

INDUCTION OF SPECIFIC TRANSPLANTATION IMMUNE REACTIONS USING ANTI-IDIOTYPIC ANTIBODIES*

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A seemingly unique property of lymphocytes is their ability to express selective immune reactivity against a given substance via their own intrinsic antigen-specific receptors. This specific immune potential of a single T or B lymphocyte can become expressed and amplified after contact with various inducing agents. These inducers may either carry specific binding properties to the antigen-binding receptors and are then called immunogens, or they may be nonspecific but endowed with intrinsic mitogenic properties for a given subset of lymphocytes, e.g., with the B- and T-cell mitogens. Very little is known about the actual molecular events leading to the triggering of lymphocytes by any of the above groups of inducing agents. It is clear, however, that collaboration between different cell types is frequently a necessary requirement for eventual triggering to occur. According to the above definition, macromolecules capable of interacting in a specific manner with the antigen-binding areas of lymphocyte receptors should be called immunogens, provided that they also express other, poorly defined features necessary for a substance to be immunogenic. In principle, two kinds of macromolecules can function as such specific immunogens: the conventional antigen, which fits properly to the antigen-binding receptors on T or B lymphocytes, or antibody molecules that are specific for the variable regions of these receptors – the so-called anti-idiotypic antibodies (1). During recent years, it has become increasingly clear that such anti-idiotypic antibodies may coexist with idiotypic receptors in the same individual (2). Thus, auto-anti-idiotypic immunity can be shown to constitute a real possibility in many systems when it is closely observed (3-5). Sophisticated theories exist discussing the possibility that idiotypes and anti-idiotypes present within the very same immune system play important roles in the actual immune cognitive and regulatory processes (6, 7). In support of such theories are results that show that anti-idiotypic antibodies administered in vivo may have specific consequences of either positive or negative nature for the specific idiotypic lymphocytes, leading to increased or decreased reactivity against the relevant antigens (8, 9).

We have been working with idiotypes and anti-idiotypes in relation to antigen-specific receptors for major histocompatibility antigens in the mouse and rat (2, 4, 5, 10-15). Here we could demonstrate the presence of idiotypic receptors on relevant, immunocompetent T and B cells, and anti-idiotypic antibodies in the presence of complement could be used to selectively wipe out reactivity against a given set of transplantation antigens (2). The idiotypic

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determinants on the T-cell receptors could be shown by several means to be of heavy-chain immunoglobulin variable gene type, with no evidence of the presence of immunoglobulin light chains (2, 16).¹ Results obtained in other systems support these findings on the characteristics of the T-cell receptor for antigen (19, 20). In experiments analyzing the impact of anti-idiotypic antibodies on mixed leukocyte cultures *in vitro* in the absence of complement, we have observed that some anti-idiotypic sera may by themselves cause increased DNA synthesis of cell populations containing relevant, idiotype-positive lymphocytes. The present article contains an analysis of the use of such anti-idiotypic antibodies in inducing specific immune reactions against histocompatibility antigens in the physical absence of these latter antigens. Here our approach was either to administer purified anti-idiotypic antibodies with adjuvant *in vivo*, or to add soluble antibodies *in vitro* to cultures of normal or immune lymphocytes. The results were quite clear-cut, showing that auto-anti-idiotypic antibodies under defined conditions can be used as a highly potent substitute for histocompatibility antigens leading to high-titered allo-antibody synthesis *in vivo*, as well as efficient induction of killer T cells *in vitro*.

Materials and Methods

Animals

MICE. Mice of the inbred strains CBA/J, C57BL/6, and DBA/2J were purchased from The Jackson Laboratory, Bar Harbor, Maine. Adult mice of either sex were used within an experiment.

RATS. Rats of the inbred strains Lewis (L), DA, BN, (L × DA)_F₁, as well as (L × BN)_F₁, were bred in our own colony. 5-12-wk-old rats of either sex were used within an experiment.

Cell Preparations. Spleen and lymph nodes were aseptically removed, and single cell suspensions were prepared by the aid of a stainless steel mesh using Dulbecco-modified phosphate-buffered saline (D-PBS)² as a medium. Cells were washed once and erythrocytes were lysed thereafter by hypotonic shock using nine parts of D-PBS followed by one part of 10 times concentrated D-PBS. Lymphocytes were washed again and resuspended in the culture medium.

Culture Medium. The culture medium for lymphocyte cultures of mice or rats was Eagle's high amino acid medium (EHHA) (19), complemented with 0.5% of fresh mouse serum for mouse cell cultures, and 0.5-1% of fresh BN rat serum for rat cell cultures.

Mixed Leukocyte Cultures (MLC). MLC for analytical purposes for mice and rats were performed in flat-bottomed microtiter plates (model M220-29ART, Cooke Laboratory Products Div. Dynatech Laboratories, Inc., Alexandria, Va.), using 0.25×10^6 responder lymphocytes and 0.5×10^6 2,000 R irradiated stimulator cells. For culture medium see above. Cultures were pulsed for 6 h with 1 μ Ci of tritiated thymidine (³H]TdR; The Radiochemical Center, Amersham, England, sp act 40-60 Ci/mmol) before harvesting as indicated in figures. Cultures were harvested using a Skatron collector (Skatron, Lierbyen, Norway) and counted in 3 ml of scintillation fluid in mini-scintillation vials.

For the preparation of large quantities of specific T lymphoblasts from MLC, MLC were cultured in tissue culture flasks (model 3013; Falcon Plastics, Div. of BioQuest, Oxnard, Calif.)

¹ H. Binz and H. Wigzell. 1977. Exclusive representation of heavy-chain Ig idiotypes on T-cell receptors for antigen. Submitted for publication.

² *Abbreviations used in this paper:* CML, cell-mediated lympholysis; D-PBS, Dulbecco-modified phosphate-buffered saline; EHAA, Eagle's high amino acid medium; FCS, fetal calf serum; [³H]TdR, tritiated thymidine; LPS, lipopolysaccharide; MLC, mixed leukocyte culture; PHA-p, purified phytohemagglutinin.

using the same medium as described above. A 15-ml cell suspension containing 1.25×10^6 responder cells and 2.5×10^6 2,000-R irradiated stimulator cells/ml were added to each flask. Cultures were harvested on days 4 or 5 after initiation of the MLC for mice, and on day 5 for rats.

Cell-Mediated Lympholysis (CML). CML was performed in V-bottomed microtiter plates in 200 μ l of EHAA medium supplemented with 5% heat-inactivated fetal calf serum (FCS). Assays were carried out in quadruplicate cultures. Each well contained 1×10^6 effector cells and 1×10^4 ^{51}Cr -labeled target cells. Plates were incubated for 6 h at 37°C in 5% CO_2 in air. Maximum release was determined by adding 100 μ l of 0.4% Nonidet P-40 detergent. Percent cytotoxicity (% CML) is expressed as

$$100 \times \frac{\text{experimental} - \text{spontaneous } ^{51}\text{Cr release}}{\text{maximum} - \text{spontaneous } ^{51}\text{Cr release}}$$

The following target cells were used: EL-4 for H-2^b, P-815 for H-2^d, and concanavalin A (Con A) blasts of CBA origin for H-2^k.

Purification of T Lymphocytes from MLC. MLC cells were pooled in 50-ml Falcon tubes and centrifuged for 10 min at 400 g. Cells were pooled and washed again in D-PBS, and resuspended in D-PBS containing 5% FCS. Cells were then applied on a linear 15–30% FCS gradient (19) using D-PBS as a diluent. The gradient was harvested after 4 h at 4°C in 15-ml tubes, and the blast fractions (determined under the microscope) were pooled, and washed four times in 50-ml Falcon tubes.

Preparation of Anti-Idiotypic Antisera

RATS. (a) Conventional method: (L \times DA)_{F1} or (L \times BN)_{F1} rats were injected i.p. with 2.5×10^7 purified Lewis T lymphocytes at 3-wk intervals. Animals were bled the first time 10 days after the fourth injection. The antisera were then screened, first in the protein-A assay for specific binding to purified Lewis T lymphocytes, and second, for their specific inhibition of MLC (20). When the antisera fulfilled these criteria, they were called anti-idiotypic antisera. (b) By autoimmunization with purified Lewis anti-DA IgG heavy chains: IgG was purified on DEAE-cellulose (DE-52; Whatman, Inc., Clifton, N. J.). The purified IgG was reduced in 0.04 M dithiothreitol in 0.55 M Tris-HCl, pH 8.2, and the separation of heavy chains from light chains was done by filtration through Sephadex G-200 in 1 M acetic acid. Fractions containing heavy chains were pooled and recycled once on the same column and then lyophilized. The resulting insoluble material was used as immunogen.

The purity of the heavy chains was assessed by analysis on 10% polyacrylamide gels. No light chains could be detected. Moreover, a rabbit hyperimmunized with this heavy-chain preparation did show anti- γ -, but no anti-light chain activity as analyzed by immune electrophoresis. Lewis animals were injected on days 0, 10, 40, 70, and 100 with 0.3 mg L \rightarrow DA (controls: normal L) γ -chains in Freund's complete adjuvant and bled 10 days after the last injection. Criteria for anti-idiotypic activity were the same as those described under (a).

MICE. Anti-idiotypic antisera of specificity anti-(C57BL/6 anti-CBA) were induced in C57BL/6 mice by autoimmunization with purified C57BL/6 anti-CBA MLC T lymphoblasts. One mouse was injected i.p. with 1×10^7 purified T blasts emulsified in 0.2 ml of Freund's adjuvant. Complete Freund's adjuvant was used for the first injection and incomplete was used for the boosters which were given in 3-wk intervals. Animals were injected four times and bled 10 days after the last injection. The antisera had to fulfill the same criteria as those described for the rat antisera. For restimulation of MLC with different agents and induction of cytolytic T lymphocytes by anti-idiotypic antisera see Results.

Purification of Anti-Idiotypic Antibodies. Anti-idiotypic antiserum of specificity anti-(Lewis anti-DA) was purified over an idiotypic Lewis anti-DA immunoabsorbant. IgG from Lewis anti-DA was coupled to Sepharose as described before (20). 10 ml of anti-idiotypic antiserum was first absorbed on Sepharose to remove naturally occurring antibodies, and it was subsequently absorbed on the idiotypic immunoabsorbant. Bound material was eluted with glycine-HCl buffer, pH 2.8, and 3 M MgCl₂. The eluate was neutralized immediately and dialysed against PBS. Purified anti-(Lewis anti-DA) anti-idiotypic antibodies were cross-linked with glutaraldehyde as described before (20).

Immunization of Lewis Rats with Purified Anti-(Lewis Anti-DA) Anti-Idiotypic Antibodies. Each of three Lewis rats received i.p. on day 0 400 μ g of purified glutaraldehyde cross-linked anti-(Lewis anti-DA) anti-idiotypic antibodies. Complete Freund's adjuvant was used for the first

injection and incomplete adjuvant for the booster injection with the same amount of protein as that given on day 20. Animals were bled on days 14 and 30 and the sera were analyzed for alloantibodies with the protein-A assay. As a control, two Lewis rats were immunized with 5×10^7 (Lewis \times DA) F_1 spleen cells on days 0 and 20. Sera were analyzed as described above.

Protein-A Assay. This was carried out in round-bottomed microtiter plates as described before (20). 7×10^6 Lewis or DA spleen cells were incubated with different dilutions of sera for 1 h at 4°C, washed three times, followed by a 1-h incubation with ^{125}I -labeled protein-A. Cells were washed again three times and bound, labeled protein-A was determined in a gamma counter.

Stimulation of Lymphocytes with Different Mitogens. Stimulation of either normal spleen cells or alloantigen-primed T cells was done with different mitogens. Con A was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden, and used at a 3- $\mu\text{g}/\text{ml}$ final concentration. Purified phytohemagglutinin (PHA-p) was obtained from Wellcome Reagents Ltd., Beckenham, England, and used at a 1- $\mu\text{g}/\text{ml}$ final concentration. Lipopolysaccharide (LPS) O55B5 from *Escherichia coli* was a gift of Dr. Jan Andersson (Department of Immunology, Uppsala University, Uppsala, Sweden) and was used at a 10- $\mu\text{g}/\text{ml}$ final concentration. 2.5×10^6 cells were stimulated in the well of a flat-bottomed microtiter plate.

Results

Mouse T cells Obtained from Primary MLC Can Be Restimulated with Anti-Idiotypic Serum in Vitro. A primary C57BL/6 anti-CBA MLC was allowed to run before harvest and restimulation with various agents. The harvest took place on day 8 and cell debris was removed by centrifugation against FCS (21). Viable lymphocytes were adjusted to $2.5 \times 10^6/\text{ml}$. 0.1 ml of this suspension was used for restimulation with various attempted stimulants using flat-bottomed microtiter plates and conditions as described in the legend to Fig. 1. Attempted stimulatory agents included 2,000 R irradiated CBA or DBA/2 spleen cells, anti-idiotypic antisera of anti-(C57BL/6 anti-CBA) specificity, or normal sera or mitogens such as Con A, PHA, and LPS. Cultures were pulsed with [^3H]TdR 6 h before the time indicated in Fig. 1. The results were clear-cut. A typically strong secondary type of MLC was induced against the CBA stimulatory cells as well as by anti-(C57BL/6 anti-CBA) antibodies with a minor reaction occurring against DBA/2 cells (in this combination sizable cross-reactions between CBA and DBA/2 stimulatory cells for purified C57BL/6 T MLC blasts are known to exist; A. B. Peck, unpublished observations). Of the mitogens used, only Con A was able to give significant values above background, indicating selection for Con A-reactive cells during MLC conditions. The mitogens used are indeed able to function properly against normal cells, and this is shown later in Fig. 5.

Thus, auto-anti-idiotypic antibodies seem able to induce specific restimulation of relevant idio-type-positive, MLC-induced C57BL/6 anti-CBA T cells. However, we had no access to other anti-idiotypic sera in the mouse as specificity controls of the observed reaction. We thus decided to try to repeat the same kinds of experiments in rats where two sets of anti-idiotypic sera were available.

Rat T Lymphocytes Obtained from Primary MLC Can Be Restimulated with Anti-Idiotypic Serum in Vitro. Experiments similar to those described in Fig. 1 were thus performed in the rat systems. Here, two anti-idiotypic sera were available: an anti-(Lewis anti-DA) and an anti-(Lewis anti-BN) serum, which were raised as described in Materials and Methods. Those Lewis anti-DA T cells to be used in restimulation experiments were harvested on day 10 after initiation of primary MLC. Otherwise, conditions were, in essence, the same as those described in Fig. 1.

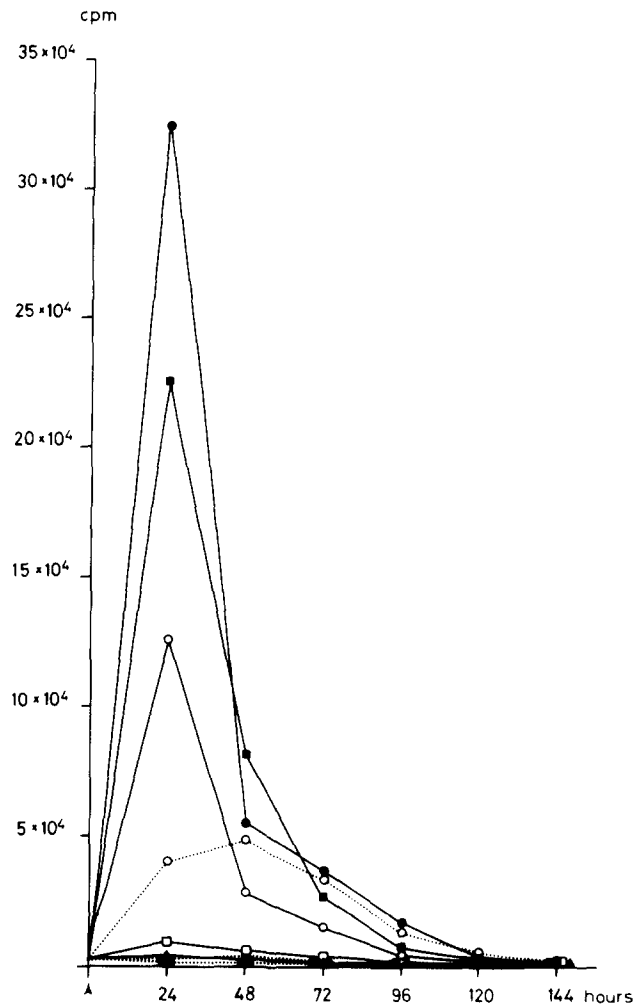


FIG. 1. Restimulation of C57BL/6 anti-CBA MLC-reactive lymphocytes with: (●), CBA cells; (■), anti-(C57BL/6 anti-CBA) serum; (○), DBA/2 cells; (□), normal C57BL/6 serum; (△), C57BL/6 cells alone; (○), Con A; (●), PHA-p; (■), LPS. Dotted lines indicate stimulation with mitogens (Con A, PHA-p, LPS).

The results obtained in the rat restimulation experiments are shown in Fig. 2. It was possible to induce a highly significant restimulation of the Lewis T cells from the anti-DA MLC with the relevant anti-idiotypic sera – in this case with the anti-(Lewis anti-DA) immune serum. This degree of restimulation was in the very same order as that obtained using the allogeneic DA cells as the stimulator agent. Also, as for the MLC of the mouse, the restimulation displayed typical second-set kinetics reaching peak values within the first 24 h, suggesting that the stimulation indeed took place in already primed T cells with anti-DA specificity. Restimulation of the same cells with the third party BN cells caused a minor cross-reactive anamnestic response. Interestingly, a similarly low degree of cross-stimulation was apparent when the anti-(Lewis

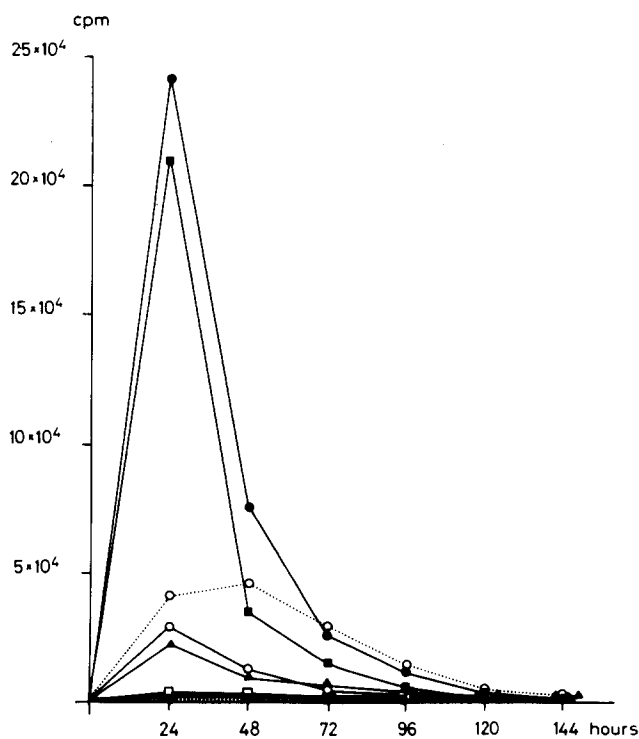


FIG. 2. Restimulation of Lewis anti-DA MLC-reactive cells with: (●), DA cells; (■), anti-(Lewis anti-DA) serum; (○), BN cells; (△), anti-(Lewis anti-BN) serum; (□), normal (L × DA)F₁ serum; (▲), Lewis cells alone; (○), Con A; (●), PHA-p; (■), LPS. Dotted lines indicate stimulation with mitogens (Con A, PHA-p, LPS).

anti-BN) serum was used as stimulant. The results obtained using mitogens were identical to those of the mouse, meaning that only Con A was able to induce a significant increase of DNA synthesis in these secondary cultures.

Lewis Anti-DA MLC-Primed T Cells Can Be Restimulated in Vitro by Anti-Idiotypic Antisera Raised Against Purified Lewis Anti-DA IgG Heavy Chains. The anti-idiotypic sera used in sections one and two were all raised against T lymphocytes or lymphoblasts as indicated. Although the specificity controls in Fig. 2 strongly indicate the anti-idiotypic nature of the stimulatory agents present in immune sera, we deemed it important to exclude possible auto-anti-blast antibodies in such sera. T lymphocytes express on their surface idiotypic determinants characteristic of the variable portion of heavy chains from Ig molecules with the same antigen-binding specificity as the T-cell receptors (15). We have thus induced specific anti-idiotypic antisera against purified heavy chains from Lewis anti-DA IgG molecules, as described in Materials and Methods. These anti-idiotypic antisera induced under autoimmune conditions must be considered highly pure and should not contain anti-cellular antibodies of types other than anti-idiotypic. Tests have shown that such antisera contain the relevant anti-idiotypic antibodies (20).

Fig. 3 depicts the ability of five individual anti-(Lewis-anti-DA γ -chain)

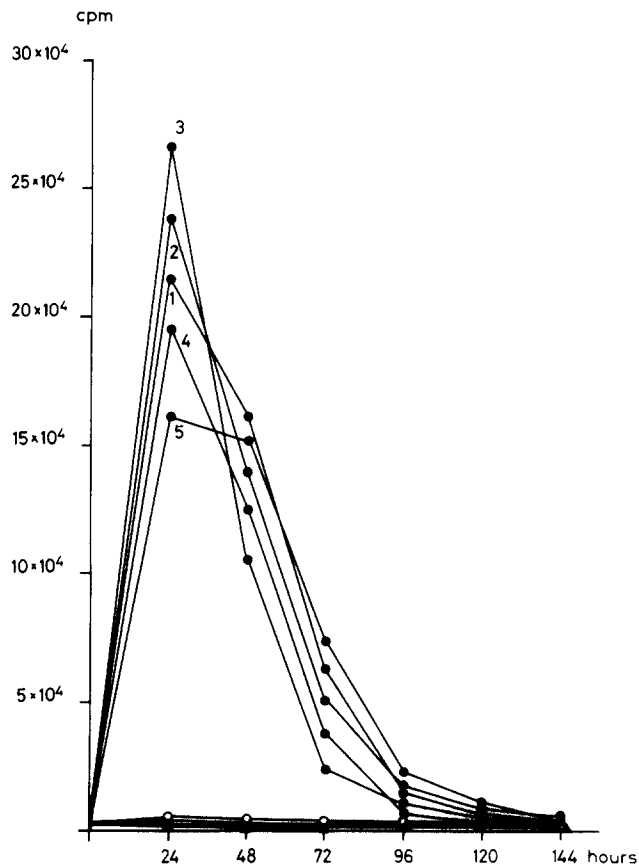


FIG. 3. Restimulation of Lewis anti-DA MLC-reactive cells with: (●), $A_1 \rightarrow A_5$ (Lewis immunized with L anti-DA heavy chains); (○), $N_1 \rightarrow N_5$ (Lewis immunized with L normal heavy chains); (Δ), normal Lewis serum.

idiotypic sera to induce a second-set restimulation of Lewis anti-DA primed T cells in vitro. Control sera from individual rats immunized with the heavy chains of IgG from normal Lewis serum had no effect. These data further support the observation that true anti-idiotypic antibodies can function as highly efficient triggers of proliferation of relevant, primed T lymphocytes.

Induction of a Primary Proliferative Response in Vitro Using Anti-Idiotypic Antibodies. Earlier results had indicated that the addition of low amounts of anti-idiotypic antiserum to primary MLC may sometimes lead to an enhanced response (data not shown). In view of these findings and the results presented in Figs. 1-3, we questioned whether or not an induction of specific proliferation of relevant normal lymphocytes might also be possible, using anti-idiotypic antibodies. Fig. 4 shows results obtained using such an approach in the rat system, whereas Fig. 5 depicts results from mouse experiments of the same kind. Two of the three anti-idiotypic sera analyzed were able to induce highly significant proliferation, namely the anti-(Lewis anti-DA) serum and the anti-(C57BL/6 anti-CBA) serum, whereas the anti-(Lewis anti-BN) serum failed in

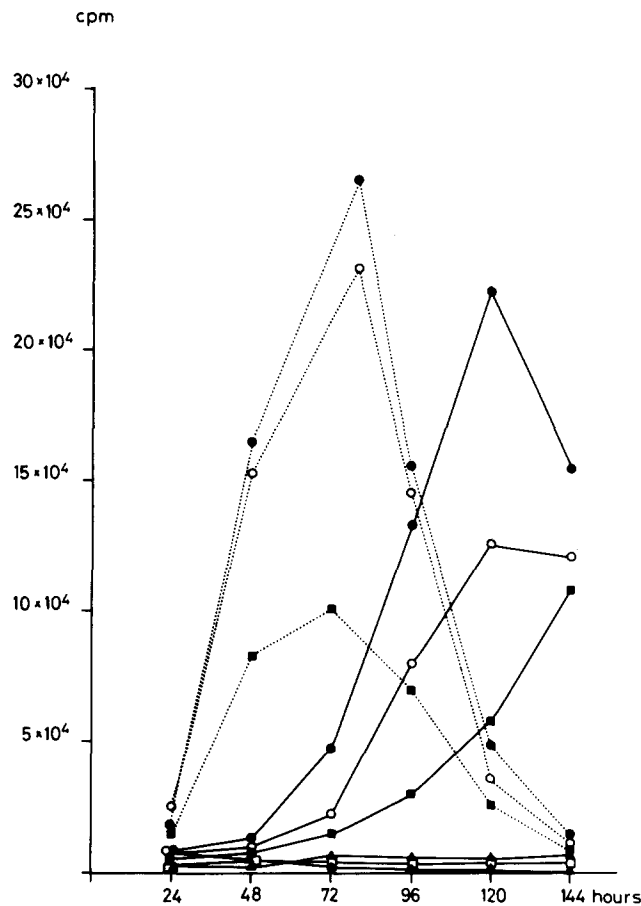


FIG. 4. Stimulation of Lewis lymphocytes with: (●), DA cells; (■), anti-(Lewis anti-DA) serum; (○), BN cells; (□), normal (Lewis × DA)F serum; (△), Lewis cells alone; (○), Con A; (●), PHA-p; (■), LPS; (▲), anti-(Lewis anti-BN) serum. Dotted lines indicate stimulation with mitogens (Con A, PHA-p, LPS).

this assay. The two positive anti-idiotypic sera both induced a proliferation in the respective cell populations which was significantly retarded, compared to the MLC induced by the corresponding allogeneic stimulator cells. This proliferation induced by the anti-idiotypic sera was taking place in the same manner irrespective of whether or not B cells were present (data not included). Mitogenic stimulation was positive as expected with all mitogens tested.

These data thus suggested that not only primed but also normal, idiotypic T cells can be triggered into proliferation via contact with the proper anti-idiotypic reagent.

Induction of Specific, Cytolytic T Cells in Normal Spleen Cell Populations in Vitro Using Anti-idiotypic Antibodies. The fact that strong proliferative responses could be induced by anti-idiotypic antisera when cultured with normal spleen cells did not prove that the actual proliferation is caused by

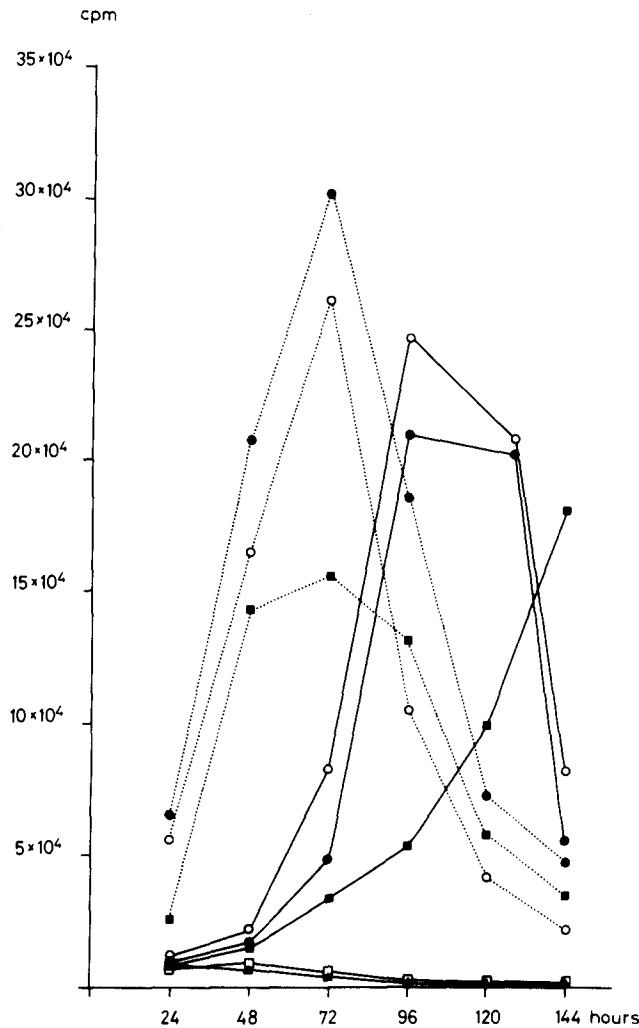


FIG. 5. Stimulation of C57BL/6 cells with: (●), CBA cells; (■), anti-(C57BL/6 anti-CBA) serum; (○), DBA/2 cells; (□), Normal C57BL/6 serum; (△), C57BL/6 cells alone; (○), Con A; (●), PHA-p; (■), LPS. Dotted lines indicate stimulation with mitogens (Con A, PHA-p, LPS).

idiotype-positive T cells present among these cells. To confirm this point, we searched for a specific change in function of such proliferating cells as a consequence of contact with the mitogenic anti-idiotypic antibodies. One possible consequence of the contact could be the induction of cytolytic T cells. We tested for presence of specific cytolytic T cells in spleen cell populations after induction into increased proliferation by anti-idiotypic antibodies. Accordingly, normal C57BL/6 spleen cells were cultured in the presence of normal C57BL/6 serum or with auto-anti-C57BL/6-anti-CBA serum. Additional controls included CBA spleen cells in the presence of the same sera. The cultures were harvested on day 7 and the surviving cells were assayed in a ⁵¹Cr release assay for presence of cytolytic cells with specificity for CBA, DBA/2, or C57BL/6 target

TABLE I
Demonstration of Cytolytic T Lymphocytes Induced by Anti-Idiotypic Antiserum

Exp.	Spleen cells derived from:	Cultured for 7 days in presence of serum:	Resulting cytotoxic T lymphocytes incubated with targets:		
			H-2 ^k	H-2 ^b	H-2 ^d
1	C57BL/6	Normal C57BL/6	0.9	% lysis 1.4	-0.5
	C57BL/6	C57BL/6 anti-(C57BL/6 anti-CBA)	71.3	-1.1	4.5
	CBA	Normal C57BL/6	0.5	1.4	-1.3
	CBA	C57BL/6 anti-(C57BL/6 anti-CBA)	1.2	1.0	0.1
	C57BL/6*	C57BL/6 anti-(C57BL/6 anti-CBA)	3.4	1.2	1.0
	2	C57BL/6	C57BL/6 anti-(C57BL/6 anti-CBA)	65.3	1.7
	C57BL/6	Ig of anti-(C57BL/6 anti-CBA)	51.9	0.9	2.9

For culture conditions see Results. CML was performed as described under Materials and Methods.

*Cytolytic T lymphocytes were incubated with anti-Thy-1.2 and complement before initiation of CML.

cells. The data in Table I show that a significant cytolytic anti-CBA effect was indeed induced in C57BL/6 spleen cells cultured with the anti-(C57BL/6 anti-CBA) idiotypic serum. Additional experiments in Table I demonstrate that the actual effector cell is a T cell (activity wiped out with anti-Thy-1.2 serum plus complement). Furthermore, since the immunoglobulin fraction of the anti-idiotypic antiserum was as effective as whole serum in inducing the cytolytic killer T cells, it gave further support to the idea that anti-idiotypic antibodies are the sole inducing factor in the system. We would conclude here that the proliferation induced in normal spleen cells by contact with anti-idiotypic antibodies as shown previously at least partially the result of specific induction of idiotypic-positive killer T cells with the expected selective lytic ability.

Induction of High Titers of Alloantibodies in Normal Rats Using Pure, Anti-Idiotypic Antibodies as Immunogen. Anti-idiotypic antibodies administered in vivo have been found to lead to either increased or reduced reactivity towards the antigen against which the idiotypic-positive lymphocytes can be shown to react (3, 9). The experiments presented so far in this article have been dealing only with induction of T-cell functions in vitro by anti-idiotypic antibodies. To investigate whether or not B cells will also be induced by the same anti-idiotypic procedure, we have included some in vivo experiments. Anti-idiotypic antibodies of specificity anti-(Lewis anti-DA) were purified using immunosorbant procedures with Sepharose-linked Lewis anti-DA antibodies (20), and they were subsequently introduced in Freund's complete adjuvant into normal Lewis rats. The animals were bled on days 14 and 30, and tested for presence of serum antibodies of anti-DA activity using the protein A assay (20). As seen in Table II, highly titered, extremely specific Lewis anti-DA

TABLE II
*Induction of Lewis Anti-DA Alloantibodies in Lewis Rats Immunized with Purified Anti-
 (Lewis Anti-DA) Anti-Idiotypic Antibodies*

Lewis rats immunized with:	Rat	Specific binding of ¹²⁵ I-labeled protein-A to											
		Lewis spleen cells						DA spleen cells					
		4*	8	16	32	64	128	4	8	16	32	64	128
(Lewis × DA) F ₁ spleen cells	1	-865	-413	-843	-1,145	-897	-1,309	21,423	19,070	17,675	13,181	7,346	5,634
	2	-298	-321	-1,294	-1,105	-607	-582	16,276	13,153	13,462	12,438	9,031	3,365
Purified anti-(Lewis anti-DA) anti-idiotypic antibodies	1	-1,506	-642	-317	-278	-713	142	7,602	6,021	4,217	4,962	3,949	3,745
	2	-267	766	-1,128	109	-349	-570	17,853	16,498	10,388	5,652	5,424	3,837
	3	-468	-909	-1,053	-400	-211	-1,143	16,410	13,555	11,366	7,893	4,364	3,679

7×10^6 Lewis or DA spleen cells were incubated with sera dilutions from bleedings on day 30 from Lewis animals as indicated above. ¹²⁵I-labeled protein-A was used as a marker (21). Figures denote mean of duplicate values after subtraction of the background obtained with two different normal Lewis sera.

*Numbers designated are reciprocal serum dilutions.

alloantibodies could be produced in this manner (for comparison see titers induced by conventional immunization procedures). Data shown are for bleedings on day 30. Thus, anti-idiotypic antibodies can also be used instead of the proper immunogen to cause the induction of antibody synthesis from the relevant, idiotypic B cells. It should be realized, however, that the actual underlying mechanisms of the present findings concerning possible requirements of T cells are poorly understood as well.

Discussion

For a substance to be recognized as an immunogen, certain minimal requirements must be fulfilled. The responding lymphocyte population must contain cells endowed with antigen-specific receptors capable of interacting with relevant sites of the molecule to allow a necessary minimum force of association. Furthermore, the immunogen would seem to require linkage with a mitogenic principle, either as an intrinsic property of the molecule itself (22) or by association with other cellular constituents, to become mitogenic in a selective manner (23, 24). The present results showing that anti-idiotypic antibodies with specificity for idiotypic, alloantigen-specific receptors can induce immunity against such alloantigens in their physical absence must be interpreted within this concept of immunogenicity.

Idiotypes and anti-idiotypes can coexist at both T- and B-cell levels in normal individuals as indicated by the fact that auto-anti-idiotypic immunity can be induced in virtually any system in which it has been observed (2, 3, 11). The present system of anti-major histocompatibility antigen reactions, in which the idiotypic T cells can be shown to constitute several percent of the normal T-cell pool, is no exception to this rule (25-28).

In this regard, the present findings of the induction of specific immune T-cell functions in vitro by anti-idiotypic antibodies supplement nicely earlier experiments using hetero-anti-idiotypic antibodies in vivo to induce specific helper or suppressor T cells (29). In these latter experiments, IgG₁ anti-idiotypic antibodies were found to prime helper T lymphocytes whereas IgG₂ anti-idiotypic

antibodies caused selective suppressor T-cell induction. These findings suggest an important role of the Fc regions of anti-idiotypic antibodies in the actual outcome of the combination with idiotypic surface receptors. Complex intercellular reactions are known to occur between various subsets of lymphoid cells during conventional immune induction (30). It is quite plausible that a particular cell type involved in such collaboration may express selective binding ability for immune complexes according to the Fc characteristics of the Ig molecules present in the complex. Such hypothetical Fc-linked cellular distribution patterns may then in a secondary manner lead to selective mitogenic properties of a given immunogenic molecule for antigen-specific lymphocytes of a particular subset. In the present system, we have made no serious efforts to analyze the immunoglobulin classes of the anti-idiotypic antibodies used as inducing agents, nor have we as yet made any detailed analysis regarding quantitative aspects. It should be noted, however, that in the present experiments, only two out of three anti-idiotypic antisera analyzed for triggering of normal T cells functioned. This is in agreement with our own negative data from earlier experiments when trying to block MLC by anti-idiotypic antibodies (2, 10, 20) and it would merely suggest additional complexity of the system. The present data showing an anti-idiotypic antiserum that failed to induce triggering of normal T cells, but which would do so with primed cells, are in line with the fact that primed T cells have triggering requirements different from those of normal T lymphocytes (29).

Several sets of observations, some of which have been published in other contexts previously (4, 20), suggest that the inducing factors in the present system are indeed the anti-idiotypic antibodies. In the present experimental system, two experiments are particularly elucidating with regard to this point. Auto-anti-idiotypic sera could be shown to function as *in vitro* inducers of efficient, specific killer T cells using normal spleen cells as starting cell suspension. Furthermore, auto-anti-idiotypic sera induced via immunization with heavy chains from IgG molecules of relevant specificity can also function as specific inducers of cellular proliferation *in vitro*. In both of these systems, any likelihood of contaminating alloantigenic fragments in the antisera used would seem remote, and the only factors carrying required specificity in such antisera should be anti-idiotypic antibody molecules. It is unknown whether this triggering is taking place via a direct triggering event following anti-idiotypic-idiotype surface reactions, or in a more complex manner via intercellular reactions. It should be recalled, however, that triggering of B lymphocytes into anti-immunoglobulin synthesis and proliferation subsequent to contact with anti-allotype sera is a well-known fact in the rabbit system (31).

Several mitogens were also tested for their ability to restimulate MLC-primed T lymphocytes. Out of the three mitogens tested (PHA, Con A, LPS), only Con A could be shown to cause such a restimulation, suggesting that in using the present systems of MLC activation, only a subset of Con A-reactive cells will multiply. Alternatively, primed T cells may express changed mitogenic reactivity. In a reciprocal system using *in vivo* alloimmunized donors, only Con A was able to induce significant triggering of alloimmune T cells (32). Although we have no further views as to how this selective Con A reactivity

comes about after MLC priming, the present findings may serve as useful information for people working in MLC systems where secondary stimulator cells are difficult to obtain.

In conclusion, the present article describes experimental conditions under which auto-anti-idiotypic antibodies may induce the proliferation and display of immune potential of the relevant, idiotype-positive T and B lymphocytes. As the present idiotypic receptors have specificity for the major histocompatibility complex-determined antigens of the species, the resulting triggering could be determined as induction of cytolytic killer T cells against H-2 antigens in vitro or the in vivo immunization leading to high titered anti-Ag-B alloantibodies in rats. The use of anti-idiotypic antibodies in this context also represents strong proof for the actual presence of idiotypic receptors on the outer surface of cytolytic T cells, an issue which has only been analyzed in part before (2, 12, 33). Another conclusion to be drawn from the present results is that idiotypic determinants typical of heavy-chain Ig variable gene products could be demonstrated on the T-cell receptors for antigen. Thus, the present data have shown that anti-idiotypic antibodies can be used as highly selective positive probes in the analysis of T-cell receptors and their role during immune triggering in vitro.

Summary

B and T lymphocytes with reactivity against major histocompatibility antigens are known to express this immune potential via a display on the outer surface of antigen-specific, idiotypic receptors. Here, we show that anti-idiotypic antibodies directed against such receptors may serve as specific triggering agents of the idiotype-positive lymphocytes in the physical absence of foreign histocompatibility antigens. This was shown in vitro using normal or immune spleen T cells where anti-idiotypic antibodies would lead to the selective proliferation and development of antigen-specific cytolytic T cells as determined by short-time ⁵¹Cr release assays. Furthermore, purified anti-idiotypic antibodies in adjuvant administered in vivo to normal syngeneic animals could be shown to lead to production of high titers of specific alloantibodies. The present experiments were in most cases carried out using auto-anti-idiotypic antibodies as triggering agents. The present results thus lend further support to the concept that idiotype-anti-idiotype reactions may be normal parts of conventional immune processes with either stimulatory or inhibitory consequences, depending upon the prevailing conditions.

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