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

II. An Analysis of the Effects Measured at the Cellular and Serological Levels*

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An approach allowing the induction of specific unresponsiveness against transplantation antigens in adult, immunocompetent individuals, would have implications of both theoretical and practical nature. We consider it possible to achieve this goal at least in certain transplantation systems via controlled autoimmune reactions directed against the idiotypic determinants present on the antigen-binding receptors of the relevant immunocompetent cells (1). Earlier results in an experimental rat system have thus shown that autologous, polymerized receptors with specificity for a given set of transplantation antigens can be used as immunogen to induce autoanti-idiotypic immunity (2).

As a consequence of this selective autoimmunity-specific unresponsiveness towards the relevant histocompatibility, antigens became apparent in the receptor-immunized animals. Attempting to extend this protocol to include clinically relevant systems, we then analyzed whether in vitro generated, antigen-specific T lymphoblasts carrying idiotypic receptors could substitute for the soluble, polymerized receptors in this system (3, 4). The results were clearcut. Thus, specific unresponsiveness as measured, both by mixed leukocyte (MLC) culture[1] and cytotoxic killer T-cell assays, could be shown to become induced with these autoblast immunization procedures (3, 4). Furthermore, in some animals, detectable amounts of autoanti-idiotypic antibodies were also induced by the blast immunization (3).

In this study we present evidence that there exists a positive correlation between the level of autoanti-idiotypic immunity and the degree of suppression in the individual animals. Furthermore, the complexity of the system as sometimes differential degrees of suppression in various T-cell subgroups are achieved will be described and discussed. Finally, we will report on attempts to use the autoblast procedures as specific abrogators

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[1] *Abbreviations used in this paper:* CTL, cytotoxic lymphocyte; D-PBS, Dulbecco modified phosphate-buffered saline; EHAA, Eagle's high amino acid; FCS, fetal calf serum; GvH, graft versus host reaction; [^{3}H]TdR, tritiated thymidine; MHC, major histocompatibility complex; MLC, mixed leukocyte culture; CML, cell-mediated lympholysis.

of already existing transplantation immunity. The theoretical and practical implications of these findings will be discussed.

Materials and Methods

Animals

MICE. Mice of the inbred strains CBA/J, CBA/H, 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 to 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 to 10 wk of either sex were used in the experiments.

Cell Preparations. Spleen and lymph node cells were aseptically removed and single cell suspensions were prepared with a stainless steel mesh by using Dulbecco's modified phosphate-buffered saline (D-PBS) as a medium. Cells were washed once and erythrocytes were lysed thereafter by hypotonic shock by using 9 parts of distilled water and 1 part of ×10 (D-PBS). Cells were washed again and resuspended in the culture medium. T lymphocytes as responder cells in MLC's were produced by using anti-Ig bead columns (5).

Culture Medium. The culture medium used for MLC and cell-mediated lympholysis (CML) both for rats and mice was Eagle's high amino acid (EHAA) medium (6), complemented with 0.5% fresh normal mouse serum for mice, and 0.5–1% of fresh BN rat serum for rats.

MLC. MLC's for mice and rats were performed in flat-bottom microtiter plates (Cook M220-29ART (Greiner, Nürtingen, Germany)), by using 0.25×10^6 responder lymphocytes and 0.5×10^6 2,000 rads irradiated stimulator cells. For the culture medium see above. Cultures were pulsed for 6 h with 1 μCi of tritiated thymidine ([^{3}H]TdR) (The Radiochemical Center, Amersham, Great Britain, 40–60 Ci/mmol) before the harvesting. Cultures were harvested by using a Skatron collector (Skatron, 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 BioQuest, Oxnard, Calif.) by 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 rads irradiated stimulator cells per ml were added to each flask. Cultures were harvested on day 4 after initiation of the MLC for mice and on day 5 for rats.

CML. CML was performed in V-bottom microtiter plates in 200 μl of EHAA medium supplemented with 5% heat-inactivated fetal calf serum (FCS). Assays were carried out in triplicate or quadruplicate. 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% NP-40 detergent (Nonidet-P40, Fluka, Buchs, Switzerland). Percent cytotoxicity (percent 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, LP-815.

Purification of T Lymphoblasts from MLC. MLC's 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 (7) by 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 was used 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.

TABLE I

Relevant Idiotypes are Present only in the MLC Responding T Cells. Evidence at the Immunogenic and Serological Level

Serum*	Target cells‡				
	I	II	III	IV	V
Anti-CBA/H-anti-DBA/2 blast	532 ± 32	227 ± 11	253 ± 58	265 ± 17	331 ± 20
Anti-CBA/H-anti-DBA/2 lymphocyte	236 ± 25	225 ± 23	233 ± 16	240 ± 11	249 ± 12
CBA normal serum	204 ± 20	226 ± 17	263 ± 35	280 ± 36	223 ± 13

*Sera from CBA/H mice immunized with blasts or small nonreactive lymphocytes from CBA/H anti-DBA/2 MLC at day 4 or 5. Sera obtained after three immunizations.

‡^{125}I-protein A assay. I = CBA anti-DBA/2 blasts, II = CBA anti-ACA blasts, III = CBA anti-DBA/2 small lymphocytes (see *), IV = CBA Con A blasts, V = CBA spleen cells.

Results

MLC-Activated T Lymphoblasts are the Sole Cells that Contain Idiotypic Receptors: Immunogenic and Serological Evidence. We have previously presented preliminary evidence that mice and rats immunized with their own, autologous MLC T blasts will produce autoanti-idiotypic antibodies against the antigen-binding receptors on these cells (3, 4). Controls did exclude the possibility that the measured antibodies were directed against stimulator alloantigens on the surface of such MLC generated T cells rather than against the idiotypic receptors. Only T blasts and not the small nonresponder T lymphocytes from the same MLC were able to induce a specific unresponsiveness in the recipients towards the relevant alloantigens (4). This suggests that the MLC blasts contain and express the idiotypic receptors of indicated type, whereas the nonresponder small lymphocytes should lack such idiotypes. Experiments as depicted in Table I were carried out to analyze whether this efficiency of T blasts to induce autoanti-idiotypic antibodies resided at the level of frequency of idiotypic receptors rather than being caused by a nonspecific superior immunogenicity of idiotypes present on blasts versus small lymphocytes. Here, it could be shown that not only were T blasts the sole cells in the MLC population that would induce specific antibodies, thereby confirming earlier findings, but they were also the only cells expressing specific binding for the presumed autoanti-idiotypic antibodies as shown in Table I. Thus, purified MLC blasts would seem to be the only cells in such an MLC population that contain cells carrying the relevant idiotypic receptors signifying reactivity against the stimulator alloantigens.

A Comparative Analysis of the Presence of Anti-Idiotypic Antibodies Versus Suppression of MLC and CML Activity in Individual Autoblast-Immunized Animals. To further analyze the relationship between anti-idiotypic immunity in the autoblast immunized animals and its relationship to the specific suppression of MLC or CML reactivity from such individuals (3, 4), a large experiment comprising 26 individual CBA mice immunized with CBA-anti-C57BL/6 T lymphoblasts was carried out. At the indicated time after autoblast immunization (see Table II), the animals were bled to death for serum and their spleen lymphocytes were analyzed in MLC and CML for reactivity against C57BL/6

TABLE II

Specific Suppression of MLC and CML of Mice Autoimmunized-Specific T Lymphoblasts and Appearance of Anti-Idiotypic Antibodies

Autoimmunized CBA mouse no.	Proliferative response to C57BL/6 stimulator cells [^{3}H]TdR incorporation	Proliferative response to DBA/2 stimulator cells [^{3}H]TdR incorporation	Mean % CML against E1-4 targets	Mean % CML against P-815 targets	Serum reaction with CBA α C57BL/6α blasts	Serum reaction with CBA α DBA/2 blasts	Serum reaction with C57BL/6 α CBA blasts	Serum reaction with CBA Con A blasts	Serum reaction with CBA normal cells
	Mean + SE	*Mean + SE*					*Mean cpm ± SE*		
1	639 ± 81	145,415 ± 4,250	4.0	10.2	8,344 ± 217	2,641 ± 89	1,109 ± 103	2,416 ± 147	2,741 ± 161
2	3,996 ± 203	151,932 ± 2,483	5.6	10.0	7,221 ± 387	2,134 ± 293	1,023 ± 210	2,251 ± 203	2,886 ± 256
3	20,848 ± 416	155,410 ± 4,086	5.9	14.3	5,455 ± 762	1,580 ± 144	1,343 ± 194	2,078 ± 116	1,934 ± 196
4	23,395 ± 3,708	153,267 ± 4,480	5.0	13.4	5,641 ± 478	2,633 ± 256	1,103 ± 53	3,019 ± 411	1,330 ± 198
5	4,898 ± 373	153,863 ± 9,226	5.0	11.5	7,790 ± 700	3,047 ± 279	1,299 ± 89	1,238 ± 142	2,200 ± 137
6	9.868 ± 1,248	157,829 ± 9,179	5.6	11.8	8,012 ± 935	4,116 ± 140	1,329 ± 210	1,013 ± 176	1,595 ± 174
7	11,493 ± 1,641	121,794 ± 8,467	5.1	11.4	2,410 ± 251	1,017 ± 101	1,198 ± 139	998 ± 72	1,795 ± 208
8	1,756 ± 514	131,456 ± 9,921	5.0	6.4	5,640 ± 141	2,458 ± 403	1,436 ± 179	2,740 ± 118	3,610 ± 181
9	4,512 ± 485	162,244 ± 10,186	4.4	9.0	6,217 ± 465	2,789 ± 257	1,046 ± 99	2,561 ± 172	2,914 ± 172
10	6,951 ± 185	153,503 ± 11,069	5.4	14.4	3,114 ± 412	1,741 ± 160	1,213 ± 114	1,317 ± 173	1,889 ± 188
11	572 ± 43	125,266 ± 12,276	4.9	11.8	8,972 ± 950	4,892 ± 371	1,125 ± 219	3,242 ± 220	2,743 ± 144
12	2,142 ± 185	130,600 ± 17,655	5.0	12.3	3,641 ± 147	1,713 ± 143	1,452 ± 167	1,376 ± 160	1,400 ± 182
13	6,929 ± 1,407	141,649 ± 11,267	4.7	21.9	2,011 ± 348	1,072 ± 75	1,230 ± 122	1,071 ± 114	1,309 ± 146
14	2,713 ± 116	131,205 ± 3,641	4.5	13.6	4,857 ± 575	1,607 ± 147	1,484 ± 401	2,117 ± 142	1,839 ± 108
15	5,448 ± 547	158,585 ± 13,576	4.8	13.3	4,230 ± 392	2,025 ± 103	1,382 ± 105	2,045 ± 129	2,710 ± 121
16	4,451 ± 487	122,089 ± 11,169	5.2	15.2	2,748 ± 190	1,590 ± 170	1,096 ± 145	1,761 ± 128	2,528 ± 142
17	5,063 ± 497	148,652 ± 11,628	4.5	13.3	6,718 ± 425	4,445 ± 392	1,275 ± 45	3,299 ± 253	2,966 ± 261
18	2,726 ± 579	107,566 ± 8,279	4.5	11.0	5,516 ± 445	3,003 ± 403	1,165 ± 154	3,358 ± 266	2,825 ± 217
19	4,493 ± 282	117,114 ± 12,225	4.0	9.0	8,051 ± 603	4,001 ± 299	1,234 ± 86	2,861 ± 244	3,053 ± 238
20	7,301 ± 1,187	154,406 ± 9,556	4.7	13.8	2,119 ± 132	1,144 ± 131	1,234 ± 128	1,859 ± 163	1,848 ± 166
21	2,263 ± 290	134,103 ± 16,198	3.1	11.6	6,089 ± 376	2,723 ± 270	1,351 ± 173	3,789 ± 226	2,226 ± 203
22	2,182 ± 542	115,648 ± 11,575	3.7	11.5	4,543 ± 556	2,450 ± 182	1,193 ± 149	2,886 ± 138	1,555 ± 181
23	1,871 ± 393	103,524 ± 8,961	3.6	11.0	3,507 ± 241	2,729 ± 148	1,118 ± 114	1,734 ± 163	1,347 ± 131
24	2,789 ± 435	103,388 ± 9,129	4.0	10.4	7,919 ± 680	3,627 ± 278	1,249 ± 183	2,330 ± 195	2,235 ± 197
25	17,929 ± 1,775	132,224 ± 3,371	3.9	11.8	1,897 ± 437	1,151 ± 140	1,171 ± 130	1,200 ± 142	1,659 ± 145
26	1,788 ± 125	115,154 ± 6,878	3.5	12.1	3,881 ± 202	1,535 ± 155	1,253 ± 257	1,595 ± 140	2,878 ± 209
Mean + SE	6,116 ± 1,185	135,688 ± 3,633	4.6 ± 1.4	12.1 ± 0.5	5,251 ± 430	2,456 ± 212	1,235 ± 24	2,159 ± 160	2,231 ± 128
Normal CBA									
1	147,468 ± 6,228	143,915 ± 4,243	33.8	14.0	1,310 ± 157	1,577 ± 226	1,088 ± 170	1,412 ± 131	1,689 ± 149
2	142,214 ± 7,662	139,734 ± 14,261	28.9	12.8	1,880 ± 270	1,839 ± 157	1,289 ± 155	1,063 ± 98	1,712 ± 178
3	148,122 ± 5,376	153,204 ± 7,901	30.4	21.5	2,633 ± 162	2,322 ± 188	1,836 ± 238	1,210 ± 169	1,451 ± 105
4	142,355 ± 1,220	148,338 ± 8,051	36.0	27.3	1,563 ± 181	1,589 ± 174	1,661 ± 387	1,341 ± 203	1,649 ± 216
+ SE	145,030 ± 1,596	146,296 ± 2,895	32.2 ± 1.6	18.9 ± 3.4	1,846 ± 286	1,831 ± 174	1,468 ± 171	1,256 ± 77	1,625 ± 60

Animals were individually killed and MLC was initiated in Falcon 3013 tissue culture flasks. 200 μl was removed on day 5 of culture and pulsed for 6 h with 1 μCi of [^{3}H]TdR. The rest of the culture was used for CML on day 6.

Serum reaction was determined by protein A assay (17). CBA anti-C57BL/6, CBA anti-DBA/2, and C57BL/6 anti-CBA blasts were purified from 5-day MLC cultures on 1 *g* FCS gradients. Con A blasts were harvested from 3-day cultures and purified in the same way. 3×10^6 blasts or normal lymphocytes were used per well.

or DBA/2 target cells. Each individual serum was tested in the ^{125}I-protein A assay for IgG antibodies against cells expected to contain idiotypic receptors of CBA/H-anti-C57BL/6 type. Furthermore, each serum was also analyzed for the possible presence of alloantibodies of anti-C57BL/6 specificity that conceivably could have been induced by stimulator alloantigens present on the T lymphoblasts used for immunization (8).

As shown in Table II, the group of experimental animals displayed the following features according to the autoblast immunization protocol: when measured at the serum level 0 out of 26 mice displayed evidence of alloantibodies aganist C57BL/6 surface antigens. On the other hand, 20 out of 26 sera contained detectable antibody activity carrying all the hallmarks of being anti-idiotypic for CBA-anti-C57BL/6 receptors. Thus, such antisera displayed strong reactivity against CBA-anti-C57BL/6 blasts and significant but weaker reactivity against normal CBA spleen cells or Con A blasts. The same sera showed only very weak binding to CBA-anti-DBA/2 blasts, thus excluding significant amounts of antiblast antibodies in these sera. Judging from earlier knowledge as to frequencies of idiotype-positive cells in various cell populations (9–14), this is exactly to be expected would the present reactions be caused by autoanti-idiotypic antibodies.

When measured for T-cell function in MLC and CML assays, a significant, highly specific reduction against C57BL/6 alloantigens was apparent in populations of cells from autoblast immune mice. Thus, all 26 suspensions exhibited significant selective reduction in MLC reactivity against C57BL/6 stimulator cells in comparison to DBA/2 control stimulators. When measuring at the level of CML activity, all autoblast populations displayed close to complete specific reduction against the C57BL/6 target cells. In occasional suspensions, CML activity was also reduced against DBA/2 (for example, animals 8, 9, and 19). In general, we consider the slight reduction in CML activity against third party DBA/2 cells due to nonspecific effects of the adjuvant therapy.

Although the protein A assay will only detect IgG antibodies in the present assay, some indications of a positive correlation of degree of suppression in MLC in relation to autoanti-idiotypic immunity expressed at the humoral level can be seen. Thus, the two animals with the highest titer of autoanti-idiotypic IgG antibodies (numbers 1 and 11) in this group of 26 mice could also be shown to be the very two animals displaying the most complete, specific suppression in MLC activity towards C57BL/6 alloantigens.

Indications of Individual Variations in T-Cell Suppression Induced by Autoblast Immunizations: An Analysis Comparing MLC, CML, and GvH Activities. T lymphocytes participating in MLC reactions are largely considered to belong to a distinct subgroup of lymphocytes reacting against a selected part of the major histocompatibility complex locus antigens, the so called Ia antigens (15–17). Cytolytic T cells, on the other hand, normally react against other antigens of the so called SD type of the MHC locus, and constitute another subgroup of T lymphocytes (15–17). As the idiotypic determinants on antigen-binding receptors vary at large according to the antigen-binding specificity, it would be reasonable to assume that T lymphocytes reactive against various parts of the MHC antigens express different idiotypes. It would thus be quite

TABLE III

Impaired MLC Reactivity of Lymphocytes Derived from Lewis Anti-DA MLC Blast-Immunized Lewis Rats

Responder cells from autoimmunized Lewis rat no.	[^{3}H]TdR incorporation of mixture with DA stimulator cells. Mean cpm of quadruplicates ± SE	[^{3}H]TdR incorporation of mixture with BN stimulator cells. Mean cpm of quadruplicates ± SE	[^{3}H]TdR incorporation of responder cells alone
Normal Lewis	64,530 ± 2,975	24,294 ± 1,135	1,976 ± 275
1	16,228 ± 1,331	24,703 ± 1,015	
2	19,393 ± 1,411	24,103 ± 616	2,376 ± 412
3	19,316 ± 1,525	20,535 ± 1,015	
4	11,755 ± 1,184	22,695 ± 1,319	

MLC was performed in Falcon no. 3013 tissue culture flasks (Material and Methods). On day 6 of culture 0.2-ml was removed from the flasks and pulsed for 6 h with 1 μCi of [^{3}H]TdR in flat bottom microtiter plates.

possible that the present autoblast immunization procedure in the in vitro activation step may selectively favor the proliferation of blasts of anti-Ia type.

Consequently, immunizations with blasts that are highly enriched for only certain idiotypes out of a given spectrum of anti-alloantigenetic receptors, may fail to lead to efficient autoanti-idiotypic immunity against the rare idiotypes among the blasts. Accordingly, individual Lewis rats immunized with autologous anti-DA T MLC blasts were analyzed in GvH, MLC, and CML assays. Tables III–V show the individual experiments of four autoblast immunized Lewis rats in these assays. Table VI presents in a summary form, by using data derived from tests with additional rats, the percentage reduction compared to the reactivity of normal Lewis spleen cells in the various assays. It would seem clear from these data that MLC and CML suppression largely went in parallel, wide scatter in reduction of GvH reactivity was apparent (see for example, rats 1, 2, and 6 in Table VI for anti-DA GvH reactivity as compared to anti-DA MLC activity). Thus, the present autoblast immunization procedure may in certain individual animals lead to elimination of only certain relevant subgroups of immunocompetent T lymphocytes.

Partial Abrogation of Existing Alloimmunity with Autoblast Immunization. Previous knowledge that idiotypic and anti-idiotypic receptors may coexist in the same individual served as a major principle when starting the present autoblast immunization scheme. Administration of one group of idiotypic receptors in a concentrated immunogenic form in normal animals would cause a shift in the balance and thus could lead to anti-idiotypic immunity. In this regard an animal already immune against foreign alloantigens should contain a higher density of idiotypic receptors (the alloantigen functioning like the anti-idiotype in shifting the initial balance) but would still be susceptible to the same regulatory forces of anti-idiotypic nature as would a normal cell population. To investigate this point, we have attempted to abrogate already existing immunity across an *H-2* barrier in the mouse by using the autoblast immunization protocol.

TABLE IV
Impaired CML Activity of Lymphocytes Derived from Lewis Anti-DA MLC Blast-Immunized Lewis Rats

Effector cells derived from autoimmunized Lewis rat no.	CML against DA target	CML against BN target
	%	%
Lewis control	15.5	19.7
1	5.9	22.0
2	6.5	22.8
3	6.1	20.8
4	6.1	20.8

Effector to target cell ratio 50:1. 6 h assay. Con A-induced blasts were used as target cells harvested on day 3 of culture.

TABLE V
Impaired GvH Reactivity of Lymphocytes Derived from Lewis Anti-DA MLC Blast-Immunized Lewis Rats

Injected cells derived from autoimmunized Lewis rat no.	Host	Mean weight (mg) ± SE of 4 popliteal nodes. Control nodes, left side	Mean weight of 4 popliteal lymph nodes. Experimental nodes, right side	Mean log ratio ± SE
			mg ± SE	
1	(Lewis × DA)F_1	52.6 ± 6.7	30.0 ± 3.9	0.24 ± 0.07
2	(Lewis × DA)F_1	48.1 ± 11.2	12.4 ± 5.8	0.66 ± 0.10
3	(Lewis × DA)F_1	45.9 ± 4.9	4.6 ± 0.4	1.0 ± 0.07
4	(Lewis × DA)F_1	33.9 ± 3.0	5.9 ± 0.6	0.76 ± 0.02
1	(Lewis × BN)F_1	29.9 ± 4.1	32.0 ± 2.2	−0.04 ± 0.03
2	(Lewis × BN)F_1	28.5 ± 2.5	28.3 ± 3.3	0.01 ± 0.02
3	(Lewis × BN)F_1	24.2 ± 1.2	25.6 ± 0.8	−0.02 ± 0.02
4	(Lewis × BN)F_1	24.6 ± 0.6	24.0 ± 1.7	0.01 ± 0.03

3×10^6 lymphocytes in 0.1 ml of D-PBS were injected into each foot-pad. As a control (control nodes) lymphocytes from normal Lewis rats were used. Lymph nodes were removed 7 days later and weighed.

The results of such attempts are exemplified in Table VII and indicate that it is indeed feasible to partially cause selective elimination of an already existing immunity by using the autoblast scheme. It is suggested from the results that the blast immunization protocol is entirely efficient in the elimination of new virgin, immunocompetent cells against the relevant alloantigens (signifies no evidence of late appearing T blasts in the MLC's from alloimmune, autoblast, immunized mice) whereas the elimination of immune memory cells, although significant, would be only of partial nature.

Discussion

Antigen-specific T lymphoblasts generated in vitro against major foreign histocompatibility antigens can be used in autologous systems to achieve

TABLE VI

Lymphocytes from Animals Autoimmunized with Antigen-Specific MLC-Induced Blasts May Show Dichotomy in Suppression When Comparing MLC and GvH Reactivity

Lymphocytes from Lewis rats autoimmunized with anti-DA blasts. Rat no.	GvH reactivity against DA as percent of control*	MLC reactivity against DA cells as percent of control*	CML reactivity against DA cells as percent of control*
1	67	4	27
2	33	3	29
3	0	27	29
4	2	15	29
5	19	15	Not done
6	55	22	Not done

* Control = 100% = reactivity obtained with equal number of Lewis normal lymphocytes in the respective assays. Values of unstimulated lymph nodes or by responder cells only subtracted before calculation. GvH test ended on day 7 after inoculation of lymphocytes; mean values calculated from four nodes. MLC test values from day 6 after initiation (= relative suppression the same on day 4). CML, see Table IV. Values demonstrating that the autoimmunized Lewis lymphocytes have normal reactivity against third party BN strain alloantigens not included.

TABLE VII

MLC Response of C57BL/6 Alloantigen Primed CBA Mice Can Be Suppressed by Autoimmunization with Specific Syngeneic MLC Blasts

Responder cells derived from animals no.	[^{3}H]TdR incorporation of mixture with C57BL/6 stimulator cells. Mean cpm ± SE of triplicates		[^{3}H]TdR incorporation of mixture with DBA/2 stimulator cells. Mean cpm ± SE of triplicates		[^{3}H]TdR incorporation of responder cells alone. Mean cpm ± SE of triplicates
	day 2	day 3	day 2	day 3	day 2
Primed 103 days before					
1	142,647 ± 10,433	103,406 ± 9,036	24,216 ± 1,096	49,989 ± 2,317	1,039 ± 174
2	167,004 ± 14,210	84,217 ± 4,246	32,403 ± 977	67,036 ± 4,233	916 ± 160
3	140,658 ± 9,244	96,104 ± 7,424	19,644 ± 2,303	39,344 ± 2,059	744 ± 188
Primed 103 days before and three times immunized					
1	44,237 ± 3,869	22,136 ± 1,860	18,727 ± 437	44,171 ± 2,977	2,468 ± 360
2	32,115 ± 1,598	19,274 ± 2,266	23,120 ± 1,781	50,881 ± 2,620	2,244 ± 288
3	28,555 ± 2,220	20,651 ± 3,280	24,777 ± 968	29,748 ± 4,030	1,934 ± 141

CBA mice were primed with 1×10^7 C57BL/6 spleen cells i.p. and either left untouched or immunized in 3-wk intervals with 1×10^7-specific CBA anti-C57BL/6 MLC blasts. Animals were tested 103 days after priming.

specific unresponsiveness against these alloantigens (3, 4). Thus, after such autoblast immunization procedures, a high percentage of animals will display partial or complete reduction in T-cell-mediated immune reactions against these very transplantation antigens (4). The actual fine mechanisms underlying the induction of this specific-immune tolerance phenomenon remain

to be proven. Based on the reasoning to be followed below, we consider it likely that all of this induced unresponsiveness is caused by autoanti-idiotypic immunity.

Alloantigen-reactive T lymphocytes carry antigen-binding receptors with idiotypic determinants denoting their binding specificity (12). Conventional anti-idiotypic antibodies in the presence of complement can, with certain sera, be shown to lead to complete selective elimination of a group of alloantigen-reactive T cells (15). Furthermore, by using soluble, polymerized idiotypic receptors as autoimmunogen, it is possible to induce autoanti-idiotypic antibodies with the above mentioned characteristics (1, 2). Finally, such autoanti-receptor-immune animals will display selective unresponsiveness against the relevant alloantigens (1, 2).

MLC purified T lymphoblasts have on their outersurface antigen-binding, idiotypic receptors as well as alloantigen of the stimulator type (8). Thus, the present system does carry an additional complication of specific nature, namely the soluble alloantigen. It is quite clear from the present findings as well as from other results (3, 4) that autoblast immunization can lead to the induction of autoanti-idiotypic antibodies. As judged from limited results, a positive correlation between the degree of T-cell suppression and autoanti-idiotypic antibody titers may exist (see Table II), but as discussed below, additional ways of autoanti-idiotypic immunity may exist. The problem of coexisting alloimmunity or sometimes even induction of alloimmunity rather than induction of suppression of reactivity is to be considered a real danger in the present autoblast procedure. Our data on this matter, although certainly incomplete, so far do not support the notion of this possibility as a fact to be commonly encountered. Thus, when analyzing for MLC reactivity, we have never seen any evidence of second-set kinetic reactions against the relevant stimulator cells and reactions do normally express normal kinetics if not close to completely suppressed. Cytolytic T cells and graft-versus-host reactions do express a similar degree of suppression, although dichotomy in single animals as to the actual degree of reduced reactivity in different assays may be noted (see below). Furthermore, when looking for the possible induction of alloantibodies by alloantigens on the autoblasts, we have again failed to detect any signs of such immunity (see for instance our failure in 26 individual mice in Table II to detect any alloantibodies of relevant specificity as well as further data given in references 3 and 4). So far, this would only exclude positive signs of alloimmunity in the blast-immunized animals. Conceivably, soluble alloantigen on blasts may lead to an efficient induction of alloantigen-reactive suppressor T cells. In experiments reported elsewhere it has been found possible to transfer suppression from autoblast-immunized mice to normal, syngeneic recipients (18). These suppressor cells could be shown to be T cells, but when analyzed for specificity they turned out to express anti-idiotypic reactivity with no detectable specificity for alloantigen. Thus, we conclude that although soluble alloantigens transferred along with the autologous MLC blasts may at least in theory create problems under certain conditions; the appearance of these problems must be a rare event using the present protocol.

The major histocompatibility complex locus products constitute from the point of view of cellular immunity two distinct antigenic groups, in the mouse

represented by the Ia gene products on one hand, and the *K* and *D* region products of *H-2* on the other (15–17, 19). Ly $1^+,2^-,3^-$ MLC-reactive cells with poor cytolytic ability are predominantly represented in the anti-Ia T blasts, anti-KD-specific T blasts are mostly Ly $1^-,2^+,3^+$ as to phenotype and are mostly efficient killer cells (20). It is likely that antigen-binding receptors on T cells reactive against either Ia or KD determinants will be different as to idiotypes in the same manner as alloantibodies directed against either Ia or KD which fail to cross-react between the two groups of antigens. This does raise the possibility that the in vitro MLC cultures generating autoblasts for immunization may be nonrandom in their selection for anti-alloantigen-reactive T cells. It is thus quite possible that certain clones of alloantigen-reactive T cells will show enough proliferative differences especially with regard to peak time kinetics during primary induction as to become comparatively diluted when harvest of MLCs take place. Along the same line of reasoning it may well occur that upon repeated stimulation of MLC responding T cells, certain clones show predominant outgrowth with time, and cause a similar dilution effect. Results pertaining to functional performance by using MLC-derived T cells undergoing repeated stimulation are suggestive that such things do occur. This may lead to relatively deficient autoanti-idiotypic immunity against the rare clones of T cells in the immunizing blast population.

In the present article we have found that MLC and cytotoxic lymphocyte (CTL) activity are frequently suppressed to a similar degree. It is dangerous, however, to conclude from this that the actual idiotypic CML precursor cells have by necessity been eliminated by the present procedure. It is well established that anti-Ia reactions may serve in vitro as helping catalysts for the induction of specific CTL's (19). Thus, absence of anti-Ia-reactive cells may in a secondary manner decrease the induction of CTL precursor cells, falsely suggesting the actual physical absence of the latter cells. Reduction of graft-versus-host reactivity in lymphocyte populations from certain autoblast immunized animals may be significantly less conspicuous than the decrease in in vitro MLC or CML reactivity of the same cells (see Table III) that are in agreement with such a select and partial induction of autoanti-idiotypic immunity. It is thus possible that similar variations in actual transplantation tolerance in autoblast immunized animals (ranging from partial to complete tolerance) (H. Wigzell, unpublished experiments) have as an underlying basis this split induction of anti-idiotypic immunity. Experiments analyzing this problem are in progress.

The present article also contains reports on attempts to abrogate an already existing allograft immunity with the autoblast procedure. This was performed for theoretical and practical reasons. Considering the balance between idiotype and anti-idiotype to be a natural part of any immune reaction (21, 22), one would assume immune animals, although biased, to still be liable to the anti-idiotypic regulatory forces. This was tested by using idiotypic receptors in a hopefully immunogenic form. The practical value would be at the level of possible future clinical attempts, where cases of already anti-HLA-immune recipients thus may become selectively treatable to eliminate their graft-preventing alloimmunity. The results obtained in this regard are, although preliminary, promising and suggest the principle validity of the above reason-

ing. It was thus possible to reduce but not completely eliminate an existing allograft immunity by using the specific blast immunization protocol. It should be noted that in this experiment primary MLC blasts were used as autoblast (see Table IV). The CBA and C57BL/6 strains that were used differed with regard to both major and minor loci. It is possible that parts of the residual activity of secondary type left after blast treatment may in fact be due to anti-minor loci reactions. Such anti-minor loci differences would not be expected to result in the production of significant frequencies of T blasts in primary MLCs. Thus, in additional experiments, we will try to further reduce existing alloimmunity, by using actual autochthonous-immune secondary MLC T blasts as autoimmunogen to eliminate this possibility.

In conclusion, the present results have further emphasized the role of autoanti-idiotypic immunity after antigen-specific, blast immunization as inducer of specific reduction in T-cell reactivity against the relevant alloantigens. This unresponsiveness would sometimes seem to be of a split nature involving only certain idiotypes and their corresponding receptor groups. It is possible that this may require the use of additional in vitro tests than MLC only when trying to assess the completeness of T-cell tolerance against entire groups of strong alloantigens encountered in haplotype differences. Procedures of this kind may have to be worked out before reproducible prognostic estimates of graft survival can be made from preceding in vitro tests in the present system.

Summary

T lymphoblasts specific for foreign histocompatibility antigens and purified via mixed leukocyte culture (MLC) and 1 *g* velocity sedimentation procedures can be used as autoimmunogen to produce specific immunological unresponsiveness in adult animals. This unresponsiveness is positively correlated to the production of autoanti-idiotypic antibodies in the blast immunized animals and no evidence of coexisting alloimmunity was found. We consider this autoanti-idiotypic immunity to be the specific inducing agent of the immune tolerance. The blast immunization procedure will lead to selective reduction in T-cell reactivity against the relevant alloantigens as measured by MLC, cell-mediated lympholysis, or graft-versus-host assays. However, in individual animals, dichotomy in suppression between two T-cell assays could sometimes be observed indicating elimination of only a select group of idiotypic functionally distinct population of T cells in these blast-immunized animals. Attempts to abrogate already immune animals by the autoblast procedure were successful, in part suggesting the use of the present procedure when trying to induce an accelerated reversion of such immunity.

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