SPECIFIC TRANSPLANTATION TOLERANCE INDUCED BY AUTOIMMUNIZATION AGAINST THE INDIVIDUAL'S OWN, NATURALLY OCCURRING IDIOTYPIC, ANTIGEN-BINDING RECEPTORS*

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Autoimmune reactions are in most circumstances considered to represent damaging or at best neutral phenomena for the individual. In the present article we would like to describe an autoimmune system, which we consider to carry great promise with regard to both theoretical and clinical immunology. No evidence exists that genes, that code for the antigen-binding sites of immunoglobulin molecules, have been under any phylogenetic selective pressure as to avoid the creation of sites with reactivity for self-components. Failure of an individual to express immune reactivity against self can thus frequently be ascribed to a phenomenon occurring during ontogeny, that is specific immune paralysis. Several levels of tolerance would seem to exist that to a varying degree involve different subsets of lymphocytes. One decisive factor in determining whether immune paralysis will occur or not is the concentration of the respective self-component in the body fluids. Here, it can be shown that the antigen-binding sites of the autochthonous immunoglobulin molecules under appropriate conditions can function as immunogens resulting in the production of auto-anti-idiotypic antibodies (1). In a few cases where difficulty has been encountered to achieve such auto-anti-idiotype antibody synthesis, this involved idiotypes being present in normal serum in unusually high concentrations (Janeway, Ch., Jr; personal communication).

We have previously been able to show that anti-idiotypic antibodies against idiotypic T- or B-cell receptors, in presence of complement, will cause the selective elimination of those cells (2, 3). Such procedures will result in the production of lymphocyte populations which from a functional point of view are perfect examples of specifically immunologically tolerant groups of cells. The system we have been working with involves the major histocompatibility locus antigens as our target antigens using inbred strains of rats or mice.

It is well known that a high percentage of T lymphocytes in a normal individual is precommitted to react across the major histocompatibility barrier of the species as

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measured by activation of function in mixed leukocyte cultures or graft-versus-host reactions (4–6). Recently, we have been able to confirm the unusual high figures in normal lymphocytes using specific anti-idiotypic antibodies (7). The high figure of precommitted cells lead us to look for the presence of such shed, antigen-binding, idiotype-positive molecules in the serum of normal adult rats. It could be shown that such molecules can be detected in low amounts in normal serum, with a low molecular weight component also being found in the urine (8).

We have isolated such molecules, defined in prelimary terms their biochemical characteristics (9, 10), and have now used them in a polymerized form trying to provoke auto-anti-idiotypic antibodies against receptors with specificity for defined histocompatibility antigens in the rat. The present article will describe how such a procedure indeed can be shown to result in the production of the relevant auto-anti-idiotypic antibodies and how this will result in the actual induction of long lasting immune tolerance to transplantation antigens. A preliminary account of these findings has been reported elsewhere (11).

Materials and Methods

Animals. Rats of the inbred strains Lewis (Ag-B[1]), DA (Ag-B[4]), BN (Ag-B[3]), and August (Ag-B[5]), as well as (Lewis × DA)F_1 and (Lewis × BN)F_1, were bred and maintained in our own colony. Adult animals of either sex were used within the experiments.

Production of Anti-Idiotypic Antisera. Anti-idiotypic antisera of specificity anti-(Lewis anti-DA) and anti-(Lewis anti-BN) were raised in (Lewis × DA)F_1 rats and (Lewis × BN)F_1 rats, respectively, against normal Lewis T lymphocytes as described before (3). The antisera were tested to fulfil the criteria to be anti-idiotypic as described before (3).

Anti-Idiotypic Immunoabsorbents. The Ig fraction (40% saturated $(NH_4)_2SO_4$ precipitate) was coupled to CnBr-activated Sepharose 4 B (Pharmacia Fine Chemicals, Uppsala, Sweden) as described previously (3). After coupling, the immunoabsorbent was washed with glycine-HCl buffer pH 2.8 containing 2 M NaCl and then with 3 M $MgCl_2$ and kept in Dulbecco's modified phosphate-buffered saline (PBS)[1] at 4°C until used.

Collection of Normal Rat Serum. Normal adult rats were anesthetized with ether and bled by heart puncture. The blood was allowed to coagulate for 30 min at room temperature after which phenylmethylsulfonylfluoride was added to a final concentration of 1 mM. After an additional 1½ h the blood was centrifuged for 20 min at 5,000 *g* and the serum sterile filtered and kept at −20°C until used.

Collection of Normal Rat Urine. Urine was collected from adult female rats maintained in "metabolic cages." The urine was centrifuged to remove sediments, dialyzed against PBS overnight at 4°C, and kept at −20°C until used.

Isolation of Normal Serum Factor. 300 ml of normal rat serum was passed at room temperature over a 1.0 × 10 cm Sepharose 4 B column and then over a 1.0 × 3 cm anti-idiotypic or control immunoabsorbant with a flow rate of 60 ml per h. The columns were washed with PBS with the same flow rate over night at 4°C. The washing fluid contained finally less than 1 μg of protein per ml. The bound material was eluted from the columns first with 10 ml of glycine-HCl buffer pH 2.8 containing 2 M NaCl followed by 10 ml of 3 M $MgCl_2$. The eluate was immediately neutralized with 0.4 N NaOH, concentrated by negative pressure dialysis and dialyzed against 0.2 M Na acetate buffer pH 5.0, and kept at −20°C until used. Idiotypic molecules were isolated in a similar way from normal urine by absorption and elution from anti-idiotypic immunoabsorbents (8).

Immunization of Normal Lewis Rats with Normal Lewis Serum Factor. Isolated normal Lewis serum factor was cross-linked with glutaraldehyde as described elsewhere (12). Linked material corresponding to 10 μg of protein in 0.5 ml of PBS was mixed with an equal amount of complete Freund's adjuvant and injected subcutaneously into the belly skin of normal 5–8 wk-old Lewis

[1] *Abbreviations used in this paper*: MLC, mixed leukocyte culture; PBS, Dulbecco's modified phosphate-buffered saline; SDS, sodium dodecyl sulfate.

rats. Some injections were administered via the foot pads. Animals were boosted as stated in the Results section with the same amount of protein with incomplete Freund's adjuvant. Boosters were given either i.p. or into the foot pads.

In a similar way rats were immunized with normal factor isolated from normal urine. Here, immunoabsorbant eluted material was mixed with 1 mg/ml (final concentration) of human IgG (Behringwerke) before the cross-linkage. Otherwise the protocol was essentially the same as described above.

Protein A Assays for Determination of Alloantibodies and Anti-Idiotypic Antibodies. These were done as described in detail before (3). Briefly: For the determination of anti-idiotypic antibodies 1×10^7 lymphocytes were incubated with 25 μl of antiserum in round bottom microtiter plates for 1 h at 4°C. Cells were washed three times with PBS and incubated with 5×10^4 cpm of ^{125}I-radiolabeled protein A. Cells were washed three to four times and counted. For the determination of alloantibodies 3×10^6 lymphocytes were used per well.

Local Graft-Versus-Host Reaction. The local popliteal lymph node assay was done as described elsewhere (13). 2×10^6 viable lymphocytes were injected into each foot pad and the popliteal lymph nodes removed 7 days later.

Mixed Leukocyte Cultures (MLC). These were performed in flat bottom microtiter plates using 2.5×10^5 responder lymphocytes and 5×10^5 2,000 R irradiated stimulator cells. Spleen and lymph node cells or purified lymphocytes from blood (Ficoll-Isopaque purified [1]) were used as responder cells and spleen cells as stimulator cells. Eagle's high amino acid medium (14) with the double amount of l-glutamine and complemented with penicillin, streptomycin, 0.5% (final dilution) of normal fresh BN rat serum, and 2.5×10^{-5} M (final concentration) of 2-mercaptoethanol was used. The cultures were pulsed as indicated in the Tables with 1 μCi of [^{3}H]TdR. The cultures were harvested on a Skatron collector and counted as described before. One series of MLCs was performed in serum-free medium (see Table VII). For the suppression of the MLC by the use of anti-idiotypic antiserum, responder cells were incubated with the antiserum or control serum and complement as described before.

Induction of Alloantibodies in the Autoimmunized Lewis Rats. Autoimmunized Lewis rats were injected i.p. with 5×10^7 DA and 5×10^7 BN spleen cells. Sera were collected 10 days later by heart puncture.

Skin Grafts. Full thickness skin grafts were performed as described elsewhere (15). Plasters were removed 5 days after grafting, and rats were examined for skin rejection every day thereafter.

Sodium Dodecyl Sulfate (SDS)-Polyacrylamide Gels. Electrophoresis was done as described in detail before (16, 17) in 10-cm columns in plastic tubes using 5% (wt/vol) acrylamide for 8–12 h (5 mA/tube). The buffer contained 0.1% SDS and 0.1 M sodium sulfate pH 7.2. Gels were frozen and cut into 1-mm slices, and radioactivity was determined in each slice.

Iodination of Isolated Material. 20 μg of isolated protein in 100 μl of PBS was mixed with 10 μl of Chloramin T (0.5 mg/ml of PBS) followed by 1 mCi of ^{125}I (Radiochemical Centre, Amersham, England) (18). The mixture was allowed to react for 1 min at room temperature under constant shaking, after which 20 μl of Na-metabisulfite (1 mg/ml of PBS) was added. Free iodine was separated by passing the mixture over a Sephadex G-25 column. The iodinated material was kept at −20°C until used.

Results

Naturally Occurring, Idiotypic, Antigen-Binding Specific Molecules in Serum or Urine from Normal Lewis Rats. We have previously shown that there exist in the serum or urine of adult normal Lewis rats molecules with idiotypic markers of Lewis-anti-DA types and with specific binding ability to DA alloantigen-positive cells (8). We believe this is due to the fact that the frequency of naturally occurring T cells with reactivity towards major histocompatibility antigens is very high in normal animals (4–7); in the present strain combinations we could directly determine the frequency to be around 6% (7).

The specificity with regard to idiotypes and antigen-binding ability of the normal anti-DA molecules are depicted in Tables I and II. In Table I inhibition

TABLE I

Presence of Naturally Occurring Idiotypic Molecules in Normal Lewis Serum and Urine

Cells		Incubated with serum	In the presence of*	Mean ± SE cpm of duplicates‡
L	T	Anti-(Lewis anti-DA)	PBS	7,326 ± 984
L	T	Normal (Lewis × DA)F_1	PBS	3,871 ± 1,016
DA	T	Anti-(Lewis anti-DA)	PBS	3,589 ± 388
DA	T	Normal (Lewis × DA)F_1	PBS	3,703 ± 451
L	T	Anti-(Lewis anti-DA)	Lewis normal serum	4,035 ± 830
L	T	"	(Lewis × DA)F_1 normal serum	7,977 ± 811
L	T	"	DA normal serum	7,560 ± 227
L	T	"	Lewis normal urine	4,273 ± 672
L	T	"	(Lewis × DA)F_1 normal urine	6,984 ± 912
L	T	"	DA normal urine	7,022 ± 755

L denotes Lewis, T denotes purified T lymphocytes.

1×10^7 T lymphocytes were added to each well.

* PBS denotes PBS.

‡ 5×10^4 cpm of ^{125}I-labeled protein A was added to each well.

TABLE II

Purified Naturally Occurring Idiotypic Molecules Express DA-Alloantigen Binding Capacity

Isolated material derived from	Absorbed and eluted from immunoabsorbant	Incubated with* Lewis spleen cells. Mean cpm ± SE of triplicates	Incubated with* (Lewis × DA)F_1 spleen cells. Mean cpm ± SE of triplicates	Incubated with* DA spleen cells. Mean cpm ± SE of triplicates
Lewis normal serum	Anti-(Lewis anti-DA)	3,328 ± 431	8,493 ± 1,016	15,989 ± 2,833
Lewis normal serum	Normal (Lewis × DA)F_1	3,099 ± 289	3,204 ± 925	3,216 ± 79
DA normal serum	Anti-(Lewis anti-DA)	4,676 ± 142	4,216 ± 337	3,982 ± 590
DA normal serum	Normal (Lewis × DA)F_1	3,741 ± 521	3,544 ± 854	3,836 ± 797

Normal Lewis or DA serum was absorbed as indicated and the eluted material radiolabeled with ^{125}I. The binding experiments were carried out immediately after iodination.

* 3×10^6 Lewis, (Lewis × DA)F_1, or DA spleen cells were incubated with 5×10^4 cpm of ^{125}I-labeled molecules.

of anti-Lewis-anti-DA idiotypic antibodies was carried out using various normal rat sera as inhibitors using the anti-idiotypic serum under suboptimal conditions. In Table II negative controls such as material from normal Lewis serum absorbed on normal (Lewis × DA)F_1 immunoglobulin immunosorbants or DA serum absorbed on the anti-(Lewis-anti-DA) immunosorbants failed to indicate any specific binding material in acid eluate and have thus been excluded. The results in Tables I and II clearly show that the material has both idiotypic and antigen-binding ability (and so has the urine factor [11]). It should be noted that the ability of the purified Lewis serum factor to bind to DA alloantigens is normally lost within 24 h after iodination even if keeping the labeled material at −70°C.

Detailed biochemical analysis of the naturally occurring Lewis molecules with specificity for foreign major histocompatibility antigens of the species has been reported elsewhere (10). However, a brief summary of the characteristics is important especially from the point of view of obtaining inhibition of idiotypic T and/or B lymphocytes using the present autoimmunization procedures.

Fig. 1 depicts the profile analysis using ^{125}I-labeled normal Lewis serum factors with specificity for DA alloantigens as obtained after immunosorbant

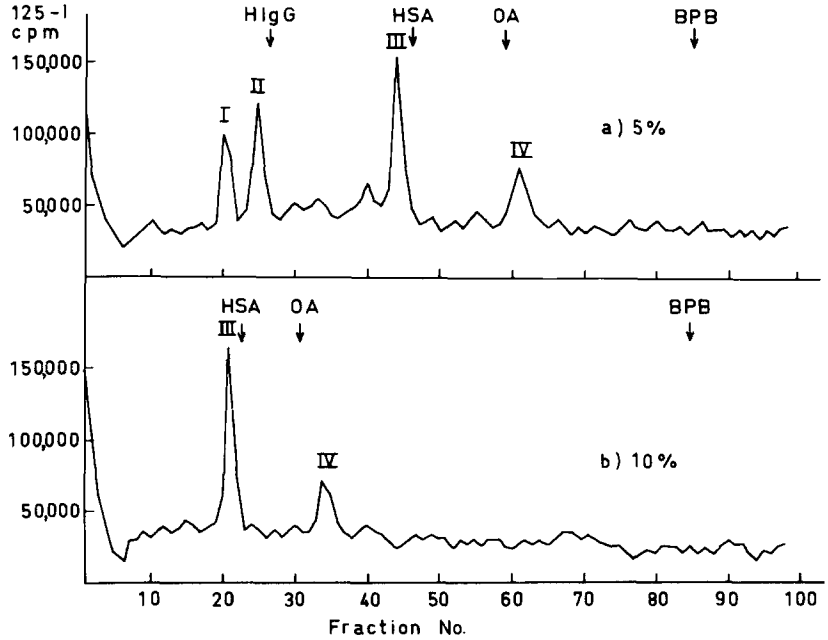

FIG. 1. Normal Lewis serum factor was absorbed and eluted from anti-(Lewis-anti-DA) anti-idiotypic immunoabsorbent, radiolabeled with ^{125}I, and analyzed on a 5 and 10% (wt/vol) SDS-polyacrylamide gel. HIgG, human IgG; HSA, human serum albumin; OA, ovalbumin. These markers were radiolabeled with ^{125}I and run on parallel gels. For further details see Results section.

purification, radiolabeling, and separation on a 5% SDS-polyacrylamide gel electrophoresis. Four major peaks can be seen: Peak I around 180,000 daltons, II at about 150,000, III at a place around 72,000 daltons, whereas peak IV varied between 30,000 to 40,000 daltons with regard to peak position. Similar profiles were obtained using factors isolated from supernates of normal Lewis T and B lymphocytes (10). Using cell fractionation techniques we have subsequently shown that peak I is from normal B lymphocytes, whereas peaks II, III, and IV are T lymphocyte derived. The actual production by these lymphocytes could be confirmed using tritiated amino acid incorporation experiments (10). Peak I could be shown to consist of "conventional" heavy and light chain immunoglobulins, which could be removed by filtration over the respective anti-Ig columns. Peak II consists of two peak III chains, but attempts to further split the peak III chain into further subunits using stronger reducing conditions have failed (6 M urea in 1 M 2-mercaptoethanol or 6 M guanidine-HCl). The single chain peak III is, however, degraded under proteolytic conditions (mere incubation with normal serum for several hours at 37°C will do to yield peak IV molecules). To achieve peak II and III profiles it is thus essential to use inhibiting conditions for proteolysis. Urine anti-DA molecules belong to peak IV (8). As to serology, the peak II, III, and IV all fail to react with anti-Ig immunosorbants (polyvalent anti-rat Ig, anti-rat IgM, anti-rat kappa [provided for by Dr. H. Bazin]). They can not be removed by DA anti-Lewis alloantibodies indicating lack of serological markers of major histocompatibility gene products on these molecules.

Finally, despite the fact that peak III and IV factors behave like single-chain molecules, they both express antigen-binding as well as idiotypic markers (10), a fact to be considered especially when using normal urine factors to obtain autoimmunization in the following paragraphs.

Induction of Auto-Anti-idiotypic Antibodies. The antigen-binding sites of autochthonous molecules can function as immunogens resulting in the production of auto-anti-idiotypic antibodies (1). In the present antigenic system, idiotypic, alloantigen-reactive receptor molecules are released in detectable form into the serum or urine of normal Lewis rats. The concentration of these molecules would not seem to reach high enough levels to induce complete tolerance as normal rats repeatedly immunized with alloantigens have been reported to produce auto-anti-idiotypic antibodies (19).

In our own approach, idiotypic, DA alloantigen-reactive, naturally occurring molecules were isolated from normal Lewis serum via anti-(Lewis anti-DA) anti-idiotypic immunoabsorbants (3). The isolated molecules were cross-linked with glutaraldehyde (12), and 20 μg of protein was injected back, together with complete Freund's adjuvant, into one normal Lewis rat on day 0. Cross-linking would be expected to yield new antigenic determinants for helper T cell to recognize, thereby breaking tolerance to the native, idiotypic sites (20). On days 30 and 121 animals were boosted with the same amount of protein together with incomplete Freund's adjuvant. Animals were bled on days 14, 38, 130, and 134. Lymphocytes isolated from blood by the use of Ficoll-Isopaque gradient were used for MLC and for the induction of graft versus host reactions. Sera were used in protein A assays to demonstrate presence of auto-anti-Lewis antibodies and to try to inhibit MLC to prove the idiotypic nature of these sera.

All seven sera from bleeding on day 130 indicated in Table III contain antibodies reacting with purified normal T lymphocytes. None of them contained any reactivity towards (Lewis × DA)F_1 T lymphocytes. To prove the anti-idiotypic nature of these antibodies, normal Lewis lymphocytes serving as responder cells in MLC against irradiated DA or BN spleen cells were incubated with these antisera and complement before the culture for reasons described before (3).

As seen in Table IV all seven autoantisera from Table III contained antibodies capable of eliminating almost completely the response of normal Lewis lymphocytes against DA alloantigens in MLC. The response to third party BN alloantigens was not touched. Four additional Lewis rat sera immunized in the same way gave similar results (experimental group 2). These data demonstrate that the autoantibodies found in Lewis rats immunized with their own spontaneously released T- and B-lymphocyte receptors are of anti-idiotypic nature. It is thus possible to produce auto-anti-idiotypic antibodies by chemical modification and/or raising the local concentration of the naturally occurring idiotypic, antigen-binding molecules present in normal rats.

We know from earlier experiments that peak no. IV (see Fig. 1) from normal Lewis serum factors as analyzed on a 5% SDS gel, and the molecules found in the urine are most likely the same. We now tried to use the idiotypic molecule from urine to induce auto-anti-idiotypic antibodies. Normal Lewis urine was absorbed and eluted from either anti-(Lewis anti-DA) or anti-(Lewis anti-BN)

TABLE III

Demonstration of Auto-Anti-Lewis Antibodies in the Serum of Lewis Rats Immunized with Naturally Occurring Idiotypic Anti-DA Molecules

Serum from auto-immunized Lewis rat no.	Mean cpm ± SE of triplicates on Lewis T lymphocytes	Mean cpm ± SE of duplicates on (Lewis × DA)F_1 T lymphocytes
1	4,366 ± 234	2,405 ± 192
2	3,743 ± 284	2,340 ± 77
3	3,814 ± 440	2,321 ± 92
4	3,379 ± 667	2,545 ± 154
5	4,004 ± 225	2,314 ± 90
6	3,967 ± 187	2,530 ± 421
7	3,629 ± 335	2,026 ± 93
Serum from normal Lewis rat no.		
1	2,313 ± 53	2,517 ± 250
2	2,353 ± 180	2,185 ± 142

1×10^7 Lewis or (Lewis × DA)F_1 T lymphocytes were added to each well.
5×10^4 cpm of ^{125}I labeled protein A was added to each well.

anti-idiotypic immunoabsorbants. The eluted material was cross-linked with glutaraldehyde and human IgG. Normal Lewis rats were then immunized with cross-linked protein together with complete Freund's adjuvant as described for normal Lewis serum factor. Animals were bled on day 0 before injection and on days 14 and 46 after immunization.

The sera were tested for their autoantibody content using radiolabeled protein A as a marker. Fig. 2 shows this experiment. Already on day 14 after immunization autoantibodies could be detected which were still around in detectable titers on day 46. The anti-idiotypic nature of these antibodies was later confirmed (see table VII). It is thus possible to use naturally occurring idiotypic molecules isolated from serum or urine to induce auto-anti-idiotype antibody synthesis.

Lack of Reactivity Against Relevant Alloantigens of Autoimmunized Rats Derived Lymphocytes as Measured in MLC and Graft Versus Host. In a second series of experiments we tried to demonstrate lack of reactivity against DA alloantigens of lymphocytes of autoimmunized Lewis rats. Lymphocytes were purified from the blood from Lewis rats at different days after immunization and used as responder cells in a MLC against DA and BN spleen cells.

Table V summarizes this experiment. Lymphocytes derived from Lewis rats immunized with the Lewis-anti-DA factors showed a highly significant lack of reactivity against DA alloantigens. The response against BN alloantigens on the other hand was normal. The reduction against DA alloantigens increased with time after immunization. In addition, when lymphocytes from autoimmunized Lewis rats were cultivated together with 2,000 R irradiated normal Lewis spleen cells a significant increase of thymidine uptake could be seen when

TABLE IV

Serum from Autoimmunized Lewis Rats Inhibit Specifically the Response in MLC

Experimental group no.	Lewis responder cells treated with serum from autoimmunized Lewis rat no.	[^{3}H]TdR incorporation of mixture with DA stimulator cells. cpm ± SE of duplicates	[^{3}H]TdR incorporation of mixture with BN stimulator cells. cpm ± SE of duplicates	[^{3}H]TdR incorporation of responder cells alone
1	1	29,510 ± 3,499 (26.3)*	31, 638 ± 7,402 (90.9)*	
	2	27,404 ± 3,314 (24.4)	32,804 ± 5,566 (94.2)	
	3	25,381 ± 6,045 (22.6)	35,976 ± 1,668 (103.4)	
	4	40,001 ± 1,931 (35.7)	31,182 ± 1,714 (89.6)	4,124 ± 157
	5	31,474 ± 10,562 (28.1)	38,276 ± 4,444 (100.8)	
	6	21,633 ± 1,908 (19.3)	35,887 ± 5,171 (103.1)	
	7	22,724 ± 1,831 (20.3)	23,477 ± 2,962 (67.4)	
2	1	8,279 ± 2,742 (6.1)	39,840 ± 3,242 (94.8)	
	2	5,431 ± 3,268 (4.0)	41,995 ± 4,649 (99.9)	
	3	13,745 ± 2,973 (10.1)	43,036 ± 2,643 (102.4)	5,984 ± 462
	4	10,288 ± 4,016 (7.5)	38,836 ± 3,964 (92.4)	
	Responder cells treated with serum from normal Lewis serum			
1	1	112,151 ± 5,622 (100)	34,811 ± 3,767 (100)	
2	1	136,784 ± 9,430 (100)	42,011 ± 4,857 (100)	

Cultures were pulsed for 4 h 96 h after setting the cultures with 1 μCi of [^{3}H]TdR. 2,000 R irradiated stimulator cells gave between 240 and 280 cpm.

* Figures in brackets denote percent reactivity compared to the response obtained with the control normal Lewis serum.

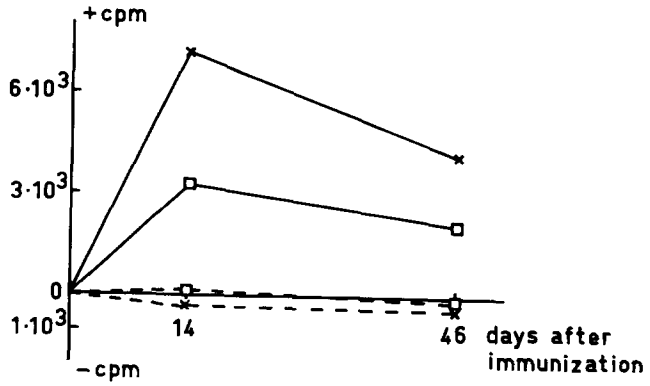

FIG. 2. Idiotypic, anti-DA or anti-BN molecules were isolated from Lewis normal urine by absorption on anti-(Lewis anti-DA) and anti-(Lewis anti-BN) anti-idiotypic immunoabsorbents. Eluted material was cross-linked with glutaraldehyde together with human IgG and used as immunogen in normal Lewis rats using Freund's adjuvants. Antisera were tested from bleedings at days indicated using ^{125}I-labeled Protein A as a marker. (×—×) immunized with anti-BN receptor and (□—□) immunized with anti-DA receptor were tested against Lewis T lymphocytes. (×---×) immunized with anti-BN receptor and (□---□) immunized with anti-DA receptor were tested against the respective (L × DA)F_1 or (L × BN)F_1 T cells. Background binding at day 0 (bleeding before immunization) is subtracted = 0 level.

compared with lymphocytes from normal, untreated Lewis rats. This autoreactivity in MLC is persisting, can be seen close to 1 yr after the last injection, and may indicate anti-idiotypic T-cell reactions. Lymphocytes from these autoim-

TABLE V

Lymphocytes Derived from Autoimmunized Lewis Rats Lack Reactivity Against the Corresponding Alloantigen as Measured in MLC

Responder cells derived from autoimmunized Lewis rat no.	[³H]TdR incorporation of mixture against DA stimulator cells. Mean cpm ± SE of duplicate wells			[³H]TdR incorporation of mixture against BN stimulator cells. Mean cpm ± SE of duplicate wells			[³H]TdR incorporation of responder cells alone. Mean cpm ± SE of duplicate wells			[³H]TdR incorporation of mixture against Lewis stimulator cell. Mean cpm ± SE of duplicate wells
	Bleeding of			Bleeding of			Bleeding of			
	Day 14	Day 38	Day 138	Day 14	Day 38	Day 138	Day 14	Day 38	Day 138	
1	29,013 ± 2,226 (24.4)*	20,943 ± 1,269 (15.6)*	14,843 ± 206 (9.5)*	37,586 ± 4,031 (88.4)*	43,179 ± 6,464 (100.5)*	41,182 ± 3,284 (76.8)*	4,222 ± 1,211	7,101 ± 1,224	9,507 ± 741	12,741 (214.6)
2	40,229 ± 4,113 (33.8)	20,094 ± 3,592 (15.0)	19,892 ± 2,036 (12.7)	41,198 ± 8,421 (97.0)	35,208 ± 3,769 (81.9)	35,488 ± 4,931 (66.2)	5,950 ± 1,066	7,297 ± 978	7,235 ± 818	12,946 ± 1,291 (218.1)
3	25,967 ± 1,412 (21.8)	18,021 ± 2,018 (13.5)	17,354 ± 1,294 (11.1)	34,604 ± 3,040 (81.4)	42,831 ± 4,420 (99.6)	39,608 ± 2,528 (73.9)	5,470 ± 2,172	8,459 ± 816	7,658 ± 786	10,298 ± 314 (173.5)
4	27,399 ± 2,403 (23.0)	25,151 ± 3,594 (18.8)	21,431 ± 2,049 (13.7)	38,077 ± 5,804 (90.0)	37,492 ± 4,220 (87.2)	43,811 ± 5,594 (81.8)	3,875 ± 127	5,418 ± 652	7,424 ± 1,366	12,581 ± 470 (211.9)
5	47,438 ± 2,347 (39.9)	20,110 ± 5,051 (15.0)	12,629 ± 496 (8.1)	42,734 ± 3,144 (100.5)	40,103 ± 2,666 (93.3)	44,145 ± 3,119 (82.4)	4,866 ± 150	5,868 ± 1,149	8,209 ± 1,792	11,618 ± 643 (195.7)
6	36,230 ± 6,621 (30.4)	19,867 ± 6,261 (14.8)	18,677 ± 2,128 (12.0)	35,613 ± 4,397 (84.0)	38,114 ± 8,681 (88.7)	33,130 ± 859 (61.8)	4,408 ± 1,205	6,098 ± 621	5,889 ± 843	11,022 ± 1,478 (185.7)
7	29,608 ± 2,221 (24.9)	20,216 ± 1,910 (15.1)	15,172 ± 3,177 (9.7)	35,617 ± 4,627 (84.0)	37,440 ± 1,454 (87.1)	38,558 ± 3,337 (72.0)	6,200 ± 1,589	7,400 ± 1,312	8,916 ± 864	11,048 ± 912 (186.1)
Responder cells derived from normal Lewis rat no.										
1	125,125 ± 7,491 (100)	115,863 ± 1,884 (100)	160,056 ± 19,238 (100)	46,293 ± 3,447 (100)	43,301 ± 4,116 (100)	66,362 ± 3,652 (100)	2,928 ± 503	4,869 ± 158	5,840 ± 901	6,100 ± 368 (100)
2	112,898 ± 6,612 (100)	151,936 ± 11,235 (100)	152,324 ± 6,352 (100)	38,713 ± 3,451 (100)	42,667 ± 2,947 (100)	40,826 ± 6,877 (100)	3,481 ± 329	5,790 ± 1,824	5,825 ± 1,193	5,771 ± 797 (100)

Cultures were pulsed for 6 h with 1 μCi of [³H]TdR on hour 112 after setting up the cultures. 2,000 R irradiated stimulator cells gave betweeen 150 and 300 cpm.

* Figures in brackets represent percent reactivity compared to the mean of reactivity of normal Lewis rats = (100%).

munized Lewis rats were also tested for graft versus host reactivity in (Lewis × DA(F_1 or (Lewis × BN)F_1 recipients. Experiments as depicted in Table VI indicate a similar specific reduction in anti-DA reactivity as seen in MLC.

In consecutive experiments we could also demonstrate that lymphocytes from Lewis rats immunized with normal urine factor lack reactivity against the relevant alloantigens as measured in MLC and graft versus host reaction. As shown in Tables VII and VIII there was a specific lack of reactivity either against DA alloantigens or BN alloantigens depending on which material Lewis rats were immunized with.

In conclusion we can thus say that lymphocytes derived from autoimmunized Lewis rats show specific lack of reactivity against the relevant alloantigen as measured in MLC and graft versus host reaction. This specific reduction is long lasting and the autoimmunity may well be self-recruiting by feeding in to the system new, "virgin" idiotype-positive lymphocytes, as immunogen.

Lack of Capacity of Autoimmunized Derived Lymphocytes to Fix Anti-idiotypic Antibodies of Corresponding Specificity. We know that a certain percentage of normal Lewis T lymphocytes have the capacity to fix anti-(Lewis anti-DA) or anti-(Lewis anti-BN) anti-idiotypic antibodies (7). T lymphocytes from Lewis rats autoimmunized against naturally occurring anti-DA molecules would be expected to lack anti-DA-reactive lymphocytes expressing anti-DA idiotype in conclusion from the MLC and graft versus host studies (see 3). On the other hand the same T-lymphocyte population should include anti-BN idiotype expressing T lymphocytes as reactivity against third party BN alloantigens was normal in MLC and graft versus host. To test this hypothesis, T lymphocytes derived from autoimmunized Lewis rats immunized against anti-DA or anti-BN naturally occurring molecules were incubated with anti-(Lewis anti-DA) and anti-(Lewis anti-BN) anti-idiotypic antisera using radiolabeled protein A as a marker.

Table IX summarizes this experiment. T lymphocytes derived from Lewis animals immunized with anti-DA normal serum factor or urine factor were deficient in binding anti-(Lewis anti-DA) anti-idiotypic antibodies. Anti-(Lewis

TABLE VI

Lymphocytes Derived from Autoimmunized Lewis Rats Lack Reactivity Against Corresponding Alloantigens as Measured in Graft Versus Host Reaction

Injected cells derived from autoimmunized Lewis rat no.	Mean weight (mg) ± SE of two popliteal lymph nodes. "experimental nodes" (left side)	Mean weight (mg) ± SE of two popliteal lymph nodes. "control nodes" (right side)	Mean log ratio ± SE
1	9.4 ± 0.70	32.0 ± 1.60	0.53 ± 0.01
2	10.6 ± 0.75	32.2 ± 2.30	0.48 ± 0.06
3	8.5 ± 2.15	29.2 ± 1.55	0.53 ± 0.11
5	13.4 ± 1.40	29.3 ± 1.30	0.35 ± 0.07
6	11.1 ± 1.40	32.3 ± 1.10	0.47 ± 0.07

2×10^6 viable cells from autoimmunized Lewis rats were injected into the left hind foot pads ("experimental nodes"), and 2×10^6 viable cells from normal Lewis rats were injected into the right hind foot pads ("control nodes"). Popliteal lymph nodes were removed 7 days later.

TABLE VII

Lymphocytes Derived from Autoimmunized Lewis Rats Against Normal Urine Factor Lack Reactivity Against the Corresponding Alloantigen as Measured in MLC

Lewis responder cell derived from	[^{3}H]TdR incorporation of mixture with DA stimulator cells. Mean cpm ± SE of triplicates	[^{3}H]TdR incorporation of mixture with BN stimulator cells. Mean cpm ± SE of triplicates	[^{3}H]TdR incorporation of mixture with August stimulator cells. Mean cpm ± SE of triplicates	[^{3}H]TdR incorporation of mixture with Lewis stimulator cells. Mean cpm ± SE of triplicates	[^{3}H]TdR incorporation of responder cells alone. Mean cpm ± SE of triplicates
Normal Lewis rat	5,466 ± 164	4,538 ± 204	5,134 ± 524	486 ± 165	359 ± 14
Lewis rat autoimmunized with anti-DA normal serum factor	1,712 ± 185	4,289 ± 388	4,388 ± 136	699 ± 239	376 ± 80
Lewis rat autoimmunized with anti-BN normal urine factor	5,917 ± 440	1,667 ± 592	5,936 ± 988	1,161 ± 290	409 ± 170
Lewis rat autoimmunized with anti-DA normal urine factor	2,040 ± 209	4,762 ± 644	4,899 ± 593	584 ± 175	278 ± 41

MLC was performed in serum-free EHAA medium (14). Cultures were pulsed for 4 h 118 h after setting up the cultures with 1 μCi of [^{3}H]TdR. Conditions otherwise as described for MLC under Material and Methods. 2,000 R irradiated stimulator cells alone gave between 100 and 150 cpm.

TABLE VIII

Lymphocytes Derived from Autoimmunized Lewis Rats with Normal Urine Factor Lack Reactivity Against Corresponding Alloantigens as Measured in Graft Versus Host Reaction

Injected lymphocytes derived from	Host	Mean weight (mg) ± SE of four to five popliteal lymph nodes. "experimental nodes" (left side)	Mean weight (mg) ± SE of four to five popliteal lymph nodes. "control nodes" (right side)	Mean log ratio ± SE
Lewis rats autoimmunized with normal anti-DA serum factor	(Lewis × DA)F_1	14.4 ± 0.8	30.7 ± 4.2	0.32 ± 0.04
	(Lewis × BN)F_1	26.4 ± 2.4	25.8 ± 2.4	−0.01 ± 0.06
Lewis rats autoimmunized with normal anti-BN urine factor	(Lewis × DA)F_1	30.8 ± 1.0	30.4 ± 0.9	−0.00 ± 0.03
	(Lewis × BN)F_1	13.1 ± 2.1	25.7 ± 1.7	0.32 ± 0.09
Lewis rats autoimmunized with normal anti-DA urine factor	(Lewis × DA)F_1	9.4 ± 1.5	39.8 ± 5.0	0.64 ± 0.12
	(Lewis × BN)F_1	31.8 ± 1.5	33.2 ± 0.6	0.02 ± 0.02

2×10^6 viable cells were injected into each foot pad. Lymphocytes derived from the experimental animals were injected into the left hind foot pad and as a control lymphocytes from normal Lewis rats were injected into the right hind foot pad. Popliteal lymph nodes were removed 7 days later.

anti-BN) anti-idiotypic antibodies on the other hand were fixed at normal levels. Conversely, Lewis T lymphocytes derived from Lewis rats immunized with anti-BN molecules fixed anti-(Lewis anti-DA) in normal amount but much less

TABLE IX

Lymphocytes from Autoimmunized Derived Lewis Rats Fail to Bind the Corresponding Anti-Idiotypic Antibody

Tested lymphocytes derived from	Incubated with serum			
	Normal (Lewis × DA)F_1	Anti-(Lewis anti-DA)	Normal (Lewis × BN)F_1	Anti-(Lewis anti-BN)
Normal Lewis rat	8,097 ± 88	21,019 ± 865	8,429 ± 373	23,635 ± 155
Normal DA rat	7,813 ± 257	8,420 ± 979	ND	ND
Normal (Lewis × BN) rat	ND	ND	8,627 ± 364	7,744 ± 688
Lewis rat autoimmunized with normal anti-DA serum factor	8,580 ± 230	14,187 ± 512	8,612 ± 435	22,481 ± 1,366
Lewis rat autoimmunized with normal anti-BN urine factor	8,030 ± 619	18,809 ± 819	8,301 ± 33	14,837 ± 1,265
Lewis rat autoimmunized with normal anti-DA urine factor	8,262 ± 580	13,974 ± 2,297	8,729 ± 882	20,189 ± 426

1×10^7 T lymphocytes were incubated with the sera as stated and ^{125}I-labeled protein A was used as a marker. Underlined values denote reaction with the two anti-idiotypic antisera.

anti-(Lewis anti-BN) anti-idiotypic antibodies. These experiments demonstrate that the relevant idiotypic T lymphocytes reactive against the corresponding Ag-B are either missing or blocked in the autoimmunized animals.

Induction of Specific Transplantation Tolerance by Immunization with Naturally Occurring Idiotypic, Antigen-Binding Molecules. Results presented so far have indicated that it is indeed possible to induce auto-anti-idiotype immunity in adult Lewis rats against their own idiotypic receptors with specificity for a given set of alloantigens. Measurements so far reported only involve tests at the serum level or testing lymphocytes in vitro from such autoimmunized rats. The eventual aim of the present procedures was to induce specific transplantation tolerance against a given alloantigen(s) by such autoimmunization procedures. Accordingly, we thus tested the ability of Lewis rats immunized with Lewis-anti-DA serum factors to reject DA or BN alloantigenic skin grafts. Table X summarizes the results of such skin grafts made to 20 adult autoimmunized Lewis rats. As seen, a drastic specific increase in survival time of DA or (Lewis × DA)F_1 skin grafts was obtained (ranging from 14 to 39 days with a mean of 24 days with grafts on control animals all being rejected between 9 to 12 days). On the other hand, BN or (Lewis × BN)F_1 skin grafts functioning as third party control grafts were rejected within the normal time period in the autoimmunized experimental rats. A highly significant degree of specific tolerance with regard to such a sensitive target tissue as a skin graft can thus be induced by auto-anti-idiotypic immunization procedures.

The eventual rejection of DA skin grafts on Lewis rats autoimmunized with Lewis-anti-DA serum factors could be due to reactions against other minor histocompatibility antigens than the Ag-B locus or to a recovery of anti-Ag-B-reactive T lymphocytes via some recruiting mechanism. We could demonstrate, however, that lymphocytes from the above autoimmunized Lewis rats after DA and BN skin graft rejection still are quite nonfunctional with regard to MLC reactivity against DA alloantigens (see Fig. 3).

TABLE X

Prolonged Survival of DA or (Lewis × DA)F_1 Skin Allografts in Lewis Rats Autoimmunized with Normal Anti-DA Serum Factor

Autoimmunized Lewis rat no.	Immunized with anti-DA normal serum factor	Day of rejection of DA or (Lewis × DA)F_1 skin grafts after transplantation	Mean	Day of rejection of BN or (Lewis × BN)F_1 skin grafts after transplantation	Mean
1	Three times	18		10	
2	"	20		11	
3	"	21		10	
4	"	31		11	
5	Twice	21		12	
6	"	35		11	
7	"	27		11	
8	"	38		13	
9	Three times	32		11	
10	"	28	24.2	10	10.8
11	"	20		10	
12	"	25		11	
13	"	16		10	
14	"	15		11	
15	"	27		11	
16	"	39		12	
17	"	14		11	
18	"	16		10	
19	"	21		10	
20	"	19		10	

(Lewis × DA)F_1 or DA skins were rejected on normal untreated Lewis rats between days 9 and 10. (Lewis × BN)F_1 or BN skins between days 10 and 12. The first four experimental animals received DA and BN skins. All the other animals were grafted with F_1 skins.

Lymphocytes were purified from blood and served as responder cells. Spleen cells from DA, BN, August, and Lewis rats were irradiated with 2,000 R and were used as stimulator cells. Fig. 3 represents four reaction patterns against these antigens. Fig. 3*a* represent autoimmunized Lewis rat no. 6 from Table XI, (*b*) no. 12, (*c*) no. 16, and (*d*) no. 19. In all four animals a secondary type of reaction can be seen against BN alloantigens and a primary reaction against August. The reactions against DA stimulator cells is drastically reduced. It has to be recalled that these animals have before already rejected a DA allograft. When these lymphocytes are co-cultivated with normal irradiated Lewis spleen cells an increased thymidine uptake can be seen over the whole culture period when compared to the thymidine uptake of responder cells alone. In conclusion we can say that these lymphocytes derived from autoimmunized Lewis rats, although confronted with DA alloantigens before, still show marked decreased reactivity against DA stimulator cells.

Production of Anti-Ag-B Alloantibodies in Autoimmunized Rats. The T-cell response against Ag-B antigens as measured in MLC was still virtually absent even after rejection of DA skin grafts to autoimmunized Lewis rats (Fig. 3). A measurable anti-DA alloantibody-producing ability was, however, demonstrable in these rats. Thus, Lewis rats autoimmune with regard to Lewis-anti-DA idiotypes that had rejected DA and BN skin grafts were injected with 5×10^7 DA

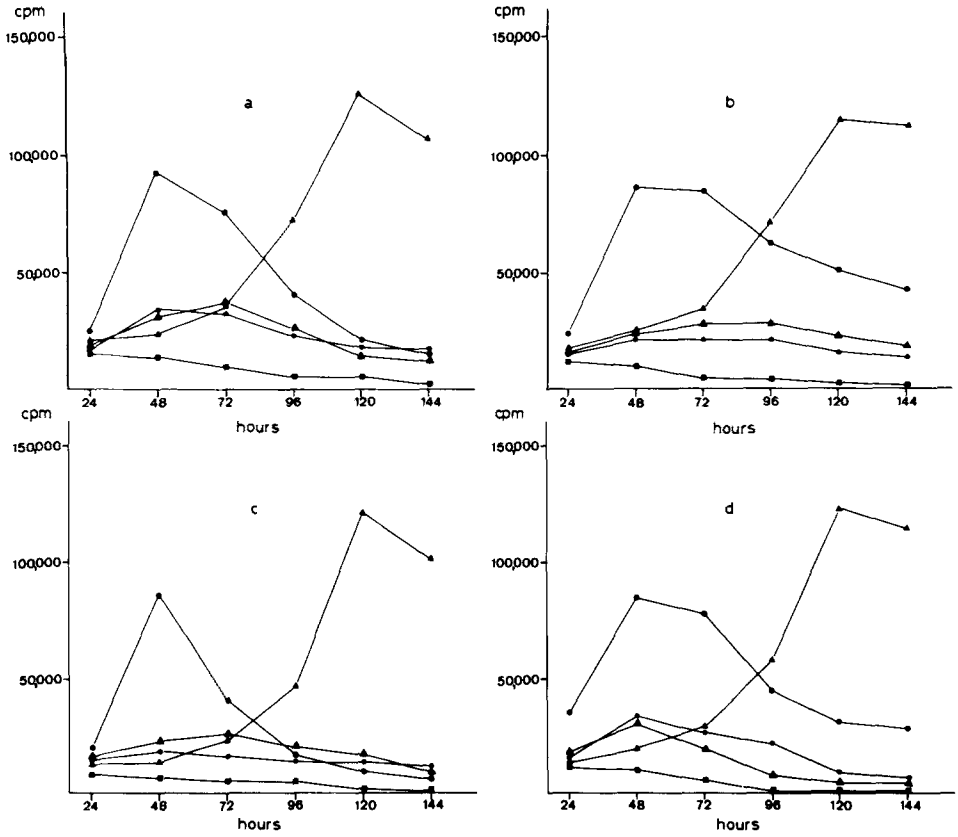

FIG. 3. MLC with lymphocytes derived from autoimmunized Lewis rats which have before rejected (Lewis × DA)F_1 and (Lewis × BN)F_1 skin grafts. Cultures were pulsed for 4 h before the times indicated in the figure with 1 μCi of [^{3}H]TdR. (●—●) Response against BN stimulator cells. (▲—▲) Response against August stimulator cells. (△—△) Response against DA stimulator cells. (○—○) Response against normal irradiated Lewis cells. (■—■) Response of responder cells alone.

and BN spleen cells, respectively, and bled for antisera at day 10. Control animals were treated in identical manner except for the autoimmunization procedures. Anti-DA or -BN alloantibodies were then tested for using the ^{125}I-protein A assay. The results are shown in Table XI and denote a significantly reduced, yet significant production of anti-DA-reactive alloantibodies in the autoimmunized Lewis rats. Anti-BN alloantibody titers were the same in the autoimmunized and the control sera. The specific reduction was in the order of a 50-fold decrease and is thus a highly significant selective depression. The fine specificity of the remaining antibodies (anti-Ag-B or minor loci antigens?) as well as whether they contain "anti-DA" idiotypic determinants of anti-Ag-B type have not been analyzed.

Analysis of Immune Status of Offsprings from Lewis Females Made "Tolerant" to DA Alloantigens via Autoimmunization. Administration of anti-idiotypic antibodies to newborn individuals has been reported to result in prolonged efficient suppression of the ability to produce antibodies carrying the

TABLE XI

Suppression of Corresponding Alloantibody Production in Autoimmunized Lewis Rats

Anti-DA normal serum factor autoimmunized Lewis rat no.	Titer of alloantibodies against DA alloantigens	Mean	Titer of alloantibodies against BN alloantigens	Mean
1	2^{7}		2^{11}	
2	2^{6}		2^{12}	
3	2^{8}		2^{12}	
4	2^{7}		2^{11}	
5	2^{7}		2^{10}	
6	2^{6}	$2^{6.75}$	2^{10}	$2^{11.17}$
7	2^{8}		2^{12}	
8	2^{5}		2^{10}	
9	2^{7}		2^{11}	
10	2^{6}		2^{12}	
11	2^{6}		2^{11}	
12	2^{8}		2^{12}	
Control Lewis rat no.				
1	2^{12}		2^{11}	
2	2^{12}	$2^{12.75}$	2^{12}	$2^{11.5}$
3	2^{14}		2^{11}	
4	2^{13}		2^{12}	

Sera were incubated with the indicated target cells. ^{125}I-labeled protein A was used as a marker. Sera were collected from autoimmunized Lewis rats and control Lewis rats which all had before (between 50 and 80 days) rejected DA as well as BN skin grafts.

relevant idiotopes (20). The autoimmune antibodies detected in the present system can be shown to contain IgG antibodies, as demonstrated by their ability to react with protein A (3) and would thus be expected to pass the placental barrier. It was thus considered worthwhile to determine whether lymphocytes from offsprings of Lewis females made tolerant to DA alloantigens via autoimmunization against Lewis-anti-DA receptors molecules would express any significant lack of anti-DA reactivity. Two litters from different autoimmunized Lewis females mated to Lewis males were analyzed as to reactivity against DA and BN alloantigens, as measured in MLC at the 5th wk of age. The results shown in Table XII indicate that the offsprings do indeed express a specific reduction in MLC reactivity against DA alloantigens. This reduction is only partial, however, and less pronounced than the MLC reduction presented by the individual mothers.

Discussion

It has been known for a few years that an individual is normally able to mount an immune reaction against idiotypic determinants present on immunoglobulin molecules produced by the individual himself (1). Our present results are in agreement with these earlier findings. They also extend the concept to contain idiotypic determinants present on T lymphocytes, even if such lymphocytes are present as a high proportion of the cell population (7). Thus, despite the fact that

TABLE XII

Partial Specific Reduction in MLC Reactivity in Lymphocytes from Offsprings of Lewis Females Autoimmunized with Anti-DA Factors

Mother no.	Child no.	Response against DA	Response against BN	Responder cells alone
1	1	40,784 ± 4,466	40,539 ± 3,894	4,291
	2	63,406 ± 2,348	37,092 ± 2,908	3,675
	3	85,355 ± 3,916	40,083 ± 2,450	3,949
	4	72,352 ± 3,291	42,862 ± 2,413	3,478
	5	66,759 ± 5,844	34,299 ± 1,725	3,280
2	6	88,666 ± 2,572	40,342 ± 1,461	3,468
	7	91,573 ± 10,111	42,356 ± 2,193	3,364
	8	83,712 ± 4,653	38,642 ± 1,968	3,900
	9	77,699 ± 1,060	40,471 ± 1,707	3,821
	Mean ± SE	74,478 ± 5,309	39,632 ± 881	
Normal Lewis	1	103,820 ± 2,994	40,159 ± 1,583	1,939
	2	94,578 ± 7,634	42,922 ± 1,068	1,913
	Mean ± SE	99,199 ± 4,621	41,540 ± 1,381	

Two mothers injected three times with naturally occurring anti-DA idiotypic molecules on day 0, 30, 121, which were grafted on day 144 with (Lewis × DA)F_1 and (Lewis × BN)F_1 skin (DA rejected on day 32, 28, respectively) got pregnant from a normal Lewis male. Mother no. 1 got five children and mother no. 2 four. The lymphocytes from the children were tested in MLC against DA and BN stimulator cells at the age of 5 wk, and the response was compared to that from normal Lewis rats of the same age.

MLC: usual procedure; Puls: for 6 h with 1 μCi at 120 h.

idiotypic receptors signifying reactivity against the Ag-B locus antigens of DA type are present on approximately 6% of normal Lewis T lymphocytes, Lewis rats are able to mount an auto-anti-DA idiotypes-associated immune response. This auto-anti-idiotypic immune response could be measured at the humoral level, that is leading to the production of detectable amounts of auto-anti-idiotypic antibodies as detected by binding or functional-inactivational assays. Whether the Lewis rats immunized with their own anti-DA receptors also mount any T-cell-mediated reaction against native anti-DA-associated idiotopes is less clear. Results in Table V suggest, however, that this may be the case as indicated by the increased DNA synthesis encountered when responder cells from autoimmunized Lewis rats were allowed to react against inactivated normal Lewis lymphocytes.

Immune alloantibodies as well as naturally occurring idiotypic, antigen-binding receptors were both used to induce the induction of auto-anti-idiotypic antibodies in the present system. Both groups of molecules did function within the dose ranges used with regard to induction of auto-anti-idiotypic antibody production and selective abolishment of the relevant T-lymphocyte-mediated immune reactivity. Within the limited experiments done for comparison we could thus not state whether immune antibodies present in serum or partially digested T-cell-derived receptor material in the urine differ in their ability to induce auto-anti-idiotypic immunity. Judging from earlier data in the present

systems it would seem likely that use of immune antibodies should be advantageous for the induction of anti-idiotypic antibodies reacting well with B-cell-derived antibodies, but maybe less well with T-cell receptors (2, 3, 9). Immunization using T-cell receptor material would yield antisera that would react equally well with the relevant antigen-binding receptors irrespective of B- or T-cell origin (3, 9). Anti-idiotypic antibodies against B-cell receptors can be shown to react against idiotopes produced by the heavy chain alone, against composite idiotypes created by heavy and light chain together, or be directed against light chain-determined idiotopes. Only the former group of idiotypic determinants seem to be expressed on the T-lymphocyte receptor for antigen (8–10). Several antisera raised against the B-cell-derived antibodies may thus react with the B but not the T-cell receptor for the very same antigen. It remains to be seen whether immunization with purified heavy chains from the relevant antigen-binding Ig molecules will lead to consistent induction of anti-T idiotypic reactive antisera.

The persistence of the present auto-anti-idiotypic immune state is unknown but is already known to exist for prolonged periods of time (see present article). It is plausible that once induced such immunity will persist for life, as constant boosting may occur in a continuous manner as stem cells will mature to lymphocytes carrying the relevant, idiotypic receptors on their outer surface. Elimination of such cells may lead to the creation of antigen-antibody complexes. No damaging impacts by the present procedure have, however, so far been noted.

The mechanism whereby the present auto-anti-idiotypic immune reaction(s) lead to selective elimination of T lymphocytes with the relevant anti-Ag-B reactivity as measured by graft versus host or MLC reactivity by skin graft survival is unknown. Direct radioimmunoassays on lymphocytes from autoimmunized rats demonstrated a selective reduction of relevant idiotypic determinants in such lymphocyte populations. It has previously been shown that anti-idiotypic antibodies in the presence of complement in vitro directly eliminate such idiotype-positive cells in an irreversible manner (3). The simplest explanation would thus seem to be that Lewis lymphocytes with idiotypic receptors and antigen-binding ability to Ag-B antigens (linkage between idiotopes and antigen-binding specificity in the present system is amply documented [2, 3]) are killed when encountering anti-idiotypic antibodies in vivo. Breakage of tolerance would thus require feeding into the immunocompetent compartment of either new, idiotype-positive cells maturing from stem cells or the amplification of rare idiotype-negative clones of lymphocytes. Such emergence of a rare clone has been reported in other idiotype-suppressed systems, but has so far not been found in the present system. We thus deem it quite likely that the present state of tolerance as induced by auto-anti-idiotypic antibodies when measured at the T-lymphocyte level will be characterized by sizeable stability and prolonged duration.

The present procedure could be shown to induce a highly significant prolongation of skin grafts of the relevant donor type, yet all grafts were eventually rejected. This is to be expected, however, as the strain combinations tested are known to differ with regard to several minor histocompatibility loci besides the major Ag-B locus. We know that our auto-anti-idiotypic antibodies to their

dominating part are directed against Ag-B reacting receptors, and this would leave reactivity in the autoimmunized rats with regard to minor antigenic differences largely intact. Skin graft prolongation of the present order is to be considered to indicate a highly significant state of immune tolerance and graft survival would be expected to be further extended when experiments can be performed on Ag-B congenic strain combinations. That the eventual rejection of skin grafts in the presented experiments very likely is due to reaction against minor loci is further indicated by the failure of lymphocytes from autoimmunized rats to react against the tolerizing donor lymphocytes, even when tested in MLC reactions after skin graft rejection.

In conclusion, we can state that it is indeed possible to procure a state of specific unresponsiveness against transplantation antigens via immunization with the individuals own, naturally occurring receptors with specificity for these alloantigens. The speed of induction is remarkable, being significant as soon as 2 wk after immunization, and the state of autoimmunity causing tolerance seems very longlasting. No negative side effects have so far been recorded. From this point of view the present system would thus seem to carry several advantageous parameters in comparison to procedures used in conventional transplantation immunology. A more selective depression of the immune reactivity as to only involve activity against the donor's antigens can be visualized, which even if only partial may significantly reduce the requirements for heavy cytostatic treatment. The system may via its active state be self-perpetuating and keep potential harmful anti-donor-reactive lymphocytes in place for maybe the life span of the individual. But there are several difficulties residing before the present procedure may be considered for clinical use. Firstly, are the findings of general applicability or restricted to certain strain combinations or to species of animals? Secondly, the procedure requires modifications to avoid the necessity of having access to anti-idiotypic immunosorbants to purify the relevant receptors, but we already have preliminary results to indicate that such alternatives are quite feasible. Thirdly, despite the fact that we have so far seen no negative side effects of this autoimmunity, only followups for prolonged periods of time will tell us whether such autoimmunized individuals in any way (lack of immune reactivity towards certain other antigens; immune complex damages) will suffer from the procedures involved. Whether these obstacles will be major or only minor hindrances in the use of a new approach to achieve transplantation tolerance only time will tell.

Summary

Serum or urine from normal adult Lewis rats can be shown to contain detectable amounts of idiotypic, antigen-binding receptors with specificity for the major histocompatibility complex locus antigens of the rat, the Ag-B locus antigens. Such purified naturally occurring receptor molecules, be they of T- or B-lymphocyte origin, can be used in a polymerized form to provoke the production of auto-anti-idiotypic antibodies when injected back into normal Lewis rats. As a consequence of this autoimmunity, lymphocytes of these Lewis rats can be shown to be depleted of cells carrying the relevant idiotypic receptors signifying reactivity against a given Ag-B locus-determined antigen(s). This specific lack of idiotypic lymphocytes is manifested as a selective loss of reactivity against the

relevant Ag-B-incompatible antigens as measured by graft versus host or MLC reactions. Furthermore, autoimmune Lewis rats display specific transplantation tolerance against the skin grafts from the relevant strain, as demonstrated by specific prolongation of graft survival. A further indication of the specific tolerence state of these rats comes from the highly reduced ability to produce circulating antibodies against the relevant Ag-B antigens. No side effects of these autoimmunization procedures have been noted so far. It would thus seem clear that a prolonged state of specific transplantation tolerance can be achieved via autoimmunization against the individual's naturally occurring idiotypic, antigen-binding receptors.

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