Differences in the *in Vitro* Induction of Thymidine-^{3}H Uptake into Leukemic Lymphoblasts by Phytohemagglutinin and Heterologous Antithymocyte Sera[1] (34872)

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The *in vitro* conversion of lymphocytes in the presence of phytohemagglutinin from the resting phase into a state of active DNA synthesis ("blast transformation") appears to be related to the state of immunological competence of the donor. Lymphocytes from patients with chronic lymphoblastic leukemia (1), disseminated Hodgkin's disease (2) and sarcoid (3) show either delayed or absent *in vitro* response to phytohemagglutinin. Such patients frequently also exhibit *in vivo* defects in delayed hypersensitivity response (4). Heterlogous antilymphocyte serum has also been found to induce *in vitro* blast transformation in sensitive cells (5). The induction of DNA synthesis appears to be related to a specific antigen (cell)-antibody (serum) reaction since the presence of heterologous nonimmune serum in itself does not induce this reaction. We wish to report on some preliminary observations on the relationship between the *in vitro* uptake of thymidine-^{3}H in response to phytohemagglutinin and heterologous antisera in lymphoblasts obtained from patients with acute leukemia at various stages of the disease.

Materials and Methods. Rabbit anti-human thymocyte serum (ATS) was prepared by hyperimmunizing rabbits with fresh human thymocytes obtained at cardiac surgery using the schedule of Levy *et al.* (6). The antisera were inactivated at 56°, and absorbed three times with washed, outdated blood-bank red cells. The hemagglutination titer was 1:1 or less. Activity of the sera was assayed by micromodifications of both agglutination and cytotoxicity tests (7). The lymphagglutination titer of the ATS was found to be greater than 1:1024 when tested against the peripheral lymphocytes obtained from a panel of 10 healthy donors. Cytotoxicity titers, although positive, were much less marked and ranged from 1:16 to 1:64 when tested against normal donors.

In vitro blast transformation was tested in a system consiting of donor lymphocytes (500 cells/mm^3) suspended in tissue-culture media (TC199 with 20% fetal calf serum plus 100 units of penicillin and streptomycin per ml) and either saline, phytohemagglutinin (1/30 dilution of phytohemagglutinin-P, Difco), or antiserum. One microcurie of thymidine-^{3}H (0.5 mmole/mCi) was added after 48 hr of incubation, and the cells were incubated for another 24 hr. The cells were then washed twice with Hanks' balanced salt solution and aliquots were taken for assay of incorporated radioactivity. The cells were combusted in an oxygen atmosphere, the evolved H_2O absorbed in ethanol, and counted in a liquid scintillation phosphor. All results are expressed as disintegrations per minute incorporated per 10^6 mononuclear cells present in the original incubating suspension and are the average of duplicate determinations.

Results. The results from a series of experiments using peripheral lymphocytes from normal donors are shown in Table I. In every instance, the addition of phytohemagglutinin resulted in a significant increase in the incorporation of radioactivity into the lympho-

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TABLE I. Incorporation of Thymidine-^{3}H by Lymphocytes Obtained from the Peripheral Blood of Normal Donors.

	DPM/10^6 cells			Ratios	
	Control	+PHA	+ATS	PHA:Control	ATS:Control
1	57.1	179.2	127.7	3.14	2.24
2	54.9	174.4	496.1	3.18	9.04
3	93.1	223.7	260.4	2.40	2.80
4	124.6	1818.0	1756.6	14.58	14.10
5	125.0	1180.0	1756.5	9.44	14.05
Average ± SD				6.55 ± 4.75	5.65 ± 6.58

cytes. The amount of increase, expressed as the ratio of DPM in the stimulated system to the control (saline) incubation suspensions, varied over a 10-fold range, but, in general, it was greater than 2-fold. Antithymocyte sera also induced an increase in thymidine-^{3}H incorporation in every instance. There was no statistically significant difference between the response of normal lymphocytes to phytohemagglutinin and antithymocyte serum.

Leukemic lymphoblasts were obtained from eight children with acute lymphoblastic leukemia. Lymphoblasts were harvested from the bone marrow where these cells comprised over 95% of the cell population. Cell viability, as measured by trypan blue exclusion, was greater than 98%. In seven of the eight patients tested during the active phase of the disease (either untreated or during relapse), the addition of phytohemagglutinin had no effect upon the uptake of thymidine-^{3}H (Table II). The addition of ATS, on the other hand, induced stimulation of thymidine-^{3}H uptake in seven of the eight patients. The difference in the response to phytohemagglutinin and ATS in these patients is significant at the 0.02 level. In two patients, studies were repeated when the patient was in complete hematologic remission. In contrast to the initial findings, the normal-appearing lymphocytes obtained at the time of remission showed a response upon the addition of phytohemagglutinin as well as to ATS, a pattern of response similar to that seen in lymphocytes obtained from normal donors.

TABLE II. Incorporation of Thymidine-^{3}H by Lymphoblasts Obtained from the Bone Marrow of Children with ALL.

	DPM/10^6 cells			Ratios	
	Control	+PHA	+ATS	PHA:Control	ATS:Control
In relapse					
1	328.4	261.7	588.7	0.80	1.80
2	431.0	198.0	895.0	0.46	2.07
3	663.0	483.0	712.0	0.73	1.07
4	740.0	630.0	1424.0	0.85	1.93
5	100.0	83.5	431.5	0.84	4.32
6	70.8	137.4	529.8	1.95	7.48
7	423.5	445.0	2550.0	1.05	6.02
8	371.4	232.3	780.5	0.63	2.10
Average ± SD				0.91 ± 0.42	3.35 ± 2.18
In remission					
3a	62.2	124.8	108.1	2.01	1.74
8a	64.7	588.0	5337.5	9.10	83.3

Discussion. Both Astaldi *et al.* (8) and Kourilsky *et al.* (9) have previously reported normal morphological response *in vitro* to PHA in lymphocytes obtained from the peripheral blood of children with acute leukemia. These studies differ in two important respects from ours: (1) the lymphocytes used were obtained from blood, the proliferative characteristics of which are known to differ from those found in the bone marrow (10), and (2) the response was judged on the basis of morphological criteria. It is probable that the cells obtained from the bone marrow represent a more homogeneous population of malignant cells.

It appears that lymphoblasts obtained from patients with acute lymphoblastic leukemia exhibit a defect in the *in vitro* response to the mitogenic agent phytohemagglutinin, but retain, in most instances, the capability of responding to antithymocyte serum. This differential response implies that the mechanism underlying the cellular response to phytohemagglutinin differs from that responsible for the increased thymidine-^{3}H uptake in the presence of heterologous specific antisera. The difference in cellular response in acute leukemia might be due either to a defect inherent in the malignant process or to the relative difference in the maturity of the two different cell populations—the lymphoblast versus the mature lymphocyte. Nevertheless, the presence of this altered *in vitro* responsiveness in lymphoblasts from patients with acute lymphoblastic leukemia, reversible by adequate antileukemic therapy, may have important implications in both the pathogenesis and the therapy of this disease.

The loss of specific antigenic or recognition sites might allow the emergence of an abnormal (neoplastic) group of cells, liberated from the normal host immunologic control mechanisms. Treatment with powerful immunosuppressive agents would add little to the long-term control of such groups of cells and may facilitate their continued emergence and proliferation (11).

Summary. "Blast transformation" (increased *in vitro* uptake of thymidine-^{3}H) of bone marrow lymphoblasts obtained from children with acute lymphoblastic leukemia (ALL) has been measured after the addition of either phytohemagglutinin (PHA) or rabbit anti-human thymocyte serum (ATS). Normal lymphocytes, or lymphocytes derived from patients with ALL in remission, show a significant increase in thymidine-^{3}H uptake when cultured with either PHA or ATS. Bone marrow lymphoblasts from patients with ALL in relapse, on the other hand, showed a significant depression in the response to PHA whereas the response to ATS appeared unaffected. This differential response to *in vitro* stimulation suggests an alteration in the immunocompetence of bone marrow lymphoblasts in ALL.

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