INDUCTION OF SPECIFIC IMMUNE UNRESPONSIVENESS USING PURIFIED MIXED LEUKOCYTE CULTURE-ACTIVATED T LYMPHOBLASTS AS AUTOIMMUNOGEN

I. Demonstration of General Validity as to Species and Histocompatibility Barriers*

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Ability to induce specific unresponsiveness against selected antigens in adult, immunocompetent individuals is a long desired goal in transplantation immunology. In the clinic it is based on the realization that heterogeneity of man with regard to major histocompatibility antigens is so great that the likelihood of obtaining completely matched grafts between nonsiblings is very small. Thus, successful grafting of organs today between human beings does normally involve various degrees of "acceptable" misfit as to histocompatibility matching. Treatment of the recipient with immunosuppressive drugs is therefore necessary. Such drug therapy is largely nonselective and is well known to have a variety of detrimental side effects as to the health of the recipient. In the present article we will describe an approach that would seem to carry great hope as to allow specific induction of immune tolerance in adult individuals towards the major histocompatibility antigens of the species.

The reasoning behind this approach goes as follows: The genes coding for the antigen-binding receptors of B and/or T lymphocytes allow the construction of antigen-binding areas reactive with foreign as well as "self"-structures. Reactivity against self is frequently controlled and prevented at several different levels, but can be circumvented in many instances. Among the self-structures against which autoimmunity can be induced are the actual antigen-binding receptor structures themselves (1). Thus, it has been possible to induce auto-anti-idiotypic immunity against receptors of either B- (2) or T-cell (3, 4) type. Such anti-receptor immunity may have varying effects depending upon the idiotypic system analyzed. In one such system it could be shown that if the receptors against which autoimmunity was induced carried specificity for a given set of histocompatibility antigens the outcome would be specific transplantation tolerance (3, 5). In these earlier experiments there was a requirement for availability of anti-idiotypic reagents to purify the soluble, idiotypic receptor material subsequently to be used as autoimmunogen (3, 5). Such antisera would be close to impossible to obtain in the hypothetical clinical situation, as they would have to be made against the recipient's own receptors with

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specificity for the potential donor's HLA antigens. Realizing this, we have attempted another, similar approach with more general applicability if successful. This system concentrates on the actual physical isolation of the idiotype-positive cells in a pure form, to subsequently be used as autoimmunogen to induce anti-idiotypic immunity. Normal T cells as well as T lymphoblasts specifically activated in mixed leukocyte culture (MLC)[1] express idiotypic receptors signifying reactivity against the relevant histocompatibility antigen. They are normally found in a few percent in the normal population (6–10) but can be greatly expanded via proliferation in MLC (11). During expansion the small lymphocytes will enlarge to lymphoblasts and such cells can now be recovered in purified form via 1 *g* velocity sedimentation procedures (12). It turns out that such specific, purified T lymphoblasts in adjuvant will constitute a powerful autoimmunogen leading to specific unresponsiveness as well as production of auto-anti-idiotypic antibodies (present article and reference 12).

The present article will describe the general validity of this autoblast immunization approach as to species and histocompatibility barriers and will discuss the theoretical and practical implications.

Materials and Methods

Animals

MICE. Mice of the inbred strains CBA/J, CBA/H, C3H/HeJ, A/J, DBA/2J, BALB/cJ, and C57BL/6J were purchased from The Jackson Laboratory, Bar Harbor, Maine, or bred in part in our own colony. Mice of uniform sex at the age of 5–8 wk were used for the experiments.

RATS. Rats of the inbred strains Lewis (Ag-B^1), DA (Ag-B^4), BN (Ag-B^3), and August (Ag-B^5) were bred and maintained in our own colony. Rats at the age of 6–10 wk of either sex were used in the experiments.

GUINEA PIGS. Outbred guinea pigs were purchased from a local breeder.

Cell Preparations. Spleen and lymph node cells were aseptically removed and single cell suspensions were prepared with a stainless steel mesh using Dulbecco's modified phosphate-buffered saline (D-PBS) as a medium. Cells were washed once and erythrocytes were lysed thereafter by hypotonic shock using nine parts of distilled water and one part of 10 times D-PBS. Cells were washed again and resuspended in the culture medium.

Peripheral blood lymphocytes were purified on a Ficoll-Paque (Pharmacia Fine Chemicals Ltd., Uppsala, Sweden). Isolated lymphocytes were washed three times in culture medium before tissue culture or tests. T lymphocytes as responder cells in MLCs were produced using anti-Ig bead columns (13).

Culture Medium. The culture medium used for MLC and cell-mediated lympholysis (CML) both for rats and mice was EHAA medium (14), complemented with 0.5% fresh normal mouse serum for mice and 0.5–1% of fresh BN rat serum for rats. Guinea pig lymphocytes were cultured in RPMI 1640 medium complemented with 5% fetal calf serum (FCS).

MLC. MLCs for mice, rats, and guinea pigs were performed in flat-bottom microtiter plates (Cook M220-29ART), using 0.25×10^6 responder lymphocytes and 0.5×10^6 2,000 R irradiated stimulator cells. For the culture medium see above. Cultures were pulsed for 6 h with 1 μCi of tritiated thymidine (^{3}H-TdR) (sp act 40–60 Ci/mmol; The Radiochemical Centre, Amersham, UK) before the harvesting as indicated in tables and figures. Cultures were harvested using a Skatron collector (Skatron, AS, 3401 Lierbyen, Norway) and counted in a liquid scintillation counter.

For the preparation of large quantities of specific lymphoblasts in MLC cells were cultured in flasks (Falcon 3013 Tissue Culture Flasks; Falcon Plastics, Div. of Becton-Dickinson & Co., Ltd., Drogheda, Ireland) using the same medium as described above. 15 ml of cell suspensions containing 1.25×10^6 responder cells and 2.5×10^6 2,000 R irradiated stimulator cells/ml were added to

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[1] *Abbreviations used in this paper*: CML, cell-mediated lympholysis; D-PBS, Dulbecco's modified phosphate-buffered saline; FCS, fetal calf serum; ^{3}H-TdR, tritiated thymidine; MLC, mixed leukocyte culture.

each flask. Cultures were harvested on day 5 after initiation of the MLC for mice and guinea pigs and on day 6 for rats.

CML. CML was performed in V-bottom microtiter plates in 200 μl of EHAA medium supplemented with 5% heat-inactivated FCS. Assays were carried out in triplicates or quadruplicates. Each well contained 1×10^6 or 5×10^5 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 figures were determined by adding 100 μl 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: For *H-2*^{b}, El-4; for *H-2*^{d}, P-815.

Purification of T Lymphoblasts from MLC. MLC 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 (15) using D-PBS as diluent. The gradient was harvested after 4–5 h at 4°C in 15-ml tubes and the blast fractions (determined under the microscope) pooled and washed four times in 50-ml Falcon tubes. The fractions containing small, MLC nonreactive lymphocytes were handled in exactly the same way.

Immunization of Animals with Purified T Lymphoblasts. As routine procedure each animal received 1×10^7 (mouse) or 2×10^7 (rat) purified T lymphoblasts in 0.2 ml of D-PBS and Freund's adjuvant intraperitoneally. For the first injection complete Freund's adjuvant was used and incomplete Freund's adjuvant for the boosters. Animals received one, two, three, or four injections in 3-wk intervals and were bled 10–14 days after the last injection. One guinea pig was injected four times in 3-wk intervals with 1.5×10^6 purified T lymphoblasts in Freund's adjuvant.

Results

Autoimmunization against Autologous Lymphoblasts Cause Specific Unresponsiveness against the Relevant Alloantigens. A pilot experiment to test for the validity of the principle was first carried out. Adult, normal CBA/J mice were thus immunized with purified specific syngeneic T lymphoblasts created in MLC against C57BL/6 stimulator cells. Each mouse received 10^7 blasts per immunization event. The animals were immunized thrice, the first time with the blasts in Freund's complete adjuvant, followed by blasts in Freund's incomplete adjuvant in 3-wk intervals. Results of the two mice depicted in Fig. 1 show the procedure to be a successful one with close to complete specific reduction in MLC against the relevant stimulator cells. Third-party reactivity was hardly touched. Thus, autoimmunization with specific MLC blasts can be used to achieve specific unresponsiveness against major histocompatibility antigens in adult mice, thus confirming preliminary, earlier data (12).

Definition of Histocompatibility Barriers and Species where Autoblast Immunization Will Lead to Specific Unresponsiveness

ACROSS MAJOR HISTOCOMPATIBILITY, *H-2*, BARRIERS IN THE MOUSE. In order to establish the conditions during which the present autoblast conditions can be shown to function with regard to species as well as across different histocompatibility barriers within a species several sets of experiments were carried out. Here, we first extended the above analysis to include a variety of mouse strain combinations all known to encompass the major locus, *H-2*, as a barrier. Figs. 2–4 depict additional combinations showing autoblasts to function as specific suppressive agents using the schedule described above. Table I adds more results on this point. All strain combinations tested were found to be subjected to

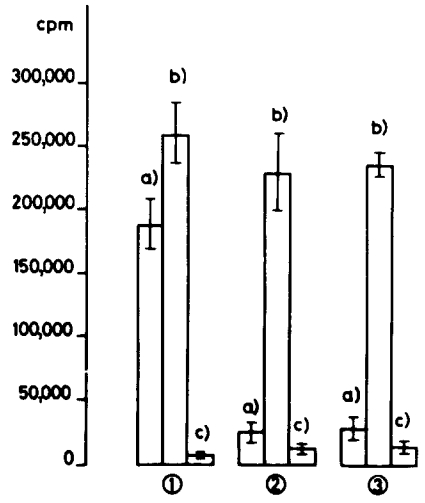


FIG. 1. Specific unresponsiveness of autoimmunized CBA/J mice against C57BL/6 alloantigens as measured in MLC. MLC was performed in flat-bottom microtiter plates as described under Material and Methods. Mice were injected three times in 3-wk intervals with purified CBA/J anti-C57BL/6 MLC T lymphoblasts (see Material and Methods) and tested 10 days after the last injection. Columns represent mean ± SE of quadruplicate cultures of purified T lymphocytes (T cells were purified on Ig-anti-Ig columns). (1) Normal CBA/J mouse, and (2) and (3) autoimmunized CBA/J mice. (*a*) Response against C57BL/6 stimulator cells, (*b*) response against DBA/2J responder cells, and (*c*) responder cells alone. Cultures were pulsed for 6 h with 1 μCi of ^{3}H-TdR and harvested 96 h after initiation of the MLC.

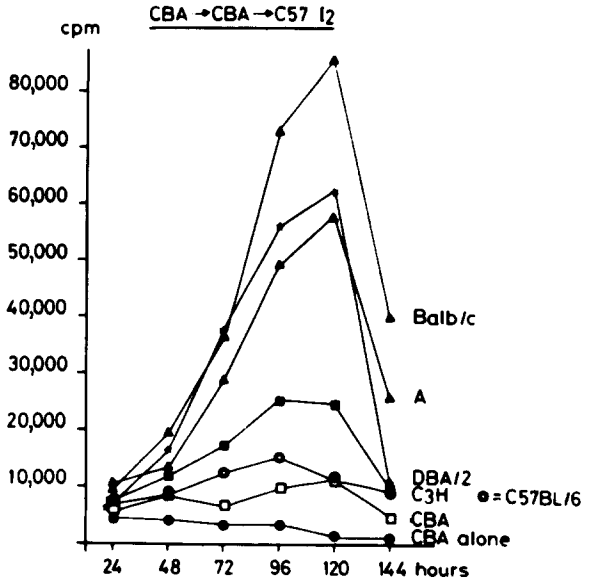


FIG. 2. Induction of specific suppression of proliferative response by autoimmunization. CBA/J mice were autoimmunized as described for Fig. 1 and tested 10 days after the last injection. Each point represents mean of triplicate cultures which were pulsed for 6 h with 1 μCi of ^{3}H-TdR. Two additional animals treated in the very same way gave similar results.

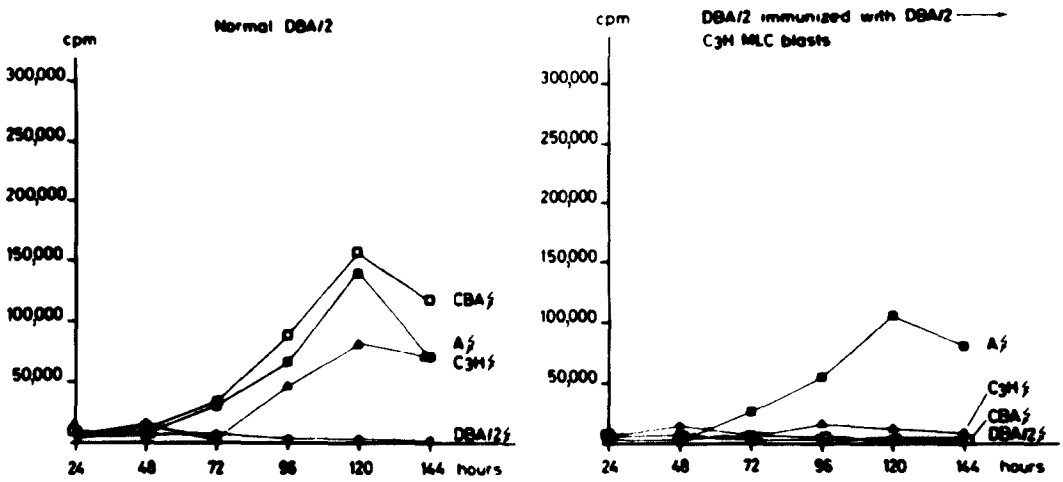
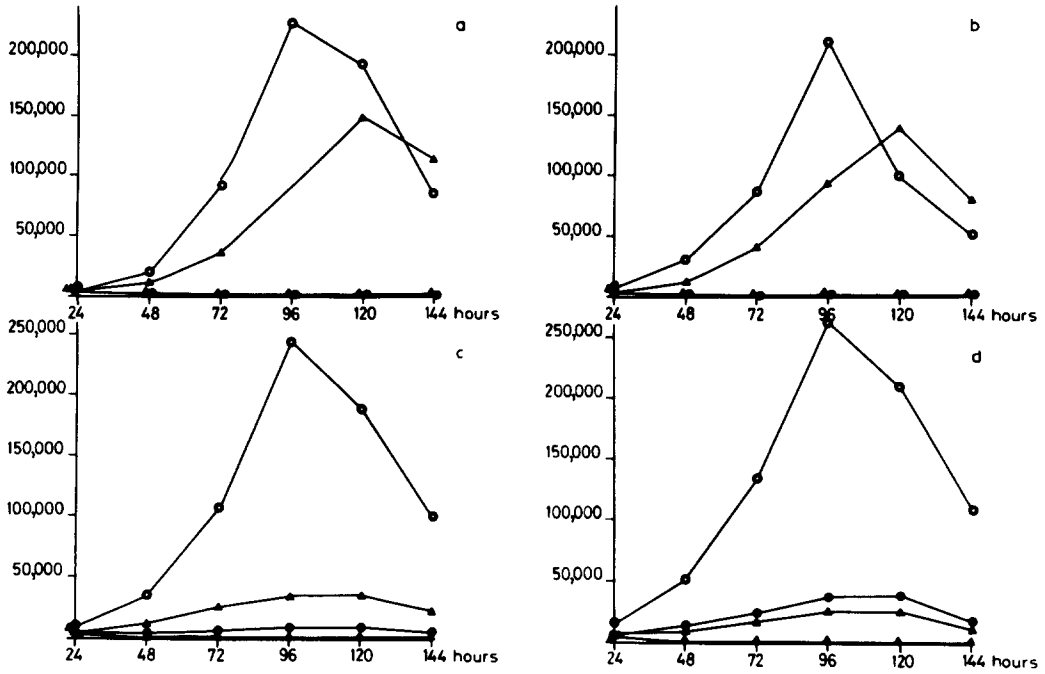


FIG. 3. DBA/2 mice were injected four times in 3-wk intervals with purified DBA/2 anti-C3H MLC T lymphoblasts and their spleen cells were tested in MLC 10 days after the last injection in comparison to normal DBA/2 mice. MLC was performed in microtiter plates as described under Material and Methods.

the specific unresponsiveness caused by the autoblast immunization, leaving third-party reactivity unperturbed. Autoblast immunization procedures would thus seem to function across probably any *H-2* barriers. It has to be noticed that lymphocytes from autoimmunized mice respond significantly stronger than normal lymphocytes to normal syngeneic stimulator cells. We don't know the exact reason behind this phenomenon. Preliminary experiments indicate that both purified B as well as purified T lymphocytes serving as stimulator cells can induce the proliferation.

ACROSS *M* LOCUS BARRIERS IN THE MOUSE. The *M* locus in the mouse represents a genetic system, distinct from the *H-2* system (16). *M* locus-determined structures can function as specific triggering antigens for a high proportion of normal, *M* locus-incompatible T cells in MLC in vitro and can thus be tested in the present system. In Figs. 5 and 6 are shown the typical results obtained using MLC blasts from reciprocal strains identical as to *H-2* but different as to *M* locus antigens. As seen, the pattern observed seemed identical to those found when using anti-*H-2* autoblasts as immunogen. Thus, specific unresponsiveness with regard to the relevant *M* locus incompatibility was induced by blast immunization with no evidence of nonspecific reduction as to third-party antigens. Autoblast immunization can thus be shown to function across *M* locus differences in the mouse.

ACROSS THE MAJOR HISTOCOMPATIBILITY, Ag-B, BARRIER IN THE RAT. Specific unresponsiveness against Ag-B-incompatible stimulator cells [including multiple, non-Ag-B barriers as well (17)] could be induced in Ag-B-incompatible strain combinations in the rat using the autoblast immunization protocol. Results of such tests are shown in Table II, depicting close to complete elimination of reactivity in several individuals with again no detectable impact as to third-party reactivity. Autoblast immunization can thus be shown to function in the rat across major histocompatibility barriers.



Legends:
a) normal A
b) A immunized with A anti-Balb/c MLC lymphocytes
c) A immunized with A anti-Balb/c
d) MLC blasts

against A
against CBA
against Balb/c
alone

FIG. 4. Mice of the inbred strain A were immunized four times in 3-wk intervals with either purified A anti-BALB/c MLC T lymphoblasts or purified A anti-BALB/c MLC small, nonresponder lymphocytes. Their spleens were tested 10 days after the last injection against irradiated stimulator cells as indicated. For MLC see Material and Methods.

TABLE I

Specific Induction of Unresponsiveness in CBA/H Mice Immunized with CBA/H-Anti-DBA/2 T Lymphoblasts

Autoimmunized donor*	DBA/2 stimulator cells‡	A.BY stimulator cells‡
	%	%
1	87	−3
2	48	11
3	67	18
4	30	−21
5	76	6
6	85	9
Mean percent inhibition	65	3

* Spleen cells from individual CBA/H autoblast-immunized mice analyzed in MLC. Cells obtained after three injections of 10^7 lymphoblasts 3 wk apart. MLC harvested at day 4.

‡ Results presented as percent inhibition as compared to control values = 0% inhibition = values obtained using spleen cells from normal CBA/H mice.

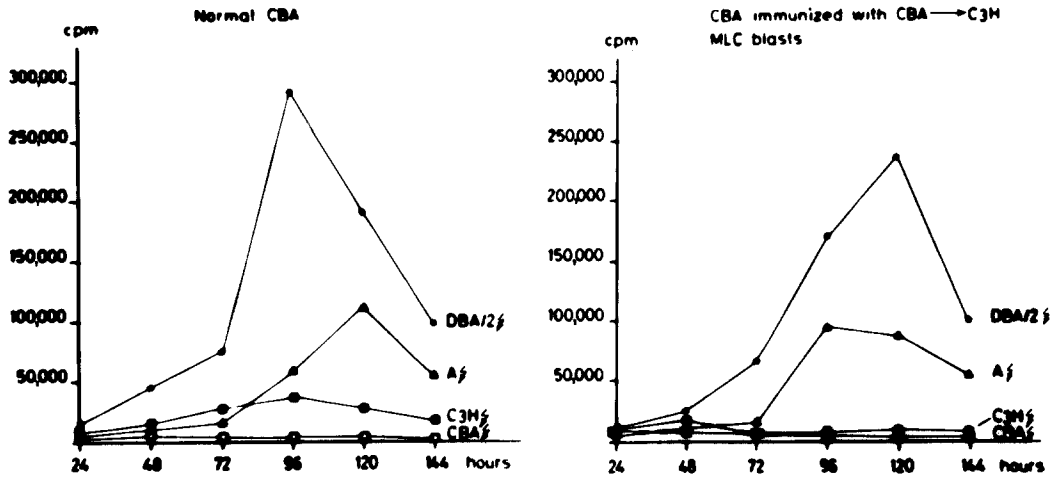


FIG. 5. CBA/J mice were immunized four times in 3-wk intervals with purified CBA/J anti-C3H MLC T lymphoblasts and their spleen cells were tested in MLC 10 days after the last injection. Conditions as described in legend to Fig. 3.

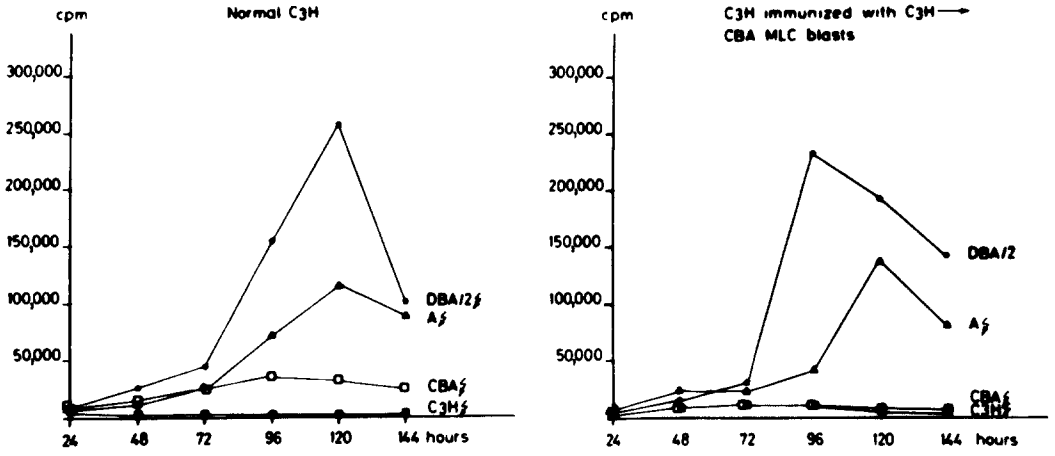


FIG. 6. C3H mice were injected four times with purified C3H anti-CBA MLC T lymphoblasts. Conditions otherwise as in legend to Fig. 5.

ACROSS "MLC BARRIERS" IN OUTBRED GUINEA PIGS. In this test we used outbred guinea pigs and are thus ignorant as to the exact histocompatibility differences involved. The guinea pigs were selected, however, to differ from each other in a three-way manner as measured by primary MLC tests. Accordingly, we assume that these tested guinea pigs have distinct differences at their major histocompatibility locus. Three outbred guinea pigs were thus tested in MLC before choosing one as the "prospective recipient" and another as stimulator of autoblasts. Blasts for autoimmunization were obtained as depicted in Material and Methods, and the immunization protocol was carried out as shown in Table III. The results were very clear-cut. A complete elimination of MLC reactivity against the relevant guinea pig was induced by the autochthonous blasts with

TABLE II

Specific Unresponsiveness Across AgB-Locus Barrier in Lewis Rats Immunized with Purified Lewis-Anti-DA T Lymphoblasts

No. of rats blast immunized*	Stimulator cells‡					
	(Lewis × BN)F_1		(Lewis × DA)F_1		0	
	Day 4	Day 6	Day 4	Day 6	Day 4	Day 6
	%	%	%	%	%	%
1	100	86	15	8	136	129
2	93	79	10	9	90	103
3	98	126	15	10	198	110
4	125	104	11	16	81	90
5	108	129	12	7	222	95
6	91	111	8	11	109	118
Mean response of normal Lewis spleens (*cpm* ± *SE*)	7,785 ± 146	14,309 ± 817	12,660 ± 1,066	25,448 ± 1,709	389 ± 19	303 ± 37

* Spleen cells from individual Lewis rats immunized with Lewis-anti-DA blasts three times.

‡ Stimulation expressed as percent reaction compared to controls.

TABLE III

Suppressed Proliferative Response of Guinea Pig Immunized with Specific T Lymphoblasts

Responder cells from guinea pig no. 1, before/after autoimmunization	^{3}H-TdR incorporation of mixture with guinea pig stimulator cell no. 2, mean cpm of triplicates ± SE	^{3}H-TdR incorporation of mixture with guinea pig stimulator cell no. 3, mean cpm of triplicates ± SE	^{3}H-TdR incorporation of responder cells alone, mean cpm of triplicates ± SE
Before	32,351 ± 2,057	29,046 ± 3,882	1,641 ± 124
After	2,338 ± 205	24,485 ± 1,100	2,688 ± 183

Outbred guinea-pig no. 1 was immunized four times with 1 anti-2 MLC lymphoblasts (see Material and Methods). Cultures were pulsed for 6 h with 1 μCi of ^{3}H-TdR 110 h after initiation of the MLC.

no effect as to MLC reactivity against the third guinea pig. We can thus conclude that the autoblast immunization protocol does also lead to specific unresponsiveness in yet another species, the guinea pig. The results do also demonstrate that it is practically possible to obtain enough lymphocytes from a donor to use in MLC activation and subsequently serve as efficient autoimmunogen in the form of purified lymphoblasts.

Analysis of MLC Factors that Can Be Used to Obtain Specific Unresponsiveness: Purified T Lymphoblasts are the Best Immunogens. Based on earlier experiments and recent studies we consider auto-anti-idiotypic immunity to be the most likely explanation as to what is causing the specific unresponsiveness induced via autoblast immunization (12). However, in the present protocol we assume that alloantigen as well as idiotypic material may play a role. Idiotypic receptor material in a soluble form can be found in the MLC supernates (12). Alloantigen can be detected both in a soluble form and frequently also on the surface of activated MLC blasts (18). To ensure that our principle reasoning as to the necessity of purified T lymphoblasts would be true we thus investigated whether concentrated MLC supernates or small nonresponding lymphocytes in MLC can be used as autoimmunogen instead of the purified lymphoblasts. A

summary of our results obtained using such approaches is shown in Table IV. As seen, the only positive outcome as to achieve specific unresponsiveness was obtained using the purified lymphoblasts.

In the same table are depicted some approaches of using pure lymphoblasts in absence of any adjuvant or attempting several forms of adjuvant treatment. As seen, only the scheme using Freund's adjuvant plus blasts was found to be efficient in inducing unresponsiveness. We have thus decided to follow the scheme outlined in the very first paragraph in the Results sections until other protocols have been shown to be functioning (see also Discussion).

Kinetics of Induction of Unresponsiveness: Influence of Dose of Blasts Administered and Time after Immunization. The number of blasts required as well as time after immunization necessary for induction of unresponsiveness to become significant are questions of practical importance in the present system. An extensive protocol would be required to study these problems in detail. Such a detailed analysis has, due to lack of sufficient mice, not yet been carried out, but several preliminary results of interest have been obtained. We would thus conclude from our gathered experience so far that already at 14 days (the earliest time after blast immunization we have tested in some individuals) there may exist partial although significant immune suppression. We have not yet attempted to speed up this reaction by repeated administration of autoblasts within a short time period. As to dose of blasts required we have failed to induce specific immune responsiveness in the mouse across *H-2* barriers in experiments using 10^6 blasts per dose whereas a 10-fold higher dose of the same blast cells has resulted in close to complete suppression. We know that repeated doses of blasts undoubtedly result in a more efficient depletion of immune specific reactivity compared to a single shot of immunization. However, a single immunization may if time is allowed to pass result in a significant reduction. In Table V are some results indicated as to the number of blast immunizations used in relation to immune suppression achieved. Finally, reduced reactivity once established in an individual has so far never been found to revert, maybe indicating a self-recruiting, suppressive system (see also Discussion).

Parallel Reduction of MLC Reactivity and Cytolytic Killer Cell Activity after Autoblast Immunization. MLC reactivity as well as the dominating cellular cytolytic activity against *H-2*-incompatible cells are considered to be caused by immunocompetent T lymphocytes. T lymphocytes reactive in MLCs are considered to represent another subpopulation of immunocompetent T cells than those acting as cytolytic killer cells (19). In the mouse there is also good evidence to indicate that the major, *H-2*-associated target structures for the two groups of cells are different: the MLC-reactive cells predominantly reacting against Ia-associated antigens while the cytolytic T cells prime targets would be the *H-2K* or *D* region structures (20, 21). As the present autoblast immunization protocol may or may not include, both subgroups of lymphocytes as efficient, immunogenic blasts we wanted to analyze the impact on cytolytic T cells after autoblast immunization procedures. Blast immunizations using mouse or rat strain combinations differing with regard to MHC antigens were used. MLC and CML were carried out at different time intervals using responder cells from normal or blast-immunized animals. In four different combinations tested (CBA/H-anti-

TABLE IV

Purified Lymphoblasts from MLC in Freund's Adjuvant are the only Inducers of Specific Unresponsiveness

Immunogen	Strain combination	No. of injections	Adjuvant	No. suppressed/total
MLC supernate 200 × concentrated	CBA/H anti-C57BL/6	2	None	0/4
"	CBA/H anti-C57BL/6	2	FCA	0/4
"	CBA/H anti-DBA/2	2	None	0/4
"	CBA/H anti-DBA/2	2	FCA	0/3
10^7 Small MLC lymphocytes	CBA/H anti-DBA/2	2	FCA	0/8
10^7 Unseparated MLC cells	"	2	FCA	0/4
10^7 Separated MLC blasts	"	2	FCA	10/12
"	"	2	None	0/4
"	"	2	FICA	1/3
"	C57BL/6 anti-CBA	3	*Bordetella pertussis*	0/4
"	C57BL/6 anti-CBA	3	None	0/4
2×10^7 Separated MLC blasts	Lewis anti-DA	3	CFA	4/4
2×10^7 Unseparated MLC cells	Lewis anti-DA	3	CFA	2/4
2×10^7 Separated MLC blasts	Lewis anti-BN	3	CFA	3/3

MLCs were performed as described under Material and Methods. Animals were tested 10–14 days after the last injection.

TABLE V

Specific Suppression of MLC in Relation to the Dose of Immunogen and Immunization Frequency

Strain combination	Dose of MLC blasts	No. of injections	Tested after last injection	Reactivity compared to normal animals (mean of two mice)		
				Against CBA/J	Against C57BL/6	Against DBA/2
			days	%	%	%
CBA/J anti-C57BL/6	1×10^6	2	14		107.0	101.0
	5×10^6	2	14		95.8	92.2
	1×10^7	2	14		19.8	87.8
	1.5×10^7	2	14		16.1	87.7
C57BL/6 anti-CBA/J	1×10^7	1	14	27.3		92.1
	1×10^7	2	14	12.9		78.1
	1×10^7	3	14	8.9		74.1
	1×10^7	4	14	6.8		73.5
C57BL/6 anti-CBA/J	1×10^7	3	90	14.2		71.3
	1×10^7	3	300	15.3		87.4

Mice were injected with purified MLC T lymphoblasts in 3-wk intervals with the dose indicated. Blasts were administered in Freund's adjuvant (see Material and Methods). MLC was performed in microtiter plates as described under Material and Methods. Cultures were pulsed for 6 h with 1 μCi of ^{3}H-TdR 90 h after initiation of the MLC.

TABLE VI
A Relative Comparison between Suppression of MLC vs. CML Activity in Autoblast-Immunized CBA/H Mice

Autoblast immunization*	MLC suppression‡		CML suppression‡	
	Anti-H-2^d	Anti-H-2^b	Anti-H-2^d	Anti-H-2^b
	%	%	%	%
Anti-H-2^d	71	21	63	0
Anti-H-2^d	93	7	100	14
Anti-H-2^d	83	26	58	47
Anti-H-2^b	0	89	11	72

* Individual CBA/H mice immunized thrice with 10^7 CBA/H anti-DBA/2 or CBA/H anti-C57BL/6 blasts, respectively.

‡ Percent reduction of activity compared to mean control values (= 100%).

DBA/2, CBA/H-anti-C57BL/6, C57BL/6-anti-DBA/2, and Lewis-anti-DA) the results were close to identical, that is MLC and cytolytic activity were reduced in a specific manner and largely in a parallel manner. Table VI shows one of several experiments of that kind, where responder cells from individual animals were compared as to ability to react in MLC and cytolytic assays. As seen, a striking positive correlation exists between the degree of specific depletion in the two kind of assays. In an additional series of experiments (data not shown) a comparison was made between the number of blast cells present in the MLCs at the time of harvesting and the cytolytic efficiency. Whereas the number of blasts were specifically reduced (see also DNA synthesis) in the relevant MLCs (the actual lytic ability per blast was found normal. Thus, the reduction of cytolytic activity achieved via autoblast immunization is not occurring via a decreased lytic activity per killer cell but is more likely due to an actual reduction in the number of cytolytic blasts.

Discussion

We present evidence that specific autoblast immunization can lead to a selective reduction or even complete elimination in immune reactivity against the relevant histocompatibility antigens. This induction of immune unresponsiveness could be shown to occur across various histocompatibility barriers and we could not detect any species as yet in which the blast immunization would fail. It was possible to induce unresponsiveness in outbred individuals using their own, autochthonous blasts as immunogen under conditions resembling those one would have to use if the present procedure in the future would be attempted in the clinical situation.

The underlying mechanism is not yet understood as to its fine details. We consider the dominating factor leading to unresponsiveness to be auto-anti-idiotypic immunity. This, we base on earlier experience when using idiotypic receptors in a soluble form as autoimmunogen (3, 5) as well as on the fact that autoblast immunization indeed induces auto-anti-idiotypic antibodies (12). As such anti-idiotypic antibodies in presence of complement can be shown to be able to eliminate in an irreversible manner the relevant immunocompetent lymphocytes (22) it would seem logical to assume a similar mechanism occurring in vivo

in the present system. The present system does, however, carry as an additional complication the possibility of carrying over alloantigen on the surface of the MLC-activated blasts (18). Conceivably, this may lead to the induction of antigen-specific suppressor functioning in an analogous manner to an auto-anti-idiotypic immunity. Although not finally excluded, preliminary experiments carried out so far have failed to indicate the actual existence of suppressor cells of such a kind in the autoblast-immunized animals.

In the present system reduction in MLC as well as in cytolytic T-cell activity occurred in parallel in the auto-blast-immunized animals. We don't know, however, whether this means that CML precursor cells have been eliminated. T lymphocytes reactive in MLC or developing into killer cells predominantly react against different antigenic structures (23). Assuming auto-anti-idiotypic immunity to be responsible for the present results, one may assume that killer and MLC-reactive cells should carry receptors with different idiotopes. We know that cytolytic T cells express idiotypic receptors at the effector stage (22, 23) but we have made no comparison as to distinguish between idiotypes on the two groups of cells. It is thus possible that the MLC-induced lymphoblasts to a predominating degree have contained "anti-Ia, idiotype-positive" blasts in a way that faulty induction in the blast-immunized animals of anti-idiotypic antibodies against receptors on CML precursors have occurred. If this was the case, the failure to develop CMLs may simply reflect the absence of MLC-reactive "helper" cells required for efficient induction of cytotoxic T cells. The necessary "three-party" experiments required to test this in the present system have not yet been carried out.

Although still requiring detailed analysis as to optimal conditions, the present autoblast immunization system carries certain attractive features as to possible clinical application. Thus, the induction on unresponsiveness is quite specific and, once established, would seem to be very long-lasting. It is actually possible that it will be life long as new idiotype-positive cells being generated from stem cells may serve as an inbuilt, self-maintaining booster device. Furthermore, the number of blasts required in the species investigated are such that, using repeated in vitro stimulation, it is indeed possible to obtain enough specific MLC blasts to use autochthonous blasts as immunogen. It should be stressed, however, that possible problems do also exist. The dominating hypothetical problem would be the creation of immune complexes if the inbuilt generation of idiotypic lymphocytes constitutes too powerful a source of antigen. Our search so far in the autoblast-immunized animals using conventional tests for immune complex damage, however, have so far been entirely negative but we consider it necessary to carry out further tests in this regard to fully exclude this possible complication.

As to the reason why autochthonous-purified T lymphoblasts can serve as autoantigen with regard to their idiotypic receptors, we can only speculate. Administration of blasts alone or with *Bordetella pertussis* have failed to induce specific suppression. Freund's adjuvant would seem essential in comparison and it is well known that such adjuvants may have several pathways of functioning. We consider the ability of the adjuvant to cause a high local concentration of idiotopes an important feature in this regard. There is also the possibility that the blasts during velocity sedimentation do pick up enough fetal bovine serum

proteins onto the surface that could subsequently serve as "carrier" antigens for the idiotypic determinants present on the same cell surface. We have so far never run the velocity sedimentations in syngeneic serum to exclude this possibility.

To conclude, we have shown that administration of syngeneic or even autochthonous-specific lymphoblasts in a pure, immunogenic form will lead to induction of specific unresponsiveness against major histocompatibility antigens. This failure to react can be induced in an adult, immunocompetent individual without detectable negative side effects so far. The immune unresponsiveness once induced would seem long-lasting and does involve at a functional level both MLC-reactive T cells as well as cytolytic T lymphocytes. The protocol would seem to be of general applicability as it was found to function in all species tested and against all histocompatibility barriers analyzed. The potential applications of the present procedure in transplantation immunology and delayed hypersensitivity would seem obvious.

Summary

Normal immunocompetent T lymphocytes can be induced into specific proliferation if confronted with the relevant alloantigen in vitro. Such mixed leukocyteculture-activated T lymphoblasts carrying idiotypic receptors on their surface can be purified using velocity sedimentation and serve as immunogen if administered in adjuvant to the autologous host. Autoblast immunization can be shown to lead to specific, long-lasting unresponsiveness against the relevant alloantigens, while leaving reactivity against third-party antigens intact. When tested as to general validity, it could be shown to function in all species analyzed (mouse, rat, and guinea pig) as well as across both major and minor histocompatibility barriers. No negative side effects have been noted so far. It would thus seem clear that autoblast immunization using the above described scheme may serve as a general tool in inducing long-lasting, specific unresponsiveness in any species and across any histocompatibility barrier.

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