

## SPECIFIC POSITIVE SELECTION OF LYMPHOCYTES REACTIVE TO STRONG HISTOCOMPATIBILITY ANTIGENS\*

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The population of thymus-derived (T) lymphocytes reactive to strong histocompatibility (H)<sup>1</sup> alloantigens (HARC) is peculiar in two respects in comparison to lymphocytes responsive to other antigens: first, the T cells reactive to any given strong H antigen outnumber by two to three orders of magnitude the T cells responsive to more conventional antigens (1-3); and secondly, the frequency of HARC to a given H alloantigen is not increased as a consequence of immunization in vivo (4-6). Just how fundamental these distinctions are between immune reactivity to H alloantigens and other antigen systems is not presently known. For example, the high frequency of HARC raises the possibility that these cells are multipotential, having the capacity to react to several different antigenic determinants. It is clear now that totipotentiality with respect to immune reactivity to H alloantigens can be excluded for HARC, since lymphocyte populations can be experimentally depleted of cells reactive to one H alloantigen, while retaining undiminished reactivity to other antigens (7-8). These "negative" selection experiments do not, however, allow conclusions concerning the ability of cells reactive to one H alloantigen to react to other H alloantigens or to conventional antigens. To examine this question it is essential to prepare pure populations of HARC with reactivity to a single H alloantigen so that the ability of these cells to react to other H alloantigens and to conventional antigens can be tested. This communication describes a procedure which appears to provide specific "positive" selection of cells reactive to chosen strong H alloantigens, and it also illustrates preliminary results obtained using this procedure.

Previous studies have shown that parental strain T lymphocytes are induced to proliferate in the mixed lymphocyte interaction (MLI) when they are mixed with alloantigen-bearing cells derived from F<sub>1</sub> animals (9), but disappear rapidly under the same culture conditions in the absence of antigen (2). This antigen-dependent survival of cells in the MLI could represent the progeny of lymphocytes specifically reactive to the priming antigens in the culture systems, and if so, these selected progeny should inherit the specific reactivity of their parents.

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<sup>1</sup> Abbreviations used in this paper: GVH, graft-vs.-host; H, histocompatibility; HARC, histocompatibility antigen reactive cells; MLI, mixed lymphocyte interaction; PBL, peripheral blood lymphocytes; TDL, thoracic duct lymphocytes.

Rather than test the immune competence of these surviving cells immediately after terminating the cultures, we elected to allow them to recover *in vivo* from their confrontation with antigen *in vitro* by "parking" them for a period of time in an immunologically inert thymus-deprived (B rat) host, a rat of the same inbred strain as the donor of the responsive HARC in the MLI. Subsequently, the degree of restoration of T-lymphocyte function in terms of MLI and graft-vs.-host (GVH) reactivity could be assessed in lymphocyte populations recovered from the host, populations containing no detectable T cells except those derived from the cultured cells.

### *Materials and Methods*

*Animals.*—The following inbred strains were used in this study: Aug (A28807; Ag-B5), HO (PVG/c; Ag-B5), as well as F<sub>1</sub> hybrids derived from Aug × DA (Ag-B4), BH (Ag-B1), or WF (Wistar Furth; Ag-B2) matings, and HO × Aug, and DA or AO (Ag-B2) matings.

*Operative Procedures.*—Thoracic duct cannulation and thymectomy of young adult rats were performed as described elsewhere (12). Cell suspensions were transferred to recipients intravenously via the lateral tail vein. Peripheral blood leukocytes were collected by cardiac puncture. All operations were performed under ether anesthesia.

*Analytical MLI Cultures.*—Culture conditions for analytical MLI were essentially as described previously (2). 10<sup>6</sup> parental strain peripheral blood lymphocytes (PBL) or thoracic duct lymphocytes (TDL) were incubated with 0.5 × 10<sup>6</sup> F<sub>1</sub> hybrid PBL in sterile disposable glass culture tubes containing 1.0 ml Eagle's MEM with glutamine (2.5 mg/ml), penicillin (100 IU/ml), streptomycin (100 mg/ml), and fresh rat serum (5% vol/vol) from BN strain donors. Cultures were exposed to tritiated thymidine [<sup>3</sup>H]TdR, 6.7 Ci/mM, 0.25 μCi/culture) for 16 h before termination at days 5 or 6. Incorporation of [<sup>3</sup>H]TdR was assessed by scintillation spectrometry and dpm were calculated from quench corrections based on an external standard.

*Preparative MLI Cultures.*—

*Multitube culture:* Large numbers of cells were obtained from active MLI cultures by pooling culture tubes set up under the same conditions as for analytical MLI except that the responding cells were always TDL and the F<sub>1</sub> hybrid cells were taken from the spleen and treated with mitomycin C (25 μg/ml for 20 min) before culture. Cultures were harvested after 6 days of incubation. At this time cell survival (determined by trypan blue exclusion) in active cultures was 40–60% of initial parental strain cell numbers compared with 1–5% inactive cultures containing 10<sup>6</sup> parental strain cells alone.

*Bulk cultures:* Bulk MLI cultures were set up in 5 cm diameter plastic Petri dishes. 50 × 10<sup>6</sup> parental strain TDL were mixed with 12.5 × 10<sup>6</sup> mitomycin C-treated F<sub>1</sub> hybrid spleen cells in 10 ml RPMI 1640 medium (Microbiological Associates, Inc., Bethesda, Md.) containing glutamine, penicillin, streptomycin, and 5% vol/vol fresh or once-thawed AO or HO rat serum. Bulk cultures were harvested after 6 days of incubation. Active bulk cultures showed a drop in pH after the 4th day of culture and cell survival (by trypan blue exclusion) on the 6th day of culture was between 35% and 50% of initial parental cell numbers in active cultures, compared with 1–5% in inactive cultures containing 50 × 10<sup>6</sup> parental strain cells alone.

*Preparation of B Rats.*—B rats were prepared essentially as described previously (12, 13). Briefly, Aug and HO rats were thymectomized when 6- to 7-wk old. 2–9 wk later they were irradiated with 900 R (Aug) or 1,000 R (HO) from <sup>137</sup>Cs (Aug) or <sup>60</sup>Co (HO) sources and reconstituted on the same day with 10<sup>7</sup> bone marrow cells from syngeneic donors that had been thymectomized previously and depleted of circulating lymphocytes via an indwelling thoracic

duct fistula over a 7-day period. B rats were used as recipients of cells from MLI cultures between 4 and 8 wk after marrow reconstitution.

*Graft Versus Host (GVH) Assay.*—The popliteal lymph node weight GVH assay was used in young F<sub>1</sub> hybrid rats of both sexes as described by Ford et al. (14). Three or four point assays were used. The dose of lymphocytes (especially T lymphocytes, see below) required to cause a popliteal lymph node enlargement to 10 mg was used as an estimate of the GVH potency of the lymphocyte populations tested.

The relative potency of a test population compared with that of a normal lymphocyte population was calculated as follows:

$$\text{relative potency} = \frac{\text{no. of (T) lymphocytes from normal populations required to cause 10 mg lymph node}}{\text{no. of (T) lymphocytes from test population required to cause 10 mg lymph node}}$$

*Estimation of T-Cell Frequency in TDL Populations.*—The approximate frequency of T lymphocytes was estimated in TDL populations by direct complement-mediated cytotoxicity using two sera directed against peripheral rat B and T cells respectively, whose source, specificity, and use are described in detail elsewhere (15). The T-cell frequency in a TDL suspension was taken as the arithmetic mean of the value given directly with the anti-T serum and the value given by difference with the anti-B serum. This procedure was adopted on the grounds that the anti-T serum is probably slightly less than 100% efficient, thus yielding a small underestimate of T-cell frequency, while the anti-B serum fails to kill both T cells and a proportion of large lymphocytes, thus yielding a small overestimate of T-cell frequency (15).

#### RESULTS

*MLI Responses.*—The plan of the first experiment is shown in Fig. 1. Aug B rats were reconstituted with  $12-48 \times 10^6$  cells from replicate multitube 6 day Aug and (Aug  $\times$  DA)F<sub>1</sub> mixed lymphocyte cultures. After such reconstitution B rats were referred to as B<sub>(MLI)</sub> rats. PBL from the B<sub>(MLI)</sub> rats were assayed in the MLI against specific (Aug  $\times$  DA)F<sub>1</sub> and nonspecific (Aug  $\times$  BH)F<sub>1</sub> and (Aug  $\times$  WF)F<sub>1</sub> stimulating cells on days 7, 14, and 28 after reconstitution, each time in parallel with PBL from normal Aug rats and from nonreconstituted Aug B rats. 35 days after reconstitution the thoracic ducts of B<sub>(MLI)</sub>, normal Aug, and Aug B rats were cannulated and their TDL also assayed in the MLI against PBL from specific and nonspecific F<sub>1</sub> hybrid donors. Cells from the reconstituted B<sub>(MLI)</sub> rats gave essentially homogeneous responses in the MLI both from rat to rat and from assay to assay, and the data are therefore combined in a single table (Table I). The data are presented as the ratio of dpm in cultures stimulated with specific (DA) and with nonspecific (BH and WF)F<sub>1</sub> cells, with the raw data also provided, for comparative purposes. The following points emerge from this table: (a) In no case was the MLI reaction of PBL or TDL from normal Aug rats to the specific antigen (DA) greater than that against BH or WF; in five or six instances, the anti-DA reaction was relatively low. (b) In all of 15 estimations of PBL and TDL reactivity from B<sub>(MLI)</sub> rats the MLI response to the specific antigen (DA) exceeded that to the nonspecific antigens (BH or WF), with a mean ratio of 3.8. (c) The proliferative responses of TDL and PBL from the B<sub>(MLI)</sub> rats in the MLI against

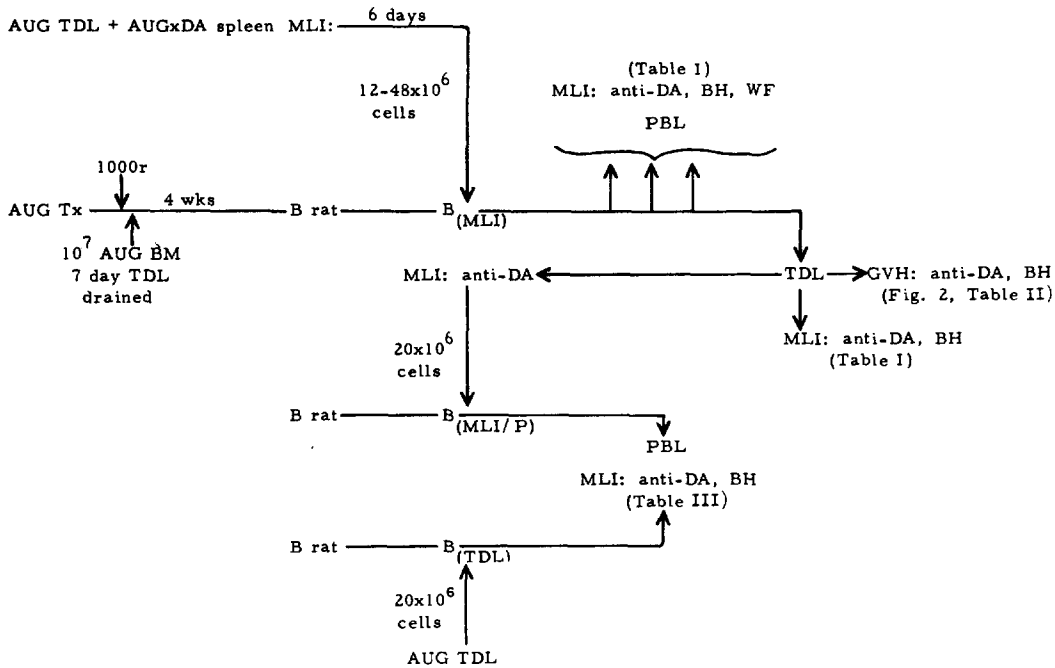


FIG. 1. Protocol for the preparation and testing of selected populations of lymphocytes reactive to a chosen histocompatibility antigen. Parental strain (AUG) thoracic duct lymphocytes are stimulated in the MLI with splenic cells from F<sub>1</sub> hybrid animals. 6 days later the cultures are terminated, the cells are collected and transferred to syngeneic recipients which have been thymectomized, irradiated, and reconstituted with lymphocyte-depleted bone marrow cells (B rats). Thereafter, the activity and specificity of peripheral blood and thoracic duct lymphocyte populations of the recipient is determined in the mixed lymphocyte and GVH reactions. The cells can also be passed by restimulating them in the MLI and transferring them to secondary B-rat recipients.

DA were similar in absolute magnitude to the response of cells from normal donors to this antigen. (d) PBL and TDL from the nonreconstituted B rats showed no demonstrable MLI activity to either the specific (DA) or the non-specific (BH) antigens. (e) The proliferative activity of unstimulated PBL and TDL populations from all the various rat donors made only an insignificant contribution to the total MLI response. Stimulation factors (stimulated culture dpm/unstimulated culture dpm) of between 100 and 1,000 are common in this assay, especially with TDL as the source of responding cells.

**GVH Reactivity.**—Thoracic duct cells from the same specifically reconstituted B<sub>(MLI)</sub> rats were also tested for their ability to elicit GVH reactions in the popliteal lymph node after injection into the hind footpads of young F<sub>1</sub> rats (14), (Aug × DA)F<sub>1</sub> being the specific recipients and (Aug × BH)F<sub>1</sub> the nonspecific recipients in this test (Fig. 2). TDL from a normal Aug, and from a nonre-

TABLE I  
*Antigen-Specific MLI Responses*

AUG suspension assayed	Ratio of dpm: anti-DA/anti-BH	Ratio of dpm: anti-DA/anti-WF	dpm anti-DA	dpm parental only
<b>PBL</b>				
normal	0.79*	0.95; 0.89	2,140-13,330	135-1139
	0.67-1.00 (3)			
B <sub>(MLI)</sub> †	3.37	3.72	2,940-9510	30-507
	1.60-6.18 (9)	3.09-4.83 (4)		
B	(0.98; 1.00)§	—	206; 400	55; 347
<b>TDL</b>				
normal	0.49	—	20,650	36
B <sub>(MLI)</sub> †	3.80; 4.32	—	22,810; 33,651	30; 186
B	(0.51)§	—	160	129

\* Mean ratio with range of results and number of animals tested.

† See Fig. 1 and text for details of these rats.

§ No response over control unstimulated cultures.

constituted Aug B rat were also tested as controls in the same assay. As with the MLI, normal Aug cells appear somewhat less reactive to DA than to BH alloantigens in the GVH assay. The specificity ratio, (number of cells required to give 10 mg node in Aug × BH)/(number of cells required to give 10 mg node in Aug × DA), for the normal Aug TDL population in the response to DA relative to BH alloantigens is approximately 0.5 (Fig. 2 *a* and Table II). In the two GVH assays performed on TDL from the B<sub>(MLI)</sub> donors, the same ratios, estimated from the anti-Aug × BH curves at 10 mg lymph node weight, were 12.1 and 2.6 (Fig. 2 *c* and *d*, and Table II). TDL from nonreconstituted B-rat donors gave no demonstrable GVH response (Fig. 2 *b*).

*Effect on Specificity of Second Passage of HARC from B<sub>(MLI)</sub> Rats.*—It is important to determine whether the persistent activity in the B<sub>(MLI)</sub> rats against the nonspecific antigens WF and BH both in the MLI and GVH was due to incomplete selection for anti-DA cells during the initial preparative MLI. If selection was incomplete, and if anti-DA specificity of the degree shown in Table I and Fig. 2 could be obtained on the first transfer, then still more impressive degrees of specificity might be obtained if an already partially specific population of HARC from the first B<sub>(MLI)</sub> rats were restimulated in the MLI against AUG × DA, and the products then transferred to other B rats (see Fig. 1). TDL collected from previously assayed AUG strain B<sub>(MLI)</sub> rats were incubated with AUG × DA splenic cells in replicate MLI tube cultures. After 6 days of culture, the cellular products were collected and transferred in doses of 20 million cells to two further AUG B recipients. PBL from these secondary B recipients (termed B<sub>(MLIP)</sub> rats) were assayed in the MLI 1 wk later for specificity against AUG × DA and AUG × BH cells (Fig. 1 and Table III). The results showed that one secondary recipient possessed anti-DA specificity similar to

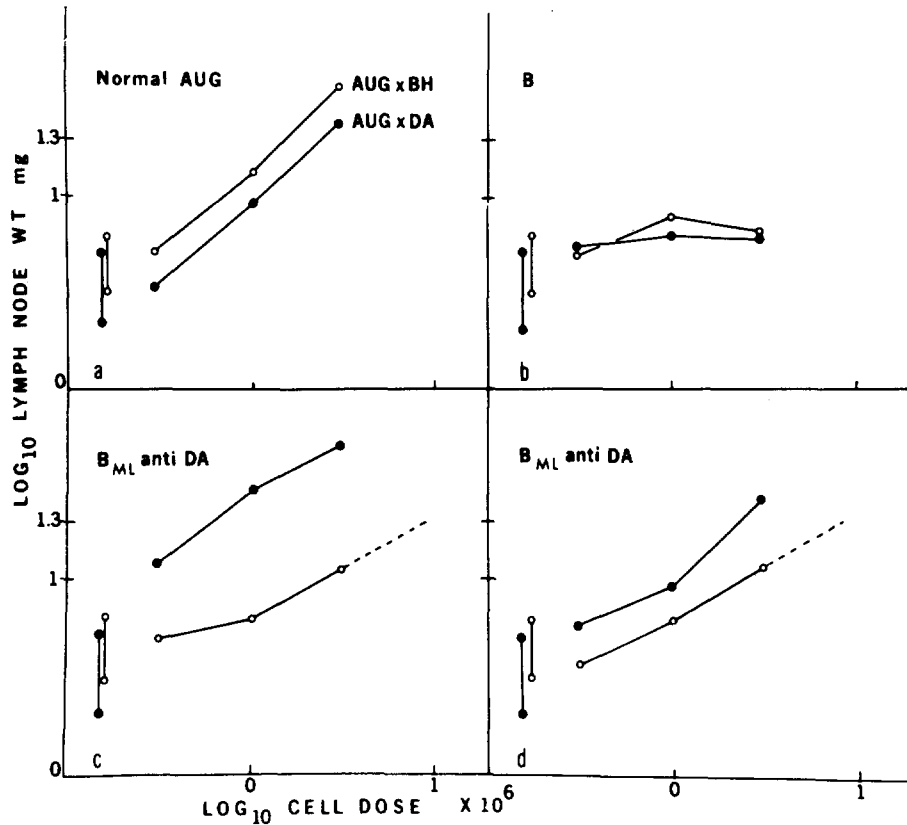


FIG. 2. GVH reactions in specific (AUG  $\times$  DA) and nonspecific (AUG  $\times$  BH) $F_1$  hybrids with thoracic duct lymphocytes from normal AUG donors, AUG B-rat donors, and B rats reconstituted with MLI cells selected against DA alloantigens.

that of the primary recipients (Table I) and the other had a considerably greater degree of specificity. These preliminary data are inconclusive concerning the issue of completeness of positive selection: they are included here to indicate that repeated passage of selected cells is a viable approach to this important question. These data also re-emphasize the conclusion that many of the descendants of HARC are by no means end cells. They can be repeatedly restimulated to react against selected H antigens, each time reproducing in their progeny the properties of their parents.

*Reconstitution Achieved in B Rats by 20 Million Normal AUG TDL.*—If the level of activity against nonspecific BH alloantigens by cells from B<sub>(MLI)</sub> rats reconstituted with anti-DA cells were due to a minority of "bystander" anti-BH HARC surviving in the original MLI and transferred with the cellular inoculum from the MLI, then 20 million unselected normal TDL should restore B rats to anti-BH activity greater than that obtained in the specifically recon-

TABLE II  
*Potency Estimates in Antigen-Specific GVH Reactions*

TDL suspension†	Percent T cells‡	T cells ( $\times 10^6$ ) required for 10 mg lymph node in:		Specificity ratio: anti-DA/anti-BH	Potency ratio normal/B <sub>(MLI)</sub> :¶	
		Aug $\times$ DA	Aug $\times$ BH		anti-DA	anti-BH
Normal Aug	68	0.77 (1.12)*	0.48 (0.71)	0.63		
Aug B <sub>(MLI)</sub> anti-DA-1	17	0.04 (0.22)	0.46 (2.70)	12.1	19.0	1.0
Aug B <sub>(MLI)</sub> anti-DA-2	12	0.11 (0.89)	0.27 (2.24)	2.6	7.0	1.8

\* Figures in parentheses indicate total number of cells (i.e., before correction for T-cell frequency) required to cause 10 mg lymph nodes in each hybrid recipient.

† TDL suspensions assayed by three point popliteal lymph node enlargement GVH assay in (Aug  $\times$  DA) and (Aug  $\times$  BH)F<sub>1</sub> hybrid recipients. Two Aug B<sub>(MLI)</sub> anti-DA donors received cells from 6 day Aug + (Aug  $\times$  DA)F<sub>1</sub> mixed lymphocyte cultures 35 days before assay (see Figs. 1 and 2).

‡ T cells estimated by direct cytotoxicity using antisera specific for T and B cells, as described in the Materials and Methods.

§ Reciprocal ratio of number of cells required to cause 10 mg lymph nodes in (Aug  $\times$  DA)F<sub>1</sub> recipients compared with (Aug  $\times$  BH)F<sub>1</sub> recipients.

¶ No. T cells from normal donors or from B<sub>(MLI)</sub> required for 10 mg lymph node weight in (Aug  $\times$  DA)F<sub>1</sub> or (Aug  $\times$  BH) recipients.

TABLE III  
*Antigen-Specific MLI Reactions (Second Passage)*

PBL	Ratio of dpm: anti-DA/anti-BH	dpm anti-DA	dpm parental only
(a) Normal	0.39; 0.69	3,000; 4,775	184; 364
(b) B <sub>(MLI/F)</sub> *	2.19; 18.8	1,700; 3,104	65; 31
(c) B <sub>(TDL)</sub> *	0.27; 0.35	202; 174	64; 73
(d) B	(0.18)‡	(9)‡	39

\* See Fig. 1 and text for details of these rats.

‡ No response over control unstimulated cultures.

stituted B<sub>(MLI)</sub> rats, where unselected anti-BH cells must be relatively less abundant in the inoculum. In this experiment, we found that 20 million normal AUG TDL would restore only feeble MLI responsiveness to B rats, of the same order of magnitude as the response to the nonspecific BH alloantigens found in recipients of selected anti-DA cells (Table III, row c). It therefore seems likely that not all the nonspecific MLI activity found in B<sub>(MLI)</sub> recipients could be due to unselected bystander HARC, and that at least some cells selected for reactivity to DA antigens must also be capable of responding to BH antigens.

*Estimates of T-Cell Potency in the GVH.*—Enrichment for T cells reactive to the selecting antigen can be measured in the GVH as a potency ratio, as defined in the Materials and Methods, in which the activity of a selected T-cell population is compared with that of a normal T-cell population. Preliminary

data of this kind were obtained from the GVH responses of TDL from the two AUG  $B_{(MLI)}$  rats already described carrying T cells selected against DA (Figs. 1 and 2) using T-cell frequency estimates calculated as described in the Materials and Methods (Table II). As anticipated, the T-cell frequency in TDL from the two  $B_{(MLI)}$  rats was substantially lower than that of the normal TDL, and thus on a per T-cell basis the two  $B_{(MLI)}$  populations were substantially more potent against DA than were the normal cells (potency ratios of 20 and 7.2 compared with normal). Curiously enough, the potency of the two selected T-cell populations against third-party antigens (BH) was equal to that of normal T cells despite the great increase in potency found against DA.

These two striking findings, high T-cell potency against the selecting antigen and normal T-cell potency against a third party H alloantigen antigen, were confirmed in a series of GVH assays on TDL from four  $B_{(MLI)}$  rats in another strain combination. In this case HO strain TDL were stimulated in bulk MLI cultures with mitomycin C-treated spleen cells from  $(HO \times DA)F_1$  donors. The 6 day MLI cells were transferred in doses of  $15-25 \times 10^6$  cells to HO strain B rats and 4 wk later the resulting  $B_{(MLI)}$  recipients were cannulated to obtain TDL. T-cell frequencies were estimated in four normal HO TDL populations and in the four  $B_{(MLI)}$  TDL populations before injection of the TDL in three or four graded doses into specific  $(HO \times DA)F_1$  and third-party  $(HO \times AO)F_1$  hybrids, for the GVH assay. The GVH response of normal and  $B_{(MLI)}$  TDL are shown in Fig. 3 *a* and *b*, already corrected for T-cell frequency. In Fig. 3 *c* the two sets of data are superimposed to emphasize both the striking increase in potency of  $B_{(MLI)}$  T cells against the third-party antigen, and secondly the near normal potency of  $B_{(MLI)}$  T cells against the third-party antigen. Potencies of  $B_{(MLI)}$  T cells relative to normal in the response to both selecting and third-party  $F_1$  hybrids are calculated from individual responses in Table IV. Combining the potency values obtained in the two experiments described in Tables II and IV gives a geometric potency of 7.6 relative to normal against the selecting antigen (DA), and 0.87 against the two third-party antigens BH and AO.

#### DISCUSSION

These experiments indicate that a lymphocyte population can be prepared which is considerably enriched in its specificity for reactivity to the alloantigenic determinant(s) of a chosen H allele. The fact that the surviving descendants of proliferating cells in the MLI appear to be largely antigen specific has also been indicated in other studies by the specificity of cytotoxic effector functions generated by mouse cells in the MLI (16). Of special interest in the present work is the finding that a consequence of the interaction between HARC and H alloantigens in the MLI is the generation of new cells which have the capacity to survive for prolonged periods in the blood and lymph of syngeneic recipients and which can react anew in the MLI and GVH reactions with specificity for the antigens which originally induced their formation. This *in vitro* antigen-specific



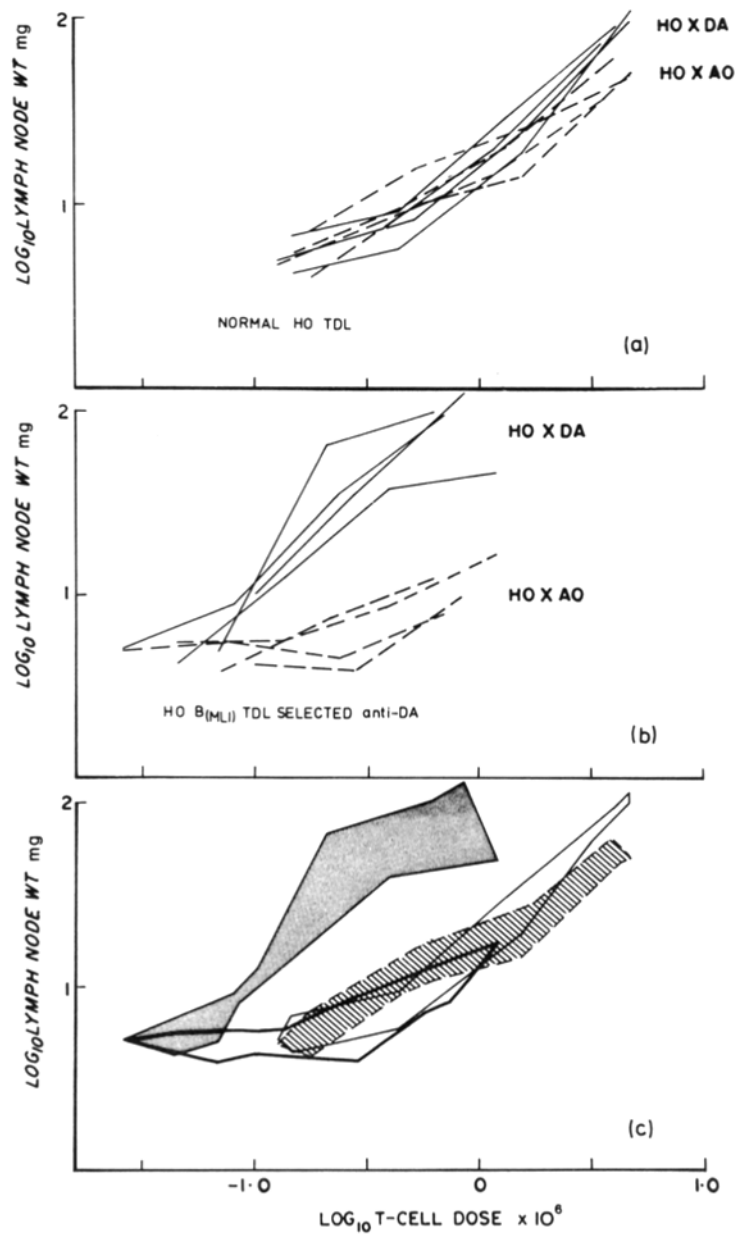


FIG. 3. GVH reaction assays for T-cell potency. TDL from normal and from HO B<sub>(MLI)</sub> rats bearing cells selected in vitro against DA strain alloantigens were injected in graded doses into the hind footpads of young (HO x DA) and (HO x AO)F<sub>1</sub> hybrids. Popliteal lymph node weights were recorded 7 days after cell injection. (a) GVH reactions of normal HO TDL, (b) GVH reactions of TDL from HO B<sub>(MLI)</sub> selected against DA, and (c) data from (a) and (b) combined to illustrate high relative potency of B<sub>(MLI)</sub> cells tested against the selecting antigens in (HO x DA)F<sub>1</sub> hybrids, and normal potency of B<sub>(MLI)</sub> cells tested against the third-part antigens in (HO x AO)F<sub>1</sub> hybrids (see also Table IV.).

TABLE IV  
*Potency Estimates in Antigen-Specific GVH Reactions*

TDL Suspension	% T cells	No. of T cells needed $\times 10^6$ for 10 mg node in:		Potency ratio normal/B <sub>(MLI)</sub> *	
		HO $\times$ DA	HO $\times$ AO	anti-DA	anti-AO
Normal HO					
1	52	0.60	0.27		
2	44	0.48	0.46		
3	52	0.78	0.51		
4	58	0.51	0.53		
Geometric mean:		0.58	0.43		
HO B <sub>(MLI)</sub> anti-DA†					
1	8	0.087	1.12‡	6.7	0.4
2	13	0.11	0.50	5.4	0.9
3	13	0.093	0.40	6.3	1.1
4	20	0.10	0.85	5.8	0.6

\* Potency ratios calculated as in Table II, using geometric mean values of normal T-cell doses in each potency comparison with individual B<sub>(MLI)</sub> rats.

† HO B rats received  $24 \times 10^6$  cells from HO anti-(HO  $\times$  DA)F<sub>1</sub> MLI cultures 4 wk before assay.

‡ Value obtained by extrapolation to 10 mg. At maximum cell dose injected a mean lymph node weight of 7.8 mg was obtained in (HO  $\times$  AO)F<sub>1</sub> recipients.

generation of new responding units similar in quality to the primary responding units, but more abundant resembles the "quantal" immunological memory for strong transplantation antigens postulated by Brent and Medawar (17); it is likely that this accounts for the effects of in vivo priming on the normal lymphocyte transfer reaction in guinea pigs. Nevertheless, this phenomenon has proved impossible to demonstrate in the MLI or GVH reactions in mice or rats after in vivo immunization (4-6). The GVH studies reported here show T-cell populations with potencies, relative to normal, of from 5 to 20 with respect to a strong alloantigen system; these figures compare with the mean factor of immunization of 1.4 which was obtained in a variety of strain combinations with various in vivo immunization schedules done by Ford and Simonsen (5).

It should be emphasized that the method used in the present experiments for calculating the potency of a selected T-lymphocyte population (see Materials and Methods) depends on estimates of the T-cell frequency in TDL suspensions using specific cytotoxic antisera. Because anti-B serum does not kill a proportion of large lymphocytes as well as T cells in TDL suspensions, this method of estimation results in a slight overestimate of T-cell frequency especially at low true T-cell frequencies, giving corresponding slight underestimates of the true potency of the T cells. The values given for potency in the GVH reaction of selected T-cell populations should therefore be considered as minimum values.

It is possible that our results are compatible with experiments of Andersson and Häyry (18) in which mouse MLI cultures, maintained in vitro for some

days after the primary response had waned, could be restimulated to respond vigorously to the priming antigen but less well to third-party antigen. The fact that cells generated in the MLI in the present experiments will respond subsequently in both the MLI and GVH is strong evidence in favor of the identity of the responding cell in these two reactions, and suggests that the two reactions are alternative manifestations of the immunological activity of this cell type, recently termed an initiator cell (19). The simplest description in cellular terms for the findings described in this paper is that the effect of immunization *in vitro* under the conditions described is to promote specific initiator cell amplifications under conditions where "nonspecific" initiator cells reactive to other antigens are selectively lost. In view of Ford's suggestion that failure to generate memory in the GVH response after *in vivo* immunization is due to disproportionate differentiation of responding lymphocytes into effector cells (20) it is of interest in the present context that exhaustive attempts to demonstrate cytotoxic effector cells in 6 day MLI cultures performed as described in this study have been completely unsuccessful, while high levels of cytotoxic activity are easily generated *in vivo* in the same strain combinations.<sup>2</sup>

It seems likely from the present data that cells selected on the basis of specificity for the products of one major H allele also react to some extent with antigenic products of other major alleles. This effect is seen most strikingly in the GVH assay, where T-cell potency can be measured reasonably well. Tables II and III, and Fig. 3 *c* show that despite the substantial increase in potency to the selecting antigen (geometric mean potency, 7.6 overall estimates) there is no concomitant decrease in potency to the two third-party antigens (geometric mean potency, 0.87). On the basis of Ford & Atkins' (3) figure of 12% T lymphocytes reactive in one rat strain to the major alloantigens of a second strain, a relative potency of 8 implies near complete selection. If these figures are approximately correct, then the persistent GVH and MLI response to the third-party antigens must be due largely to the activity of cells with specificity also for the selecting antigens. It is not at present possible to decide what the basis for this cross-reaction may be but in any event it seems to dispose of total exclusiveness among cells reactive to different major H antigens as suggested by Ford and Atkins (8) and thus reduces to some extent the threatened "over occupancy" of the T-cell pool by cells reactive to major H antigens. This concern arose because of the repeated finding that the frequency of reactive to a given H alloantigen was very high (1-12% according to various estimates [1-3, 21]), so that reactivity at that frequency to all alloantigens of the species would require the commitment of more T cells than any individual possessed. An unanswered but crucial question is whether cells selected on the basis of reactivity to a major H alloantigen also include populations reactive to determinants of conventional antigens.

<sup>2</sup> Howard, J. C., and D. B. Wilson. Manuscript in preparation.

## SUMMARY

Selected populations of thymus-derived (T) rat lymphocytes having specific immunological reactivity to chosen histocompatibility (H) alloantigens are found among the cellular products of the mixed lymphocyte interaction (MLI). Such specific selection seems to depend on (a) the antigen-induced proliferation of specific H antigen reactive cells (HARC), and (b) the disappearance of non-reactive cells from the cultures. When the surviving cells from this lymphocyte-antigen interaction are transferred into thymectomized, X-irradiated, marrow-reconstituted syngeneic recipients (B rats) which lack detectable T-lymphocyte functions, the lymphocyte populations subsequently recovered from the hosts possess the capacity to react in the MLI and in the graft-vs.-host (GVH) reaction, and the reactions have specificity for the original priming alloantigens. In addition, these findings identify the cell that reacts in the MLI with the GVH reactive cell.

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