

T-LYMPHOCYTE PRECURSORS

I. Synergy Between Precursor and Mature T Lymphocytes in the Response to Concanavalin A*

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Thymus-derived lymphocyte precursors (pre-T cells)¹ are found in adult mammalian bone marrow. The precursor nature of the cells can be demonstrated by their ability to reconstitute the thymus of irradiated recipients (1). This procedure is tedious, not easily quantitated, and only rarely applicable to humans. Fortunately, another assay for pre-T cells has recently been described: in the presence of certain "inducers," notably thymus humoral factors, pre-T cells rapidly express surface antigens characteristic of more mature, thymic cells (2). The number of such induced cells can be quantitated by a cytotoxic assay (2) or by immunofluorescence (3). Whether all pre-T cells are detected in this way is unknown.

If pre-T-cell maturation is analogous to that of other hematopoietic precursor cells, there will be several maturation stages between the uncommitted hematopoietic stem cell and the final pre-T cell, that is, the cell that enters the thymus and begins the intrathymic differentiation process. The assay using induction of surface markers is of limited use in distinguishing different maturation stages. We have been looking for functional markers of pre-T-cell maturation, and here report on a novel property of certain pre-T cells: the ability to enhance the response of mature T cells to the phyto mitogen concanavalin A (Con A).

Materials and Methods

Mice. Most experiments reported here used male CBA/J mice obtained from The Jackson Laboratory, Bar Harbor, Maine. They were purchased at 5 wk of age and kept in our animal house at least 3 wk before use, to minimize possible stress-induced sequestration of T cells in their bone marrow. Male CBA/CaJ and CBA/H-T6J mice were also obtained from The Jackson Laboratory. Male AKR/J mice, 8-wk old, were a gift of Dr. P. A. Campbell. Nude (athymic) mice bred on a C57BL/6 background were also supplied by Dr. Campbell.

Lymphocyte Preparations. Bone marrow (BM) was flushed from tibias and femurs with Hanks' balanced salt solution (HBSS), passed once through a 25-gauge needle, washed in HBSS, and resuspended in tissue culture medium (TCM) consisting of 5% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y., lot number 730703—the importance of using a selected lot will be discussed) in RPMI 1640 with 100 µg/ml streptomycin and 100 U/ml penicillin. Cortisol-

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¹ *Abbreviations used in this paper:* BM, bone marrow; Con A, concanavalin A; CRT, cortisol-resistant thymocytes; [³H]TdR, tritium-labeled thymidine; HBSS, Hanks' balanced salt solution; PHA, phytohemagglutinin; pre-T, precursors of T cells; TCM, tissue culture medium.

resistant thymocytes (CRT) were prepared from the thymuses of mice given 2.5 mg of hydrocortisone acetate (Cortril, Pfizer Inc., New York) intraperitoneally 2 days previously. The thymuses were disrupted by gentle tapping on 100-mesh wire screens, and the cells were passed once through a 25-gauge needle, washed in HBSS, and resuspended in TCM. Cell suspensions were similarly prepared from normal lymph nodes and spleen. Total nucleated cells were counted on a hemocytometer.

Peritoneal cells were obtained by washing the peritoneums of normal CBA/J mice with 3 ml of HBSS; recovery was about 2×10^6 cells/mouse. 3T3 cells, harvested as they approached density-dependent growth inhibition, were kindly supplied by Dr. John Lehman. Glass-adherent cells were prepared from spleen by the method of Mosier (4).

Cells with surface immunoglobulin (Ig) were removed from bone marrow by passage through a column of polymetacrylate beads to which mouse Ig and rabbit anti-mouse Ig had been bound (5).

Lymphocyte Cultures. When it was intended to measure proliferation, cultures were set up in Microtest II plates (Falcon Plastics, Oxnard, Calif.) in a total vol of 200 μ l. In our standard conditions for demonstrating interactions, 6×10^5 BM cells and 2×10^6 CRT cells were used, either separately or mixed. One set of triplicate cultures had no further additions, and Con A (final concentration 2 μ g/ml) was added to another set. The plates were incubated at 37°C in humidified 5% CO₂ in air for 48 h; 0.5 μ Ci of tritiated thymidine (³H]TdR, Schwartz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y., 6.7 Ci/mM) were added 6 h before harvest. Incorporation of the label into trichloroacetic acid insoluble material was determined by liquid scintillation counting.

In some experiments blast transformation was determined. Aliquots of cultures were diluted in 2% acetic acid and examined by phase contrast microscopy. Those cells with diameters at least thrice that of a small lymphocyte and with enlarged nuclei with visible nucleoli were counted as lymphoblasts. Other aliquots were stained with (a) rabbit anti-mouse immunoglobulin labeled with fluorescein isothiocyanate (RAMIG-FITC, Behring Diagnostics, Inc., Woodbury, N. Y.), (b) anti-Thy-1.1, followed by RAMIG-FITC, or (c) anti-Thy-1.2, followed by RAMIG-FITC. Cells bearing Thy-1 were enumerated by subtracting the percentage of cells labeled with RAMIG-FITC alone (B cells) from the percentage staining with anti-Thy-1 and RAMIG-FITC. This method has been described (3).

Similar cultures were set up using CRT and BM from CBA/H-T6J and CBA/Ca mice. After 48 h of culture 1 μ g of colchicine was added per ml of culture, and 1.5 h later the cells were swollen, fixed, and examined for metaphases by the method of Ford (6). 100 metaphases were counted in each mixture.

Results

Synergistic Responses of Lymphocyte Mixtures to Con A. BM cells at a concentration of 6×10^5 /well (3×10^6 /ml) respond poorly to Con A (Table I). CRT at 2×10^5 cells/well (10^6 /ml) also respond poorly, in this case because the concentration of cells is suboptimal. The response of CRT is variable from experiment to experiment, as can be seen in Tables I and IV.

When BM and CRT are mixed in culture, the response to Con A is greatly enhanced (Table I). A "synergistic index" can be derived by dividing the observed uptake of [³H]TdR in the mixture by the sum of the uptakes by BM and CRT incubated separately. Indices of 3–40 have been observed. Other cell mixtures also give synergistic responses. Table I lists several of these: spleen + CRT, BM + spleen, BM + lymph node.

Most of our studies have centered on the BM-CRT interaction. We have found that the optimum concentration of Con A (2 μ g/ml) is the same for the mixtures as for the separate populations. Similarly, the peak response is not shifted, being on day 2 in this system. The optimal proportions of BM to CRT were examined in experiments presented in Fig. 1 and 2. Fig. 1 shows the response of

TABLE I
Synergistic Responses of Lymphoid Cell Mixtures to Con A

Exp.	Cells	Cells/Well $\times 10^{-5}$	Con A response <i>cpm</i>	Additive	Synergistic index*
1	BM	6	1,120	2,340	7.5
	CRT	2	1,220		
	BM + CRT	6 + 2	17,600		
2	Spleen	6	45,800	55,900	3.4
	CRT	2	10,090		
	Spleen + CRT	6 + 2	190,200		
3a.	BM	6	4,370	7,200	2.9
	Spleen	1	2,820		
	BM + spleen	6 + 1	20,840		
3b.	BM	6	4,370	12,510	4.0
	Lymph node	1	8,140		
	BM + lymph node	6 + 1	50,320		

* Con A response of mixture divided by sum of Con A responses of individual members of mixture.

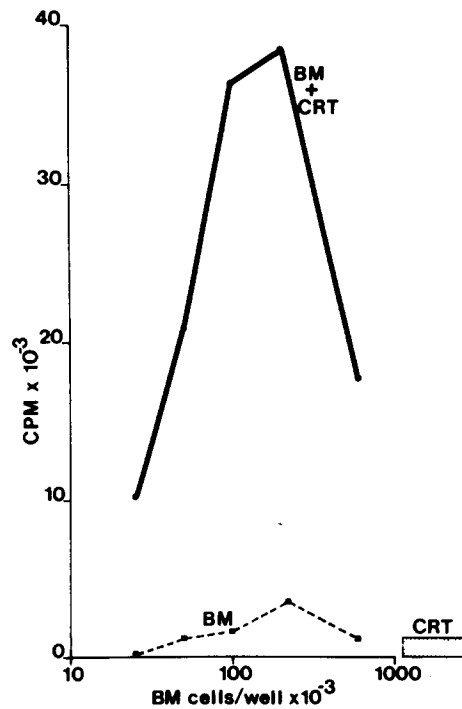


FIG. 1. Response of 2×10^5 CRT to Con A (stippled bar) and response of the same number of CRT in the presence of variable numbers of BM cells (upper curve). The lower curve shows the response of BM cells alone.

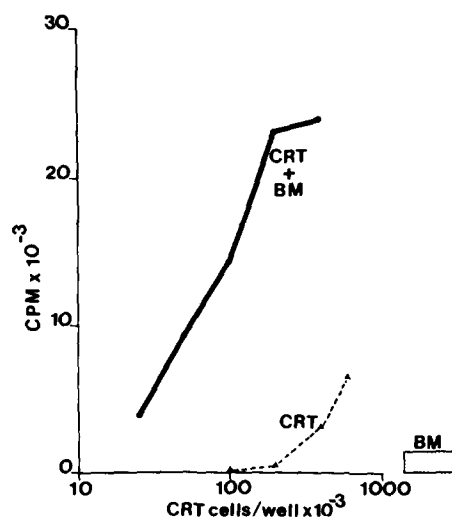


FIG. 2. Response to Con A of variable numbers of CRT alone (lower curve) and in the presence of 2×10^5 BM cells (upper curve). The response of 2×10^5 BM cells alone is also shown (stippled bar).

a constant number of CRT in the presence of variable numbers of BM cells. There is a sharp increase in [^3H]TdR incorporation as the BM dose is increased from 0.25 to 1.0×10^5 cells/well; the slope of a log:log plot of this part of the curve is 1.0 . After a plateau, there is a marked decrease in synergy at high BM concentrations. Different batches of CRT and BM have different optima; in general, best synergy is seen with a BM:CRT ratio of $1:1$ – $3:1$.

Fig. 2 shows typical dose-response curves of CRT, both alone and in the presence of a constant number (2×10^5) of BM cells/well. The slope of log CRT number vs. log cpm is 2.2 for CRT alone (mean in seven experiments was 2.3 ± 0.22 standard error [SE]). Similarly plotted, the slope for CRT in the presence of constant BM was 0.9 (mean 0.83 ± 0.05 SE).

BM Induces CRT to Respond to Con A. The next series of experiments was carried out to determine which partner (BM or CRT) actually contributed most of the synergistic [^3H]TdR uptake in the mixture. BM and CRT were prepared from both CBA/Ca and CBA/H-T6J mice, mixed reciprocally, and stimulated with Con A. Spreads of metaphase cells were examined at day 2 for normal and T6T6 karyotypes. Using T6T6 BM and normal CRT (Table II, experiment 1), 86% of classifiable metaphases were of CRT type; in the reciprocal mixture, 100% of classifiable metaphases were of CRT type. The difference between the two results is probably technical, it being easier to establish that a metaphase is T6T6 than that it is definitely not. Thus, on the average, 93% of the metaphases we could identify were of CRT origin.

A second approach to the same question involved mixing CRT and BM of AKR/J and CBA/J donors. These cells are matched at *H-2* but differ at *Thy-1*. They also differ at the *M* locus, but in our hands give no detectable MLC (probably due to the immaturity of BM and CRT and to a suboptimal incubation time). Cells that underwent blast transformation were identified by surface

TABLE II
Cells with T6/T6 and Normal Karyotypes in Mixtures of CBA/H-T6 and CBA/Ca Bone Marrow and Cortisol-Resistant Thymocytes

Exp.	Bone marrow	CRT	Metaphases		
			T6/T6	Normal	Unclassified
1	T6/T6	Ca	12	71	17
2	Ca	T6/T6	88	0	12

TABLE III
Lymphoblasts Bearing Thy-1.1 and Thy-1.2 in Con A-Stimulated Cultures of BM and CRT from CBA/J and AKR/J Donors

Bone marrow	CRT	Synergistic index*	Thy-1.1	Thy-1.2
			%	%
CBA (Thy-1.2)	AKR (Thy-1.1)	11	92	4
AKR	CBA	19	20	74

* Total blasts/culture in mixtures divided by the sum of blasts/culture in separate BM and CRT cultures.

markers; surface Ig, Thy-1.1, and Thy-1.2 were detected by immunofluorescence. From Table III it can be seen that there was good synergy for blast transformation. No Ig-positive blasts were observed, and 94–96% of total blasts bore either Thy-1.1 or Thy-1.2. A mean of 83% of blasts were of CRT origin.

What is the "Stimulator" Cell in BM? The following experiments were designed to determine if a specific cell type in BM were responsible for inducing the enhanced response of CRT to Con A. Since our first thought was that any cell might work, possibly by a feeder effect, we added contact-inhibited 3T3 cells to CRT. Not only was there no synergy, there was profound inhibition of [³H]Tdr incorporation (Table IV). Similarly, neither normal peritoneal cells nor purified splenic adherent cells enhanced the response of CRT. Thus the possibility of CRT being deficient in adherent cell function could be eliminated.

BM contains considerable numbers of B lymphocytes, which could conceivably be responsible for the synergy. To test this, BM was run through an anti-Ig column (Table IV). The unprocessed BM contained 30% small lymphocytes, of which 20% were surface Ig positive. The column-passed cells were also 30% small lymphocytes; of these, fewer than 0.5% were Ig positive. The column-passed (B depleted) BM synergized slightly better than did the unseparated BM, whose synergistic index was 6.3. Nylon wool columns (7) also failed to remove the CRT-stimulating activity (data not shown, because the removal of B cells was incomplete).

Although BM contains few T cells (that is, cells that are Thy-1 positive and post-thymic), it does contain some, and T-T interaction was a possible explanation of our results. Accordingly, BM and spleen from nude mice were mixed with CRT and synergy measured. Neither spleen nor BM responded significantly to Con A by themselves, and yet both enhanced the Con A response of CRT.

TABLE IV
Different Cell Populations as Stimulators of Con A Response of 2×10^5 Cortisol-Resistant Thymocytes

Exp.	Cell population tested	CRT	Con A response	Additive	Synergistic index
			<i>cpm</i>		
1.	3T3 (6)*	0	50	5,080	0.02
	—	+	5,030		
	+	+	90		
2.	Peritoneal washings (6)	0	7,570	13,460	0.4
	—	+	5,890		
	+	+	5,600		
3.	Adherent cells (3)	0	1,300	11,400	0.9
	—	+	10,900		
	+	+	10,300		
4.	Anti-Ig column-passed BM (6)	0	0	890	9.7
	—	+	890		
	+	+	8,670		
5a.	Nude bone marrow (6)	0	860	860	5.8
	—	+	0		
	+	+	5,040		
5b.	Nude spleen (6)	0	610	610	59
	—	+	0		
	+	+	35,780		

* Number of cells/well $\times 10^{-5}$.

Studies were done on the T-cell precursor content of nude BM and spleen. Suspensions of these cells were incubated overnight in TCM containing 200 $\mu\text{g}/\text{ml}$ mouse thymosin, fraction 3 (8), or 2×10^{-5} M 8-(*p*-chlorophenyl)thiocyclic AMP. They were then stained with anti-Thy-1.2 by the indirect technique. The results with both inducers were the same: 16% of the small lymphocytes in BM and 20% in spleen became Thy-1 positive. Since this BM contained 10% small lymphocytes and the spleen 80%, the overall pre-T content of BM was 1.6%, and of spleen, 16%. The correlation with the synergistic indices obtained with BM (5.8) and spleen (59) is striking.

Is the Stimulating Cell in BM a Pre-T Cell? We discovered fortuitously that BM incubated for 48 h in TCM before mixing with CRT and Con A lost its ability to synergize. We were thus able to see if we could add substances to maintain synergy. In Fig. 3 it can be seen that thymosin is such a substance. Other experiments, not shown here, have demonstrated that 8-(*p*-chlorophenyl)thiocyclic AMP will also maintain the synergistic ability of BM in vitro. In both cases the BM cells were adjusted to 3×10^6 cells/ml in TCM, and thymosin (final concentration 200 $\mu\text{g}/\text{ml}$) or cyclic AMP (final concentration 2.5×10^{-5} M) added. After 48 h of incubation the cells were washed twice, reconstituted to the original volume, and mixed with CRT in Microtest plates as described.

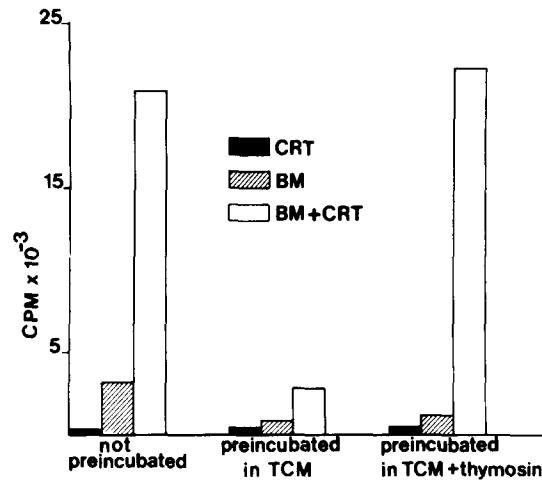


FIG. 3. Ability of 6×10^5 BM cells to stimulate the Con A response of 2×10^5 CRT. Bone marrow was used fresh or after 48-h incubation in TCM, or TCM containing 200 $\mu\text{g/ml}$ thymosin.

Discussion

When CRT and BM cells are mixed and Con A added, a response is seen that is much greater than the sum of the responses of CRT and BM cultured separately. This is true whether the response is measured by $[^3\text{H}]\text{TdR}$ incorporation or by counting transformed lymphocytes. Although our standard mixture contains three times as many BM cells as CRT, the bulk of the response is by the CRT cells. This was established both with chromosomal markers (Table II) and with surface antigens (Table III). These experiments showed that between 79 and 100% of identifiable responding cells were of CRT origin; and Table III also shows that 94–96% of all responding cells bore the Thy-1 antigen. We conclude, then, that when BM and CRT are mixed, the BM cells in some way enhance the response of T cells in CRT to Con A.

A possible explanation is that CRT are deficient in macrophages (or macrophage-like function), since it has been shown that macrophages are necessary for lymphocytes to respond well to mitogens (9, 10). However, added macrophages in the form of peritoneal washings or adherent cells (Table IV) failed to enhance the response of CRT to Con A. These experiments, and the one using 3T3 cells, also rule out the possibility that virtually any cell might stimulate CRT's response, possibly by presenting Con A to the T cells in a more efficient way.

The interaction of various numbers of CRT and BM cells is shown in Fig. 1 and 2. As few as 25×10^3 BM cells cause a marked increase in the Con A response of 2×10^5 CRT (synergistic index 2.4). The curve is nearly linear at lower concentrations of BM, with a log:log slope of 1.0. A similar slope is obtained when the concentration of CRT is varied in the presence of a constant number of BM cells (Fig. 2); the slope of CRT alone is about 2.3. The simplest explanation of the change in slope is that a minimum of two cells interact when CRT alone respond to Con A; both these cells are in limiting concentration (11).

The slope of 1.0 when CRT is titrated in the presence of BM might suggest a lack of cell-cell interaction; however, we think it more likely that this is an example of a pseudo-first order process and really represents the interaction of two or more cells, only one of which (the responsive CRT cell) is now limiting. Whatever the mechanism, it is clear that a given number of CRT respond to Con A far more vigorously in the presence of BM cells than in their absence. Whether this represents more cells being triggered into cycle, or the same number of cells being triggered into more rapid cycle, remains to be determined.

The phenomenon of synergy is not restricted to BM and CRT. Table I shows other combinations in which synergy has been observed. Thus, BM can synergize with spleen or lymph node. Using BM and spleen or lymph node from CBA and AKR mice in appropriate combinations, and staining the transformed cells as we did for BM-CRT mixtures, we have found that the majority of responding cells are T cells of spleen or lymph node origin in BM-spleen and BM-lymph node mixtures, respectively (unpublished). It appears that as a general principle, BM will induce mature T cells to respond to Con A better than they otherwise would.

The nature of the cell in BM which interacts with mature T cells in the Con A response is of considerable interest. We approached the question of its identity by a process of elimination. We have already discussed how we eliminated a nonspecific trephocytic effect and the possibility that CRT is deficient in macrophage (adherent cell) function. The B cell was eliminated as a candidate for synergizer because removal of B cells on an anti-immunoglobulin column did not diminish the BM's ability to synergize. Mature T cells were also eliminated by showing that nude BM or spleen could synergize with CRT, although neither contained detectable Thy-1 positive cells. (Normal BM treated with anti-Thy-1 serum and complement, and BM from neonatally thymectomized mice, also synergized with CRT; results not shown.)

Having ruled out T cells, B cells, and macrophages as synergizers, we were left with lymphoid precursors and other hematopoietic cells as possibilities. Our further studies have implicated a precursor of T cells. First, there is the striking correlation between content of pre-T cells in nude spleen and BM (as judged by the number of cells induced to express Thy-1 by thymosin) and their ability to stimulate the response of CRT to Con A. This evidence is circumstantial, however, and requires independent support, which is provided by the type of experiment shown in Fig. 3. BM, which was capable of normal synergy with CRT when fresh, lost virtually all this capacity after incubation for 48 h in TCM. If thymosin were added to the preincubation medium, however, synergy was maintained or enhanced. A similar maintenance was obtained if the BM was preincubated in TCM containing 2.5×10^{-5} M 8-(*p*-chlorophenyl)thio-cyclic AMP. The phosphodiesterase inhibitor RO20-1724 (12) did not maintain the synergistic property. On the other hand, a B-cell function, the ability to respond to lipopolysaccharide, was enhanced by preincubation with RO20-1724 or cyclic AMP, but not by thymosin.² This is evidence for the pre-T-cell specificity of our thymosin and further support for the view that thymosin works by raising the intracellular concentration of cyclic AMP (13). We believe these data indicate that there is a pre-T cell in BM and spleen which can interact with CRT and

² MacMillan, R. G., and J. J. Cohen. Manuscript in preparation.

other mature T cells in such a way that the T cells' response to Con A is greatly enhanced. This pre-T cell dies or becomes inactive on *in vitro* incubation unless it is maintained by thymic hormone. There is an elegant parallel to this hypothesis in studies of erythropoiesis. Murine proerythroblasts rapidly die in culture, unless incubated with erythropoietin, which maintains them and induces hemoglobin formation (14); in other words, differentiation is necessary for survival of precursors in culture.

The batch of fetal calf serum used in the TCM in which BM is preincubated is critical. About half of the batches we have screened maintain the synergistic ability of BM for 48 h as well as does thymosin. These batches have been shown to contain pre-T-cell inducer activity in a variety of other systems;³ the activity may be due to thymus hormone itself.

Interaction between subpopulations of T cells is a familiar concept in immunology, but to our knowledge this is the first reported interaction of T cells with precursors of T cells. There have been several systems described in which a similar phenomenon may have taken part. Tridente et al. (15) found that thymus and BM synergized for phytohemagglutinin (PHA) response, but only if the thymus derived from new-born mice; the cell in BM which synergized was not a macrophage. Winkelstein et al. (16) found an increased response to PHA in thymus-BM mixtures, but concluded that the increased response was contributed by BM cells. We too have seen BM-CRT synergy in PHA responses, but it is much less than with Con A and works best at a BM:CRT ratio of approximately 1:7 (unpublished results), while optimum for Con A response is at a BM:CRT ratio of from 1:1 to 3:1. These differences in magnitude and optimal proportions for Con A and PHA may be due to peculiarities of the culture system, or may reflect the fact that Con A and PHA seem to stimulate substantially separate T-cell populations (17).

The availability of a new test for pre-T-cell function raises several interesting questions. We would like to know, for example, how the pre-T function we have described here relates to the pre-T cell which becomes Thy-1 positive on incubation with thymic hormones (2, 3, 13), and to the pre-T cell which can repopulate an irradiated thymus (1). We would also be very interested to know if a similar pre-T cell exists in man and if a similar test can be used to reveal it. A recent report by Delespesse et al. (18) describes striking synergy between purified human T cells and what are described as B cells. We note, however, that 15–48% of cells in their B-cell fraction are unidentified; it is tempting to speculate that among them are pre-T cells which are the actual synergizers. Should this prove to be true, it would mean that human pre-T cells can be evaluated by a relatively simple technique, the application of which will extend our knowledge of normal lymphopoiesis, immunodeficiency disease, and possibly lymphatic neoplasia.

Summary

When mouse bone marrow cells are mixed with cortisol-resistant thymocytes and stimulated *in vitro* with concanavalin A, the mitogenic response observed is much greater than additive, that is, it is synergistic. Between 94 and 96% of

³ Fairchild, S. S., and J. J. Cohen. Manuscript in preparation.

responding cells could be identified as T cells (Thy-1 positive) and of these, 79–100% derived from the cortisol-resisant thymocyte population, not from the bone marrow. Purified macrophages could not replace bone marrow; and marrow depleted of mature T or B cells worked as well as normal marrow. Thus, T and B cells and macrophages were ruled out as the synergizing cell of bone marrow. Nude spleen contained 10 times as many precursors of T cells as did nude marrow and was 10 times better at synergy with cortisol-resistant thymocytes. This implication of the pre-T cell as synergizer was supported by the finding that the synergistic activity of marrow was lost on preincubation, but maintained if the preincubation medium contained thymosin or cyclic AMP. Thus, the ability to enhance the response of relatively mature T cells to Con A is a property of pre-T cells. It is anticipated that this property will allow more detailed studies of T-cell precursor development in mice, and possibly in man.

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References

1. Ford, C. E., H. S. Micklem, E. P. Evans, J. G. Gray, and D. A. Ogden. 1966. The inflow of bone marrow cells to the thymus: studies with part-body irradiated mice injected with chromosome-marked bone marrow and subjected to antigenic stimulation. *Ann. N. Y. Acad. Sci.* 129:283.
2. Komuro, K., and E. A. Boyse. 1973. In-vitro demonstration of thymic hormone in the mouse by conversion of precursor cells into lymphocytes. *Lancet.* 1:740.
3. Cohen, J. J., and C. K. Patterson. 1975. Induction of theta-positive lymphocytes and lymphoblasts in mouse bone marrow by mitogens. *J. Immunol.* 114:374.
4. Mosier, D. E. 1967. A requirement for two cell types for antibody formation in vitro. *Science (Wash. D. C.).* 158:1575.
5. Wigzell, H., K. G. Sundgrist, and T. O. Yoshida. 1972. Separation of cells according to surface antigens by the use of antibody-coated columns. Fractionation of cells carrying immunoglobulins and blood group antigens. *Scand. J. Immunol.* 1:75.
6. Ford, C. E. 1966. The use of chromosome markers. In *Tissue Grafting and Radiation*. H. S. Micklem and J. F. Loutit, editors. Academic Press, Inc., New York.
7. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* 3:645.
8. Goldstein, A. L., F. D. Slater, and A. White. 1966. Preparation, assay, and partial purification of a thymic lymphocytopoietic factor (thymosin). *Proc. Natl. Acad. Sci. U. S. A.* 56:1010.
9. Levis, W. R., and J. H. Robbins. 1970. Effect of glass-adherent cells on the blastogenic response of purified lymphocytes to phytohemagglutinin. *Exp. Cell Res.* 61:153.
10. Lipsky, P. E., J. J. Ellner, and A. S. Rosenthal. 1976. Phytohemagglutinin-induced proliferation of guinea pig thymus-derived lymphocytes. I. Accessory cell dependence. *J. Immunol.* 116:868.
11. Coppleson, L. W., and D. Michie. 1966. A quantitative study of the chorioallantoic membrane reaction in the chick embryo. *Proc. R. Soc. Lond. B. Biol. Sci.* 163:555.
12. Sheppard, H., and G. Wiggan. 1971. Analogues of 4(3,4-dimethoxybenzyl)-2-imidazolidinone as potent inhibitors of rat erythrocyte adenosine cyclic 3':5' phosphate phosphodiesterase. *Mol. Pharmacol.* 7:111.

13. Scheid, M. P., M. K. Hoffman, K. Komuro, U. Hammerling, J. Abbott, E. A. Boyse, G. H. Cohen, J. A. Hooper, R. S. Schulof, and A. L. Goldstein. 1973. Differentiation of T cells induced by preparations from thymus and by nonthymic agents. The determined state of the precursor cell. *J. Exp. Med.* 138:1027.
14. Cantor, L. N., A. J. Morris, P. A. Marks, and R. A. Rifkind. 1972. Purification of erythropoietin-responsive cells by immune hemolysis. *Proc. Natl. Acad. Sci. U. S. A.* 69:1337.
15. Tridente, G., G. Biasi, L. Chieco-Bianchi, and L. Fiore-Donati. 1971. Thymus-marrow cell interaction evaluated by phytohemagglutinin stimulation and graft-versus-host activity. *Nature (Lond.)*. 234:105.
16. Winkelstein, A., N. A. Rangoowala, and S. M. Zimkus. 1972. Thymus-marrow synergy in response to phytohemagglutinin. In Sixth Annual Leucocyte Culture Conference. M. R. Schwarz, editor, Academic Press, Inc., New York. 471.
17. Niederhuber, J. E., J. A. Frelinger, M. S. Dine, P. Shoffner, P. Dugan, and D. C. Shreffler. 1976. Effects of anti-Ia sera on mitogenic responses. II. Differential expression of the Ia marker on phytohemagglutinin- and concanavalin A-reactive T cells. *J. Exp. Med.* 143:372.
18. Delespesse, G., J. Duchateau, P. Gausset, and A. Govaerts. 1976. In vitro response of subpopulations of human tonsil lymphocytes. I. Cellular collaboration in the proliferative response to phytohemagglutinin and concanavalin A. *J. Immunol.* 116:437.