SYNERGY AMONG LYMPHOID CELLS MEDIATING THE GRAFT-VERSUS-HOST RESPONSE

III. Evidence for Interaction between two Types of Thymus-Derived Cells

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The graft-*versus*-host (GVH)[1] reaction is an example of a cell-mediated immune response (1). The reaction can be elicited by grafting lymphoid cells from an adult animal of parental strain to a neonatal F_1 hybrid recipient. A previous study showed that appropriate mixtures of lymphoid cells from certain tissues of mice interacted synergistically in the induction of GVH reactions (2). For example, peripheral lymph node (PLN) cells and thymus cells from BALB/c mice inoculated together into BALB/c × C57BL/F_1 newborn recipients produced GVH reactions greater than the sum expected from separate reactivity of the two cell populations alone, as judged by the Simonsen spleen weight assay. Synergy did not occur unless both cell populations had been obtained from donors allogeneic to the host (2). The results were interpreted as indicating that at least two types of specifically reactive lymphoid cells participate in GVH reactions.

The present study evaluates the effects of heterologous anti-thymocyte serum and neonatal thymectomy upon the interacting cells, using a quantitative assay. One component cell population was obtained from the circulating lymphoid pool, peripheral blood lymphocytes (PBL), and is presumably analogous to PLN cells. The second component was found in greatest concentration in the thymus and spleen. The data indicate that both cells are thymus-derived (T) lymphocytes, and that the T cell present in excess in the thymus and spleen (T_1) recirculates slowly, if at all, and is the precursor of the cell which inflicts immunologic injury; this activity is amplified by a rapidly recirculating T lymphocyte (T_2) present in excess in the peripheral blood and lymph nodes.

[1] *Abbreviations used in this paper:* ATS, anti-thymocyte serum; BM, bone marrow; GVH, graft-*versus*-host; PBL, peripheral blood lymphocytes; PLN, peripheral lymph nodes; T, thymus derived.

Materials and Methods

Animals.—10–12-week old male BALB/cAnN (*H-2*^{d}), C57BL/6N (*H-2*^{b}), and F_1 hybrids of these strains, as well as litters of 1–4-day old F_1 mice, were obtained from the Rabbit and Rodent Production Section, National Institutes of Health, Bethesda, Md.

Cell Suspensions.—Thymi were excised from the mediastinum, gently rinsed with Hanks' balanced salt solution, and the cells teased into suspension as previously described (3). PBL were obtained after sedimentation of erythrocytes from the whole blood using 2–½% dextran solutions according to the method described by Wilson et al. (4). The leukocyte-rich supernate was centrifuged at 1500 rpm for 10 min. Leukocyte yields with this procedure were 50–55% of the original content. Between 90 and 95% of the leukocytes obtained were lymphocytes. This represented about a 20% enrichment of these cells compared to normal blood. Intraperitoneal injections were made in a constant volume of 0.05 ml into individual mice. Combined cell populations were injected in the same volume and by the same route as the uncombined cell suspensions. Cells were combined just before inoculation.

Graft-Versus-Host Assay.—A modification of the spleen weight assay described by Simonsen (1) was performed. 9 days after grafting of cells, litters were killed and a spleen weight to body weight ratio was determined both for the inoculated recipients and control uninoculated littermates. An index of spleen enlargement was computed by dividing the spleen weight to body weight ratio of the injected animals by that of their untreated littermates as previously described (3, 5). In all experiments in which the potency of mixtures of cells was tested, some litters were also injected with each cell population alone. In several experiments, individual litters were used to test the relative potency of cell mixtures and each component of the mixture, using a modification of the spleen weight assay suggested by Mitchie (24). This protocol was used to determine if standard curves were sufficiently reliable to employ in measurements of relative potency. No differences were seen between standard reactivity curves and the "internal" controls. These litters were divided into three groups: the first group received the mixture of cells; the second, one of the cell components; and the third served as uninoculated controls.

Anti-Thymocyte Serum (ATS).—Rabbit anti-thymocyte serum was prepared as previously described (6), using the method of Levey and Medawar (7). A pool of serum from eight rabbits was made after determination that each of the individual sera could produce at least 90% suppression of the GVH reactivity of spleen cells 2 days after administration. ATS was heat inactivated at 56° C for 2 hr and absorbed four times with mouse erythrocytes (MRC) at a ratio of one part packed MRC to 20 parts ATS.

Thymectomy.—Neonatal thymectomy was performed during the first 24 hr of life according to a method previously described (8).

Double-Transfer Experiments.—Parental cells to be tested were inoculated into 1–4-day old F_1 recipients. 9 days later, the spleens from these recipients were excised and cell suspensions prepared. Appropriate aliquots of these cells were inoculated into newborn (1-day old) BALB/c and C57BL/6 recipients as described above. Spleen indices in these second recipients were measured 9 days after transfer.

RESULTS

Reactivity of Peripheral Blood Lymphocytes (PBL) and Thymus Cells from Adult BALB/c Mice.—GVH reactions produced by different numbers of PBL and thymocytes are shown in Fig. 1. Each point on the reactivity curves was determined using at least four litters in the case of PBL and 10 litters in the case of thymus. As expected, there was a linear relationship between the spleen index determined 9 days after administration of cells and the logarithm of the

number of cells inoculated. The smallest number of PBL which would produce a detectable reaction (spleen index > 1.3) was approximately 3.5×10^5; the smallest number of thymocytes which would produce such a reaction was approximately 5.5×10^6. Since the reactivity curves for PBL and thymus were nearly parallel, the relative reactivity of these two tissues could be calculated. PBL were approximately 20 times as reactive as thymocytes.

Reactivity of Combinations of PBL and Thymocytes.—Fig. 1 shows the results of combining different numbers of PBL with 5, 2.5, or 1×10^6 thymocytes. It can be seen that 3×10^5 PBL combined with 5×10^6 thymocytes

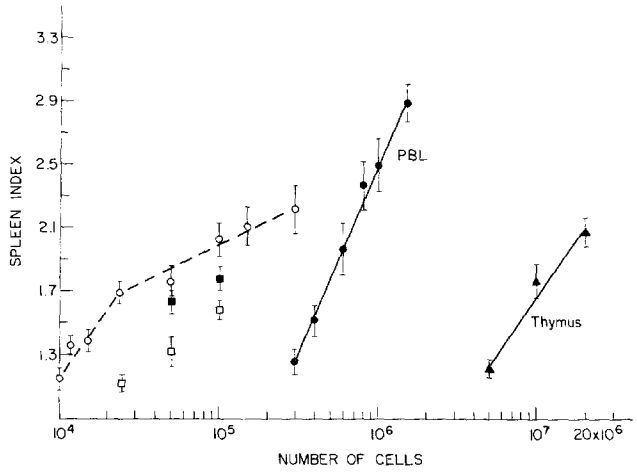

FIG. 1. GVH reactions of PBL, thymus, and PBL-thymus mixtures: the effect of varying the number of PBL in the mixture. Mean spleen indices in litters of C57BL/6N × BALB/c F_1 neonatal recipients after injection of different numbers of thymocytes (▲) or PBL (●) are shown on the right-hand side of the graph. On the left, reactions produced by different numbers of PBL (inactive alone) mixed with 5×10^6 thymocytes (○), 2.5×10^6 thymocytes (■), or 1×10^6 thymocytes (□) are shown. Each point represents data from four to ten recipient litters; vertical bars denote the limits of one standard error. Spleen indices are plotted against the logarithm of the number of cells injected.

produced a reaction in excess of that expected by adding the reactivities of the separate components. (3.5×10^5 PBL + $\frac{1}{20} \times 5 \times 10^6$ thymocytes = $3.5 \times 10^5 + 2.5 \times 10^5 = 6 \times 10^5$. The expected spleen index is 1.7; the observed spleen index is 2.3.) Reduction in the numbers of PBL resulted in a gradual loss of reactivity in the mixtures. Over most of the range tested there was a linear relationship between the spleen index and the logarithm of the number of PBL added to any fixed inoculum of thymocytes. Fig. 2 shows that a similar relationship was observed when the numbers of PBL were fixed and the numbers of thymocytes varied; there was linear regression of the spleen index to the logarithm of the number of thymocytes added to each

fixed number of PBL. Reactions produced by appropriate combinations of cells such as 5×10^6 thymocytes and 10^5 PBL, or 2.5×10^6 thymocytes and 5×10^4 PBL, were greatly in excess (between two- and threefold) of those expected by adding separate reactivities.

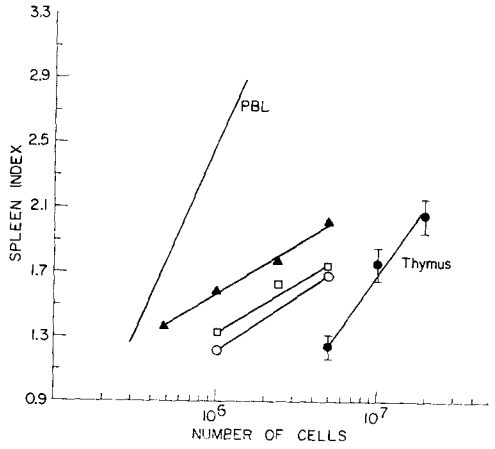

FIG. 2. GVH reactions produced by mixtures of PBL and thymus cells: the effect of varying thymus cells in the mixture. GVH reactivity curves obtained with PBL-thymus mixtures where a constant dose of either 10^5 (▲), 5×10^4 (□), or 2.5×10^4 (○) PBL was mixed with different numbers of thymocytes. Standard reactivity curves for thymus alone (●) or PBL are shown for comparison. Vertical bars denote the limits of one standard error.

TABLE I

Failure of F_1 Thymocytes or PBL to Substitute for Parental Cells in the Production of GVH Reactions in F_1 Hosts

Donor of 10^5 PBL	Donor of 5×10^6 thymocytes	Individual spleen indices					Mean spleen index ± SE
BALB/c	BALB/c	2.33	2.33	2.20	1.82	2.02	2.17 ± 0.07
		2.15	2.09	2.41			
BALB/c	C57BL/6	1.80	2.28	1.40	1.92	2.26	2.37 ± 0.18
		2.04	2.46	3.32	3.44	2.00	
		3.24	2.26				
BALB/c	F_1	0.98	1.02	1.10	1.39	1.00	1.12 ± 0.07
		1.22	1.20	1.10	1.08		
F_1	BALB/c	1.02	0.95	1.75	1.05	0.82	1.08 ± 0.08
		1.12	1.11	0.89	1.02	1.10	

Failure of PBL or Thymus Cells from C57BL × BALB/c F_1 Hybrid Donors to Interact with Parental Strain Cells.—It was shown in a previous study that combinations of parental lymph node cells and thymocytes produced synergistic interactions in the GVH reaction in F_1 recipients (2). A similar inter-

action was observed when cells from these two tissues of BALB/c origin were inoculated into C57BL/6 newborn recipients. In the latter situation, substitution of either adult F_1 thymocytes or adult F_1 lymph node cells failed to produce increased reactions. Table I shows the results of substituting adult F_1 thymo-

TABLE II

Failure of Marrow Cells from BALB/c Mice to Produce Synergy with PBL or Thymocytes

BM $\times 10^{-6}$	T $\times 10^{-6}$	PBL $\times 10^{-6}$	Individual spleen indices				Mean spleen index $\pm$ 1 SE
8	—	—	0.97	1.02	0.77	1.21	1.00 $\pm$ 0.06
			1.08	0.97			
1	5	—	0.72	1.09	0.85	1.08	0.98 $\pm$ 0.06
			1.07	1.12	0.77		
4	—	0.1	1.16	1.16	1.35	1.32	1.25 $\pm$ 0.05
6	—	0.1	1.26	1.23	1.16	1.39	1.13 $\pm$ 0.04
			1.19	0.94	1.17	0.99	
			0.97	1.11	1.05		

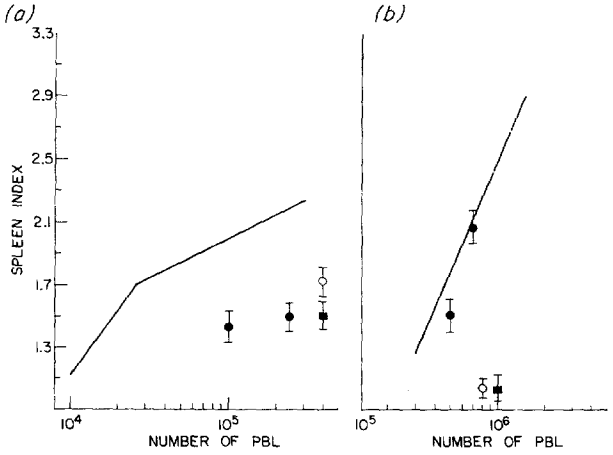

FIG. 3. (*a*) Effect of different doses of ATS upon capacity of PBL to produce synergistic reactions with thymocytes. The standard reactivity curve for 5×10^6 thymocytes and decreasing numbers of PBL is shown. A substantial loss of activity was noted if the PBL component had been obtained from mice treated with either 0.05 ml (●), 0.15 ml (○), or 0.25 ml (■) of ATS. Vertical bars denote the limits of one standard error; each point represents the mean of three to seven recipient litters.

(*b*) Effect of different doses of ATS upon GVH reactivity of PBL. The standard reactivity curve for PBL is shown. Virtually no decrease in activity was seen 3 days after the administration of 0.05 ml of ATS (●). By contrast, a severe depletion of reactivity was seen after the administration of either 0.15 ml (○) or 0.25 ml (■) of ATS.

cytes or PBL for parental cells in F_1 recipients. It is again clear that no synergy results if one of the components is not able to recognize host histocompatibility antigens.

Failure of Adult Parental Bone Marrow Cells to Interact with Thymocytes of PBL in the GVH Reaction.—Table II shows the results of combining bone marrow (BM) cells either with PBL or thymocytes. No significant splenomegaly was produced in either case.

The Effect of Prior Treatment with ATS on the Capacity of PBL to Interact with Normal Thymocytes.—PBL were obtained from BALB/c mice that had received either 0.05, 0.15, or 0.25 ml of ATS 3 days previously. The effects of

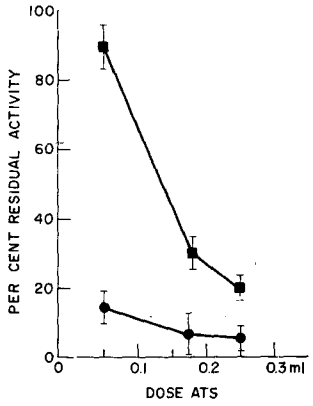

FIG. 4. The differential effects of ATS upon inherent GVH activity and synergistic activity of PBL. The per cent residual GVH activity in PBL is shown 3 days after the administration of different doses of ATS (■). The lowest dose (0.05 ml) has practically no effect on GVH activity. By contrast, all three doses shown severely impair the ability of PBL to produce synergistic responses with added thymocytes (●). The vertical bars denote the limits of one standard error.

these three doses of ATS on the GVH reactivity of PBL are shown in Fig. 3 *b*. Almost no reduction in reactivity was seen after injection of 0.05 ml of ATS. In contrast, treatment with the higher doses of ATS resulted in a marked reduction in GVH activity; PBL obtained from animals treated with either of the two higher doses suffered at least a 70% loss of activity. The effects of ATS on directly measured GVH activity are summarized in the top curve of Fig. 4.

PBL obtained from mice treated with these doses of ATS were tested for their capacity to interact synergistically with normal thymocytes. PBL from mice treated with 0.05 ml of ATS, as well as PBL from mice treated with the higher doses of ATS, produced much smaller spleen indices in combination with 5×10^6 thymocytes than did PBL from untreated animals. The capacity of each of these populations of PBL relative to the capacity of normal PBL

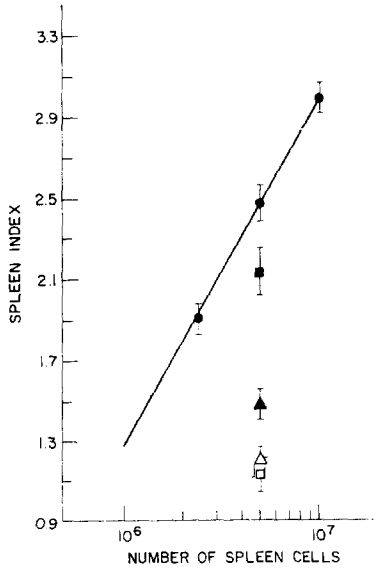

FIG. 5. GVH reactivity of spleen cells after treatment with ATS: the restorative effects of PBL. The standard reactivity curve for normal spleen cells is shown (●). The reaction produced by 5×10^6 spleen cells 3 days after the administration of 0.25 ml of ATS (□) is compared with the reaction produced when 1×10^5 PBL was added to the inoculum (■). The reaction produced by 5×10^6 spleen cells after 0.50 ml of ATS (△) is compared with the response when 1×10^5 PBL was added to the inoculum (▲). The dose of PBL was not active alone (see Fig. 1).

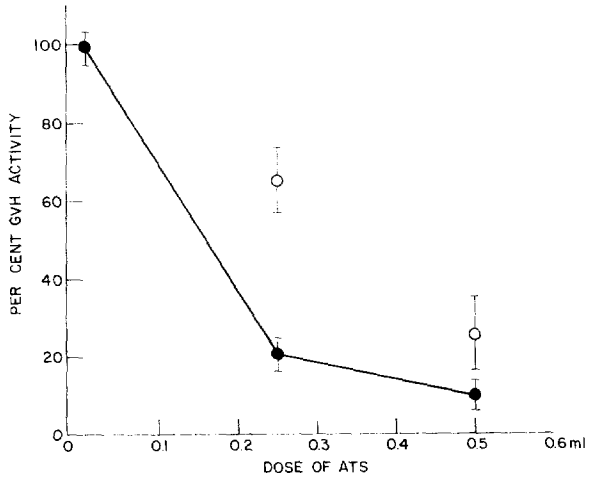

FIG. 6. GVH activity of spleen cells after treatment with ATS: restoration by PBL. The per cent residual GVH activity of spleen cells 3 days after the administration of different doses of ATS is shown (●). The effects of adding 1×10^5 PBL to these cells is also shown (○). Vertical bars denote the limits of one standard error.

to interact with thymocytes can be obtained by reference to the dilution curves in Fig. 1. The lower curve in Fig. 4 shows the relative reactivity of identical populations of PBL combined with 5×10^6 thymus cells. It can be seen that the capacity to interact with thymus cells was lost somewhat more rapidly than the capacity to produce a directly measured reaction.

GVH Activity of Spleen Cells after Treatment with ATS.—The open symbols in Fig. 5 show the spleen indices produced by 5×10^6 spleen cells from mice

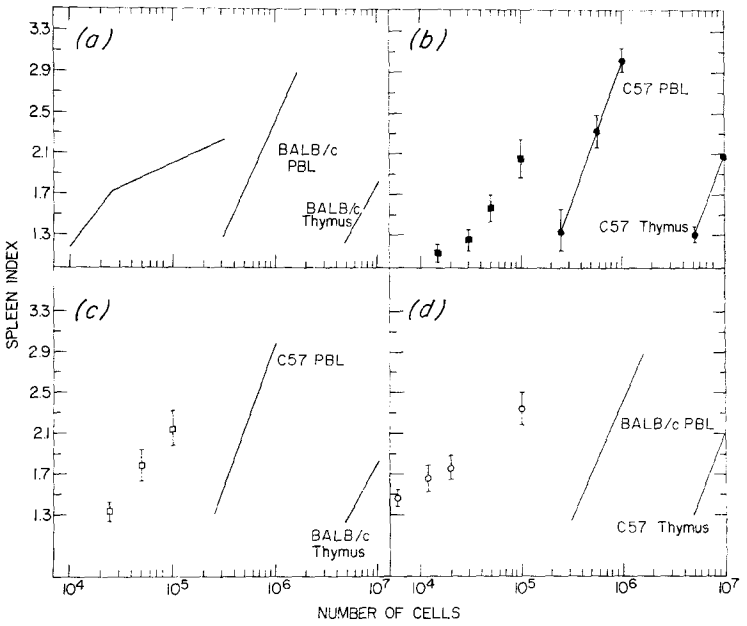

FIG. 7. GVH reactions produced in F_1 hybrid recipients by combinations of PBL and thymocytes from both parents. (*a*) Shows the standard reactivity curves for PBL, thymus, and different numbers of PBL combined with 5×10^6 thymocytes from BALB/c mice. (*b*) Shows similar reactivity curves produced by PBL and thymus cells obtained from C57BL/6 donors. (*c*) and (*d*) Show the synergistic GVH responses produced by a mixture composed of C57 PBL and BALB/c thymocytes (*c*) or BALB/c PBL and C57 thymocytes (*d*).

treated 3 days previously either with 0.25 ml or 0.5 ml of ATS. Previous work has shown that these points will fall on lines parallel to the reactivity curve for normal spleen (7). These doses of ATS produced at least an 80% suppression of GVH activity. The closed symbols show the spleen indices produced by these cells after the addition of 10^5 PBL. Substantial restoration of activity was observed with the spleen cells obtained from mice that had been treated with a lower dose of ATS. Little restoration was seen with the population obtained from mice treated with the higher dose. The curve in Fig. 6 shows the reduction in GVH activity of spleen cells as a function of the dose of

ATS, when the reactivity is measured directly (6). The open circles show the activity of spleen cells from treated mice combined with 10^5 PBL relative to normal spleen cells. Directly measured GVH activity was lost at lower doses of ATS than activity in combination with PBL.

GVH Reactions Produced in F_1 Hybrid recipients by Combinations of PBL and Thymocytes from Both Parents.—Fig. 7*a* shows standard reactivity curves for thymocytes, PBL, and different numbers of PBL combined with 5×10^6 thymocytes from BALB/c mice. These curves are taken from Fig. 1 and are shown for reference. Fig. 7 *b* shows spleen indices produced by identical numbers and similar combinations of cells obtained from C57BL donors. Both thymocytes and PBL from C57BL mice appeared slightly more reactive in the same F_1 hosts than did those of BALB/c mice. Synergistic interaction between PBL and thymocytes of this strain were observed and are shown by the

TABLE III

Lack of Synergy in Mixtures of BALB/c and C57 Spleen Cells

BALB ($\times 10^{-6}$)	C57 ($\times 10^{-6}$)	Individual spleen indices					Mean spleen index ± SE	Expected* spleen index
2	2	3.05	2.82	2.44	2.86	1.87	2.57 ± 0.12	2.74
		2.25	2.66	2.45	2.73			
1	0.1	1.50	1.66	1.85	1.78	2.02	1.74 ± 0.008	1.82
		1.63	1.40	2.05				
0.1	1	1.40	1.44	1.52	1.34	1.38	1.57 ± 0.05	1.50
		1.64	1.71	1.78	1.69	1.79		

* Calculated by adding the separate reactivities of the constituent cells.

closed squares. Figs. 7 *c* and *d* show reactions produced by combining C57BL PBL with BALB/c thymocytes or C57 thymocytes with BALB/c PBL. Reactivity curves for the uncombined populations are shown for reference. The reactions produced were somewhat larger than those found with combinations of C57 thymocytes and C57 PBL.

It was necessary to establish that the allogeneic combinations shown in Fig. 7 did not produce synergy by virtue of mutual immunization. Table III shows spleen indices produced by different proportions of spleen cells from C57BL and BALB/c donors inoculated together into F_1 recipients. It is clear that no increase in reactivity was observed. In fact, some decrease over expected reactivity may be present.

Double-Transfer Experiments.—The results of three such experiments, as outlined in Materials and Methods, using thymocytes from C57BL adult donors and PBL from BALB/c adult donors are summarized in Table IV. 10 million or 30×10^6 cells from recipients of 25×10^6 thymocytes produced

positive GVH reactions (spleen indices greater than 1.3) in 9 of 17 secondary BALB/c recipients. No reactions were produced by these cells in C57 recipients. 10 million cells from recipients of 1–1.5 × 10^6 BALB/c PBL produced positive reactions in 1 of 13 secondary C57BL recipients and none in BALB/c recipients. When the first recipients received the combination of 10^5 BALB/c PBL and 10^7 C57 thymocytes, cells from their spleens produced reactions in 21 of 30 BALB/c secondary recipients but in only 2 of 15 C57 secondary recipients.

Effects of Neonatal Thymectomy on GVH Reactivity of Spleen Cells either alone or in Combination with PBL from Normal Mice.—Fig. 8 shows the reactivity of 5 × 10^6 spleen cells obtained from BALB/c mice thymectomized on the day of birth. Cells were obtained at 10–11 wk of age. The mice employed had no overt signs of wasting, and inspection of their mediastina showed no residual thymus. A broken line parallel to the reactivity curve for normal spleen is drawn through this point, since it has been shown previously that reactivity curves obtained with different numbers of spleen cells from thymectomized BALB/c mice are parallel to those obtained using cells from normal animals (2). The open circle shows the reaction produced by combining 3.5 × 10^6 of these spleen cells with 10^5 PBL from normal mice. A slight increase in

TABLE IV

Secondary Transfer

Exp. No.	Group No.	Donor cells (× 10^{-6})		Cells from primary recipient	Reaction in secondary recipient	
		BALB PBL	C57 Thym.		BALB	C57
1	1	0.75	—	7.5	—	0/8 (1.08)
	2	—	10.0	7.5	0/9* (1.03)	—
	3	0.1	5.0	15.0	3/4 (1.40)	0/6 (0.93)
	4	0.1	5.0	7.5	6/7 (1.40)	1/3 (1.15)
2	1	1.5	—	10.0	—	0/7 (1.03)
	2	—	25.0	10.0	3/6 (1.25)	—
	3	0.3	10.0	30.0	3/4 (1.38)	0/4 (0.97)
	4	0.3	10.0	10.0	3/8 (1.20)	0/6 (0.99)
3	1	1.5	—	10.0	0/8 (1.02)	1/6 (1.01)
	2	—	25.0	10.0	3/7 (1.31)	0/6 (1.04)
	3	—	10.0	10.0	0/6 (1.04)	0/7 (1.10)
	4	0.1	10.0	10.0	6/7 (1.38)	1/6 (1.08)

Summary:

1	BALB-PBL → F_1 → C57	1/13 high dose; 0/8 low dose
2	C57 − T → F_1 → BALB	6/13 high dose; 0/15 low dose
3	BALB-PBL + C57 − T → F_1 → BALB	21/30
4	BALB-PBL + C57 − T → F_1 → C57	2/25

* Fraction = No. > 1.3 of total; numbers in parentheses = mean spleen index.

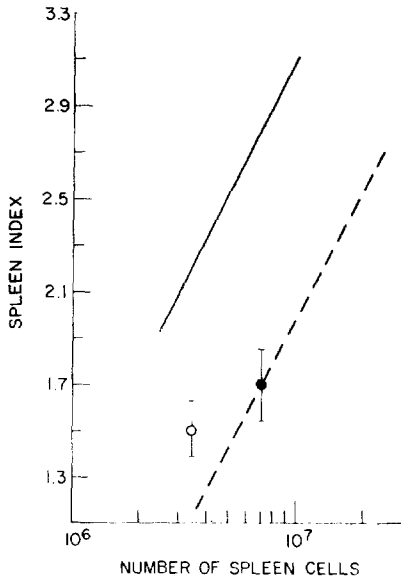

FIG. 8. Effects of neonatal thymectomy on the GVH reactivity of spleen cells alone or in combination with PBL from normal mice. The solid line represents the standard reactivity curve obtained for spleen cells from normal mice. The GVH reactivity of 5×10^6 spleen cells obtained from mice that had been thymectomized on the day of birth is also shown (●). The reactions produced by 3.5×10^6 of these cells combined with 1×10^5 PBL from normal animals is also shown (○). A slight increase (<10%) was observed over the expected reactivity of this cell mixture. Vertical bars denote the limits of one standard error.

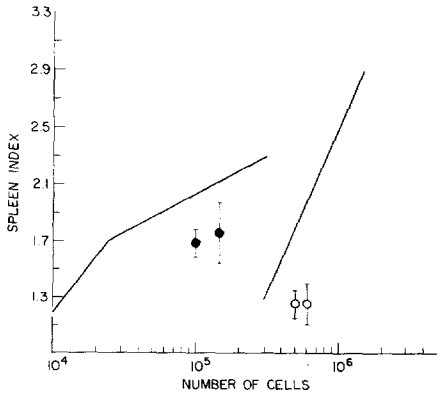

FIG. 9. Reactivity of PBL from neonatally thymectomized mice. Standard reactivity curves for normal PBL alone and in combination with 5×10^6 thymocytes are shown. No significant reactions were produced by 5×10^5 and 6×10^5 cells obtained from neonatally thymectomized donors. When 1.5×10^5 or 1.0×10^5 PBL from thymectomized mice were combined with 5×10^6 thymocytes, substantially reduced reactions were noted (see text). Vertical bars denote the limits of one standard error.

reactivity was observed. It was calculated that this increase did not exceed by more than 10% the expected reactivity of this combination of cells.

Reactivity of PBL from Neonatally Thymectomized BALB/c Mice Measured either alone or in Combination with 5 × 10^6 Thymocytes from Normal Mice.— The right-hand part of Fig. 9 shows reactions produced by PBL from thymectomized mice. A standard reactivity curve for normal PBL is shown for reference. Significant spleen indices were not produced by either of the two doses of PBL used, 5 × 10^5 or 7 × 10^5. The closed circles in the left-hand side of this figure show GVH reactions produced by combining different numbers of these PBL with 5 × 10^6 thymocytes from normal mice. The reactivity curve for 5 × 10^6 normal thymocytes combined with different numbers of normal PBL is shown for reference. Since the two doses of PBL tested gave reactions which fell on a line parallel to that produced by combining different numbers of PBL with thymocytes, it can be estimated that thymectomy at birth resulted in a reduction of the capacity to interact with thymocytes of about 50–75%.

DISCUSSION

This paper presents a further analysis of the synergistic interaction that can be observed when appropriate combinations of lymphoid cells are inoculated into allogeneic recipients. The data suggest that two classes of T lymphocytes interact in this cell-mediated response. One class, T_1, was found mainly in the spleen and thymus and was characterized by a resistance to the in vivo effect of ATS. A second class, T_2, was extremely susceptible to ATS in vivo and was found mainly in the peripheral blood and lymph nodes.

The major lesion produced by ATS in moderate doses appears to be in the pool of recirculating lymphocytes (9, 10). The importance of this pool of cells in the production of GVH reactions in rats has been shown in studies of thoracic duct cells by Gowans et al. (11). In accord with this idea, we have reported previously that in mice splenic GVH activity was markedly reduced after drainage of only 20–60 × 10^6 cells from the thoracic duct, suggesting that GVH activity in fixed tissues such as the spleen was also a function of itinerant recirculating lymphocytes (3). This idea must now be reconsidered in light of the present observation that the loss of GVH activity in spleen after treatment with modest doses of ATS could be restored substantially by small numbers of cells from the circulating pool of untreated mice. This finding suggests that a second residual cell which is relatively resistant to ATS, and which probably recirculates slowly or is sessile in lymphoid tissues, also may participate in the GVH response together with recirculating cells. Moreover, the observation that very low doses of ATS produced a modest reduction in the direct GVH activity of circulating lymphocytes, but produced a striking decrease in the capacity of these cells to interact with thymocytes, indicates that an excess of the interacting, recirculating, component exists in the blood. These findings

present an interesting parallel to studies of antibody responses which show that T helper activity is sensitive to ALS (12) and recirculates rapidly (13) while B precursors of antibody-forming cells do not. This parallel suggests, but of course does not prove, that the class of cell interacting with B cells in the antibody response and the class of cells interacting with thymocytes, and spleen cells from mice treated with moderate doses of ATS, belong to a single functional group.

The existence of this kind of parallel, for example an interaction among T cells similar to that observed between T cells and B cells, has been questioned. Doubt has been raised because it is known that the enlargement of the spleen seen in the host in GVH reactions is in part the result of an inflammatory response (14). Studies have shown that irradiated mice, which are incapable of mounting such an inflammatory response, and therefore show no splenomegaly, are capable of reactive splenomegaly after restoration with bone marrow cells of either syngeneic, hemiallogeneic, or allogeneic origin (15, 16). It is most unlikely that this artifact accounts for the synergy seen in the present study. First, bone marrow from allogeneic donors failed to increase the reactivity either of thymocytes or PBL. Apparently, in unirradiated mice, the inflammatory response of the host is sufficient to react to the immunologic insult produced by allogeneic lymphoid cells without the addition of B cells. Second, both the specificity controls reported previously (2) and those reported in this paper show that both cell components must be allogeneic to the host if synergy is to be observed. Third, synergy between PBL and thymocytes can be demonstrated in a mortality assay.[2] This latter finding probably makes speculation concerning requirements for inflammatory cells irrelevant. Finally, it seems unlikely that the results obtained could be explained by "thymic function" (17), an apparently hormonal factor which expands T cell activity. Such effects have not been observed with dissociated thymocytes as prepared in these experiments, they do not require specific reactivity to the host, and they do not seem relevant when combinations such as PBL and spleen cells from animals treated with ATS are used.

The secondary transfer experiments described in this paper demonstrated two points. First, activation of parental thymocytes in primary F_1 hosts occurred, since cells from these hosts, presumably of donor origin, could mediate a second GVH reaction when transferred to secondary newborn hosts allogeniec but not syngeneic to the donor of the thymocytes. Second, this activation was clearly increased when thymocytes were combined with PBL. The results indicate that, for the mixtures used, the cell in excess in the thymus determines the specificity of the immunologic injury leading to enlarged spleens and in this

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[2] Tigelaar, R. E., and R. Asofsky. 1972. Synergy among lymphoid cells mediating the graft-*versus*-host reaction. IV. Synergy in the GVH reaction quantitated by a mortality assay in sublethally irradiated recipients. *J. Exp. Med.* In press.

case was the precursor of GVH effector cells; the cell in excess in the PBL appears to amplify the reactivity of this first cell.

Since both activities were strikingly reduced 10 wk after neonatal thymectomy, it may be concluded that both activities are thymus dependent and reside in T lymphocytes. This is in agreement with the recent finding that both activities, as measured in the spleen and lymph node, are sensitive to the in vitro cytotoxic action of anti-theta antiserum and guinea pig complement (18). It is worth noting that the effect of neonatal thymectomy is in certain respects opposite to the effect of heterologous ATS. The latter reagent produced a marked deficit in amplifier activity, but only a modest deficit in precursor activity unless very high doses were used. By contrast, neonatal thymectomy resulted in almost complete loss of precursor activity with preservation of 25–50% of amplifying activity.

It is clear from this series of papers that GVH reactions are mediated, at least in part, by an interaction among T lymphocytes that may be analogous to the interaction between T lymphocytes and the precursors of antibody-forming cells in many humoral antibody responses (19–21). The two types of T lymphocytes which engage in GVH reactions show different distributions among the different lymphoid compartments, different sensitivity to ATS in vivo and anti-theta in vitro (17), and are differently affected by neonatal thymectomy. Two hypotheses have been put forward to explain the observations. The first states that both the interacting T lymphocytes belong to a single differentiated line but differ in their degree of maturation. In this scheme, T_1 cells differentiate from thymocytes within the thymus, and then further mature to T_2 cells within the thymus or more commonly in the periphery. T_1 cells are immature, short-lived, nonrecirculating (and thus resistant to ATS in vivo), are present in large numbers in the spleen but in relatively low numbers in the blood, and are more sensitive to anti-theta than T_2 cells but less so than thymocytes (17). T_2 cells would be more mature, would recirculate rapidly, and would therefore be sensitive to small doses of ATS. Further, they would be long-lived and capable of amplifying cellular responses via interaction with T_1 cells and antigen as seen here, or humoral antibody responses via interaction with B cells. Evidence for this hypothesis of T cell maturation is discussed more fully elsewhere (21–23). The alternative hypothesis states that there are at least two separate, differentiated lines of T lymphocytes in peripheral tissue and thymus. The observed properties of amplifiers and precursors are thought, in this view, to be properties belonging to each of these lines. Each hypothesis makes specific, and usually mutually exclusive predictions. For example, the first hypothesis suggests that if the supply of new T cells in the adult is removed by thymectomy, amplifiers will arise at the expense of precursors; this effect is suggested by the effects of neonatal thymectomy reported here. The second hypothesis suggests that in this situation amplifying and precursor activity will both be lost, although perhaps at some-

what different rates. As yet, the data do not permit definitive exclusion of either hypothesis.

SUMMARY

Two types of thymus-derived (T) lymphocytes have been shown to cooperate in the induction of graft-*versus*-host responses. One cell type is found in highest concentrations in the peripheral blood and lymph node, is extremely sensitive to anti-thymocyte serum (ATS) in vivo, and is probably part of the recirculating lymphoid cell pool (3). The second cell type, found in highest concentrations in the thymus and spleen, is relatively resistant to small doses of ATS in vivo. Both cell types are substantially depleted after neonatal thymectomy.

Moreover, since synergism was also obtained using appropriate mixtures of cells from either parental strain in F_1 hosts, it was possible to show that the nonrecirculating cells determined the specificity of the response and were probably the precursors of effector cells in this response. The recirculating T cell appeared to amplify this response. The implications of these data are discussed.

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