denote the mean of three–four tubes/group in Exp. 1 and 2, and ten tubes/group in Exp. 3 and 4.

ND, not done.

to a significant degree only in populations containing B cells. In fact, uptake of the antialloantibody was proportional to the percentage of B cells of the relevant strain in the tested cellular population. In a further attempt to find idiotypic determinants on relevant T cells, parental thymocytes were inoculated into irradiated F_1 hybrid animals for activation *in vivo*. These cells were subsequently recovered from the spleen, filtered through anti-Ig columns to remove

possible contaminating B cells (11, 12) leaving a T-cell population known to be highly pure with regard to T cells and with excellent specific immunocompetence of T-cell type (13). However, even such immune T cells failed to express any selective binding of the relevant antialloantibody as shown in Table II. Thus, as judged by uptake of radiolabeled antialloantibody, receptors with the idiotypes of alloantibodies were only to be found on B lymphocytes.

Cytotoxicity of Antialloantibody Ig and Complement on Lymphocytes Carrying Idiotypic Markers of the Alloantibody.—To show that the idiotypic determinants present on B lymphocytes were produced by these cells, treatment with anti-alloantibody Ig and complement was carried out on normal parental strain lymphoid cells. Anti-Ig and complement had previously been found frequently to be selectively cytotoxic for B lymphocytes (14). Incubation with anti-

TABLE II
Uptake of ^{125}I -Labeled Antialloantibody Ig by Activated Thymocytes

Labeled antialloantibody	Uptake of ^{125}I by thymocytes activated for:	
	L antigen*	DA antigen*
	<i>cpm</i>	
Anti-(L anti-DA)	992 ± 60	2,638 ± 82
Anti-(DA anti-L)	1,160 ± 69	2,525 ± 43

* DA or Lewis thymocytes were inoculated i.v. into 1,000 (DA × Lewis) F₁ rats. 5 days later spleens were harvested and the cells filtered through anti-Ig columns whereafter the ^{125}I uptake was measured. Results denote mean ± SE of triplicate measurements using 5×10^6 cells/tube.

alloantibody Ig in presence or absence of complement was carried out. Thereafter, the cells were treated with trypsin to remove antigen-antibody complexes from the cell surfaces and were subsequently allowed to recover overnight at 37°C, whereafter [^{125}I]antialloantibody was added to study the presence or absence of idio-type-containing cells in the population. The results, as shown in Table III, clearly demonstrate that the antialloantibody in the presence of complement (but not in its absence) caused a specific elimination (most likely cytolytic) of cells with the relevant idiotypes.

Further evidence that idiotypic markers similar to those of alloantibodies were present on B lymphocytes in such a way as to make these cells sensitive to the cytolytic action of antialloantibodies and complement came from experiments in which rabbit antisera directed against either rat Ig or T cells were included in the cytolytic assays. Treatment with anti-Ig in the presence of complement caused an elimination of the idio-type-containing cells, whereas this was not the case when the anti-T serum was used.

The Uptake of [^{125}I]Antialloantibody Ig on Normal B Lymphocytes is Due to the Presence of Idiotypic Receptors with Specificity for Alloantigens.—We applied

TABLE III
Cytotoxicity of Antialloantibodies for Lymphoid Cells. Comparison of Uptake of ¹²⁵I Anti-alloantibody Ig by Cells after Treatment with Antialloantibody Plus Complement and Antialloantibody Alone

Cells	Treatment	Labeled antialloantibody	Uptake of ¹²⁵ I cpm ± SE*
L	Anti-(L anti-DA)	Anti-(L anti-DA)	<i>5,239 ± 363</i>
L	Anti-(L anti-DA)	Anti-(DA anti-L)	<i>2,328 ± 52</i>
L	Anti-(L anti-DA) + C'	Anti-(L anti-DA)	<i>1,825 ± 97</i>
L	Anti-(L anti-DA) + C'	Anti-(DA anti-L)	<i>2,086 ± 108</i>
DA	Anti-(DA anti-L)	Anti-(DA anti-L)	<i>6,992 ± 289</i>
DA	Anti-(DA anti-L)	Anti-(L anti-DA)	<i>2,159 ± 42</i>
DA	Anti-(DA anti-L) + C'	Anti-(DA anti-L)	<i>3,757 ± 129</i>
DA	Anti-(DA anti-L) + C'	Anti-(L anti-DA)	<i>3,865 ± 296</i>

* Italicized values indicate groups where specific uptake occurred. If only specific uptake minus nonspecific uptake is compared, none of the antialloantibody and complement-treated cells displayed any surviving idiotype-positive cells (−261 cpm for Lewis cells and −108 cpm for DA cells, respectively).

immunosorbent techniques with monolayers (15) to demonstrate that the cells with idiotypic receptors expressed the expected antigen-binding capacity. Normal lymphoid cells of DA or Lewis origin were incubated at 37°C for 90 min on DA or Lewis fibroblast monolayers in the presence of normal F₁ serum, of the relevant antialloantibody serum or of rabbit anti-T or rabbit anti-Ig serum. The lymphocytes in the supernate and the bound, EDTA-eluted cells were subsequently trypsin-treated, left overnight to recover and then assayed for presence of idiotype-containing cells using [¹²⁵I]antialloantibody Ig.

As shown in Table IV, normal F₁ serum did not inhibit adsorption of idiotype-carrying cells to the monolayers, as evidenced by the fact that the unadsorbed cells fixed little radioactive antialloantibody, whereas the eluted cells, representing a population enriched in the specific receptor, fixed much more. Anti-alloantibody of the proper specificity, however, prevented adsorption, yielding higher radioactivity in the unadsorbed fraction and lower radioactivity in the eluted fraction. The same effect could be induced with rabbit anti-Ig serum, but not with rabbit anti-T serum. This suggests that the bulk of radioactivity must have been taken up by B cells, and that these cells, when carrying idiotypic determinants present in alloantibodies of a given specificity, also expressed the same antigen-binding specificity at the cellular level.

Lack of Temperature Influence on the Binding of Idiotype-Positive Cells to Relevant Monolayers.—Whereas it is easy to demonstrate in other immunosorbent systems that B cells carrying receptors with a certain antigen-binding specificity bind as well at 4°C as at 37°C to the relevant antigen (15), this is not the case for T cells. Here, for reasons which are poorly understood, significant binding to the relevant monolayers used for immunosorbents will occur only at

TABLE IV
*Idiotypic Determinants on Surface Receptors for Allogantigens Present on Normal B Cells.
 Binding to Allogeneic Monolayers and its Interference with Antialloantibody IgG.*

Lymphoid cells	Fibroblast monolayers from strain	Inhibitors	Labeled antialloantibody	Uptake* of ^{125}I by cells:	
				Remaining in the supernate	Eluted from the monolayers and allowed to recover
				<i>cpm</i>	
L	DA	Normal F ₁ serum		997	3,893
		F ₁ anti-(L anti-DA)	Anti-(L anti-DA)	4,020	1,386
		Rabbit antirat Ig		5,146	1,221
		Rabbit anti-T		1,182	3,908
L	L	Normal F ₁ serum		3,777	1,022
		F ₁ anti-(L anti-DA)	Anti-(L anti-DA)	4,536	2,540
		Rabbit antirat Ig		5,081	3,901
		Rabbit anti-T		4,053	2,056
DA	DA	Normal F ₁ serum		5,064	2,503
		F ₁ anti-(DA anti-L)	Anti-(DA anti-L)	5,968	1,985
		Rabbit antirat Ig		5,930	2,761
		Rabbit anti-T		4,999	3,099
DA	L	Normal F ₁ serum		1,923	3,350
		F ₁ anti-(DA anti-L)	Anti-(DA anti-L)	6,832	1,910
		Rabbit antirat Ig		7,974	1,590
		Rabbit anti-T		2,687	6,323

* Italicized values indicate experiments where specific interference of binding to allogeneic monolayers occurred. Uptake of a nonspecific kind on supernatant cells was 1,813 for [^{125}I]-anti-(DA anti-L) on L cells and 1994 for [^{125}I]anti-(L anti-DA) on DA cells. Each value in the table represents the mean of triplicates.

20°C or above (15). In the present system, idiotype containing cells could be shown to bind well to relevant monolayers at 4°C (see Table V), thus further emphasizing the B nature of these cells.

DISCUSSION

F₁ hybrid rats, when inoculated with alloantibody raised in one parent against the other parent, regularly produce antibodies directed against the idiotypes present on the alloantibodies injected (2). Such antialloantibodies can be radio-labeled and shown to exhibit specific binding with lymphocytes capable of producing alloantibodies of the relevant antigen-binding specificity (3). In the serum of F₁ hybrid rats inoculated with alloantibodies specific factors capable of inactivating the GvH reactivity of lymphocytes from the strain of alloantibody production are also present (7). It is not known, however, if the anti-idiotypic antibodies as revealed by the radioimmunoassay and the specific

TABLE V
Binding of Idiotypic-Carrying Lymphocytes to Allogeneic Monolayers at 4°C

Cells	Monolayers	Labeled antialloantibody	Uptake* of ¹²⁵ I by cells:		Ratio†
			Remaining in the supernate	Eluted from the monolayers and allowed to recover	
			<i>cpm</i>		
DA	L, 4°C		<i>310</i>	<i>1,626</i>	0.19
DA	DA, 4°C	Anti-(DA anti-L)	551	436	1.26
L	L, 4°C		1,019	224	4.55
L	DA, 4°C	Anti-(L anti-DA)	<i>179</i>	<i>1,262</i>	0.14

* Italicized values indicate combinations where idiotype-containing cells would be expected to adsorb.

† Ratios below 1 indicate that cells carrying the relevant idiotype were depleted from the supernate and were enriched in the eluate.

factors capable of selective inhibition of GvH reactivity are the same molecules. As the GvH reaction is considered to primarily involve T-cell activity (16), we considered it essential to analyze if indeed T and B lymphocytes reactive with alloantigens used similar if not identical recognition sites. In another system, the "product of antigen recognition" or PAR assay, evidence has been accumulating that lymphocytes and alloantibodies from the same donor carry identical recognition structures for alloantigens (17), but it is not yet clear whether this applies equally to B and/or T cells.

Our first approach was to purify T and B lymphocytes from the strains of rats used for production of the alloantibodies. The procedures used yielded highly purified B and T lymphocytes, and we knew from other studies that the purification procedures did not affect the specific immune capacity of such cells (14). Now, when antialloantibody Ig labeled with ¹²⁵I was tested for its capacity to bind to the various cell populations, significant binding to cells of the proper genotype only occurred *if* these cells contained B lymphocytes (see Table I). In fact, the binding of radiolabeled anti-idiotypic antibodies was proportional to the percentage of B lymphocytes in the population, leaving no evidence for idiotypic receptors firmly bound on T cells of the same genotype. When trying to increase the binding of antialloantibody Ig to T cells by using selectively preimmunized T blasts purified with regard to B cells, we reached the same conclusion: there was no evidence that by the present radioimmunoassay we could detect idiotypic receptors for alloantigens on T cells.

Furthermore, we could show that the antialloantibody serum in the presence of complement was cytolytic for the relevant idiotype-containing lymphocytes. The same was true when using a rabbit antirat Ig serum and complement, whereas a specific rabbit anti-T serum, although cytotoxic for T cells, left the idiotype-positive cells intact. These findings would thus further stress the linkage of the idiotypic determinants to the B lymphocytes.

Idiotypes on humoral antibodies are frequently linked to the antigen-binding specificity of the antibody (18) although exceptions of different degrees have been reported (19, 20). By the use of allogeneic cellular monolayers as immunosorbents we could demonstrate in the present article that all cells with the relevant idiotypic determinants on their surface (as measured by the radioimmunoassay) also expressed the expected antigen-binding capacity. They would thus bind selectively to the monolayers with the "right" alloantigens, and the binding of the cells could be inhibited specifically with the correct anti-alloantibody serum or less specifically with rabbit antirat Ig serum (see Table IV). Here also, anti-T antibodies were completely inefficient in achieving any blocking. Using cellular monolayers containing allogeneic cells as immunosorbents, it was possible to remove immunocompetent B and T cells reactive with the foreign antigens (15). However, whereas B cells could bind to immunosorbents at 4°C as well as at higher temperature (15), T-cell binding to specific immunosorbents has only been found to take place at around 20°C or higher, being negligible at 4°C (15). In the present assay system, the idiotype containing lymphocytes could be shown to bind very well to the relevant monolayer at 4°C, reinforcing their B-cell character.

As T lymphocytes could not be shown to bind to any detectable extent the present anti-idiotype antibodies, it remains quite unclear how the same serum (containing the anti-idiotype antibodies) could also inhibit the GvH reaction of parental lymphocytes in a specific way (7). This is remarkable as there exists good evidence that T lymphocytes are the major cell type involved in provoking GvH reactions (16). In the following article, we have therefore continued our analysis of the action of antialloantibody sera on T lymphocytes as studied by direct cytotoxicity, inhibition of binding of specific T lymphocytes to allogeneic monolayers, and elimination of GvH reactivity. The data obtained in these systems clearly confirm the earlier findings (7) that F₁ sera obtained from animals immunized with alloantibodies indeed contain antibodies or other specific factors capable of interfering with specific T lymphocytes. A discussion covering the effects on T vs. B lymphocytes using the present antisera will be placed in the second article of this series. It should be stressed, however, that the uptake of radiolabeled antialloantibody as used in the present paper might not be a sensitive enough assay for cell-surface idiotypes. If T cells (now assuming a similar frequency of specific B and T cells) carried at any given time less than $\frac{1}{100}$ the numbers of receptors present on B cells, any specific binding of anti-alloantibody by T cells would have remained below the threshold of detectability.

SUMMARY

Antialloantibodies were prepared in F₁ hybrid rats by immunization with alloantibodies from one parent raised against antigens of the other parent. The Ig fraction of such antialloantibodies was iodinated and used to investigate the nature of idiotype-carrying normal lymphoid cells. Lymphoid cell populations

of the proper genotype fixed radioactive antialloantibody in proportion to their B-cell content. Activated T-cell populations, when depleted of B cells, did not fix significant amounts of radioactivity. Idiotype-carrying cells were sensitive to rabbit antirat Ig serum lysis and to antialloantiserum lysis, but not to rabbit anti-T-serum lysis. Also, the normal idiotype-containing B cell could be shown to have the expected antigen-binding specificity of its receptor. This was shown by the use of fibroblast cell monolayers that adsorbed specifically those cells capable of fixing the proper antialloantibody.

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