Check for updates

# The Transfer RNA Methylases of Human Lymphocytes. II. Delayed Induction by PHA in Lymphocytes From Patients With Chronic Lymphocytic Leukemia

*By* DAVID H. RIDDICK AND ROBERT C. GALLO

**Treatment of lymphocytes from three patients with chronic lymphocytic leukemia (CLL) with phytohemagglutinin (PHA) resulted in a fivefold increase in transfer RNA (tRNA) methylase activity. Evidence was obtained which suggests that the increase included enzyme species not present prior to the induction. As with normal lymphocytes, the time of induction coincided with the formation of fully transformed PHA cells, which is markedly delayed compared with that occurring in normal lymphocytes stimulated with PHA. Although all three patients had an identical pattern of tRNA methylase induction, initial and induced absolute values were higher in the cells of one patient who had 10 per cent immature peripheral blood lymphocytes. The levels of transfer RNA methylase activity were similar in normal and CLL lymphocytes. These data suggest that PHA induces quantitative and qualitative changes in tRNA methylase enzymes of CLL lymphocytes similar to those previously reported in normal lymphocytes but that the sequence of events of PHA interaction with CLL lymphocytes leading to the subsequent enzyme induction is delayed.**

Downloaded from http://ashpublications.net/blood/article-pdf/37/3/293/575601/293.pdf by guest on 29 May 2024

LYMPHOCYTES FROM PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) undergo delayed blastogenesis in culture with phytohemagglutinin (PHA).[1] In the preceding paper we reported the induction by PHA of transfer RNA (tRNA) methylases in normal peripheral blood lymphocytes.[2] The comparison of the tRNA methylase activity in CLL and normal lymphocytes and the demonstration of delayed PHA induction of tRNA methylase activity in CLL lymphocytes are the subjects of this report.

## MATERIALS AND METHODS

Fifty ml. of peripheral blood from each of three patients with untreated CLL were collected in a syringe containing 1 ml. heparin (1000 units/ml., The Upjohn Co.). Erythrocytes were sedimented with 6 per cent dextran for 1 hour at 37°C. The plasma containing the leukocytes was withdrawn and centrifuged at 175 × g. No attempt was made to purify the lymphocytes further since all patients had greater than 95 per cent lymphocytes on peripheral blood differential count.

Cells were suspended to $3 \times 10^6$ per ml. in RMPI Medium 1640 (Industrial Biological

*From the Section on Cellular Control Mechanisms, Human Tumor Cell Biology Branch, National Cancer Institute, Bethesda, Md.*

*Submitted August 5, 1970; revised October 5, 1970; accepted October 15, 1970.*

DAVID H. RIDDICK, M.D.: *Hematology Section, University of Virginia Medical School, Charlottesville, Va.; formerly Clinical Associate, Section on Cellular Control Mechanisms, Human Tumor Cell Biology, National Cancer Institute, Bethesda, Md.* ROBERT C. GALLO, M.D.: *Head, Section on Cellular Control Mechanisms, Human Tumor Cell Biology Branch, National Cancer Institute, Bethesda, Md.*

**Table 1.—Clinical Information on Patients With Chronic Lymphocytic Leukemia**

| Patient | (1) P.W., Age 58 (Male) | (2) H.C., Age 62 (Male) | (3) J.G., Age 60 (Male) |
|---|---|---|---|
| History | 9 months, no therapy | 2 months, no therapy | 6 months, no therapy |
| Complications | None | None | Herpetic skin infections |
| WBC/cu. mm. | 75,000 | 85,000 | 157,000* |
| Hgb (Gm. Per Cent) | 5.8 | 12.5 | 11.7 |
| Platelets/cu. mm. | 21,000 | 119,000 | 116,000 |
| Lymphadenopathy | Marked | Slight | Moderate |
| Hepatomegaly | Slight | None | None |
| Splenomegaly | Marked | None | Slight |
| Immunoglobulins | Low IgA, IgM | Low IgM | Low IgA, IgM |
| Abnormal antibodies | None | None | Direct Coomb's |
| Bone marrow | Lymphocytosis | Lymphocytosis | Lymphocytosis, 15 per cent large immature lymphoreticular cells |

* 10 per cent young lymphocytes on peripheral smear.

Laboratories) containing 30 per cent fetal calf serum (Grand Island Biological Co.) and 6500 units each of penicillin and streptomycin.

Cell cultures and harvests, preparations of cell extracts and undermethylated tRNA, and tRNA methylase assays were performed as previously described.[2]

## Results

### *Chronic Lymphocytic Leukemia Patients*

Clinical data on CLL patients from whom lymphocytes were obtained are summarized in Table 1. All three patients had untreated, "high count" (greater

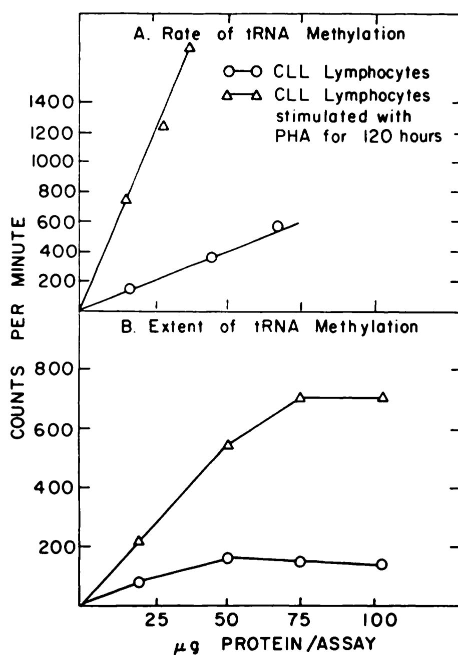


**Fig. 1.**—Rate and extent of tRNA methylation in CLL lymphocytes. Extracts of lymphocytes from Patient 2 (H.C.) incubated with and without PHA for 120 hours were assayed for tRNA methylase activity. Details of the assay have been described.[2] Rate assays were performed with excess *E. coli* undermethylated tRNA (60 μg.) and limiting enzyme, and extent assays with limiting tRNA (1 μg.) and excess enzyme.

Downloaded from http://ashpublications.net/blood/article-pdf/37/3/293/575601/293.pdf by guest on 29 May 2024

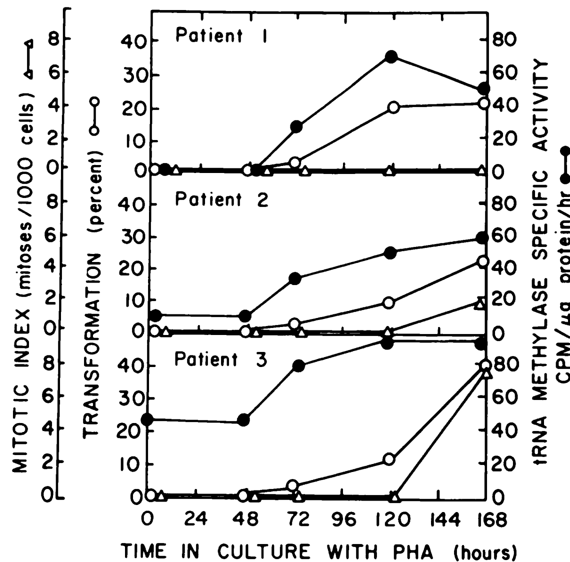


**Fig. 2.**—Time of tRNA methylase induction by PHA in CLL lymphocytes and relationship to the time of transformation and mitosis. Peripheral leukocytes from three patients with CLL were incubated for various times with PHA. Per cent transformation and mitotic index were determined from smears of the cultures. Details of the assay have been described.[2] Patient 1, P.W.; Patient 2, H.C.; Patient 3, J.G.

than 50,000 WBC/cu. mm.)CLL of less than one year's duration. Patient 1 (P.W.) and Patient 2 (H.C.) had morphologically mature lymphocytes in their peripheral blood and bone marrow. Patient 3 (J.G.), in addition to a predominance of mature lymphocytes, had immature lymphoid cells in his bone marrow (15 per cent of total nucleated cells) and peripheral blood (10 per cent of total lymphocytes).

### *Rate and Extent of tRNA Methylation*

Extracts of PHA transformed CLL lymphocytes had higher rates and extents of methylation than those of the nonstimulated controls. The results of an experiment in which tRNA methylase activity was measured in lymphocytes of Patient 2 (H.C.) incubated with and without PHA for 120 hours are illustrated in Fig. 1. Cells incubated in cultures without PHA were small, mature lymphocytes, whereas 8.2 per cent of cells in cultures with PHA were typical, large transformed cells. In extracts from PHA-treated lymphocytes the specific activity of the tRNA methylases was approximately 500 per cent and the tRNA methylase capacity (extent of methylation) was 400 per cent of control values. Similar elevations of activity were observed in transformed lymphocyte extracts from Patient 1 (P.W.) and Patient 3 (J.G.), but, as will be discussed, the absolute values were higher in those of Patient 3. These data suggest that PHA induces quantitative and qualitative changes in CLL lymphocyte tRNA methylases, and the magnitude of the response is comparable to the response of normal lymphocytes.[2]

### *Transformation of CLL Lymphocytes by PHA*

Blastic transformation occurring in CLL lymphocytes cultured with PHA is delayed compared to that in normal lymphocytes.[1] Figure 2 reveals that a small percentage of cells derived from all three patients had transformed after

Downloaded from http://ashpublications.net/blood/article-pdf/37/3/293/575601/293.pdf by guest on 29 May 2024

72 hours in culture with PHA and that maximum transformation did not occur until 168 hours had elapsed. This is in contrast to maximum transformation of normal lymphocytes after 40 hours' culture with PHA under identical culture conditions.[2]

*Time of PHA Induction of tRNA Methylase Activity in CLL Lymphocytes*

The per cent of transformed cells, the mitotic indices, and tRNA methylase activities were measured in CLL lymphocytes cultured for various times with PHA (Fig. 2). Only tRNA methylase specific activity (rate) is shown, but increases in tRNA methylase extents paralleled the increases in specific activity (Fig. 1). Elevation of tRNA methylase activity was apparent at 72 hours, a time at which morphologic transformation had already developed. Activity was maximal at 120 hours, whereas the largest per cent of cells transformed was at 168 hours. Thus, although tRNA methylase elevations were associated with the formation of fully developed PHA cells, in contrast to the situation in normal lymphocytes,[2] the two were not strictly proportional. The most likely explanation is that the criteria used for counting cells as transformed excluded marginally transformed cells in 72-hour PHA cultures.

The pattern of PHA induction of tRNA methylase activity in lymphocytes from all three CLL patients was identical. As mentioned previously, both initial and induced tRNA methylase activities were higher in lymphocytes from Patient 3 (J.G.) than in those from the other two patients. The presence of immature lymphoid cells in this patient's peripheral blood (Table 1) is the most likely explanation, since we have previously shown that tRNA methylase activity is high in lymphoblasts.[3]

In Fig. 3 the PHA induction of tRNA methylase activity previously reported in normal peripheral blood lymphocytes[2] is compared with an idealized composite of tRNA methylase activities in the three patients with CLL. It is apparent that induction coincides with transformation in both; however, in CLL it is markedly delayed, correlating with the delayed transformation.

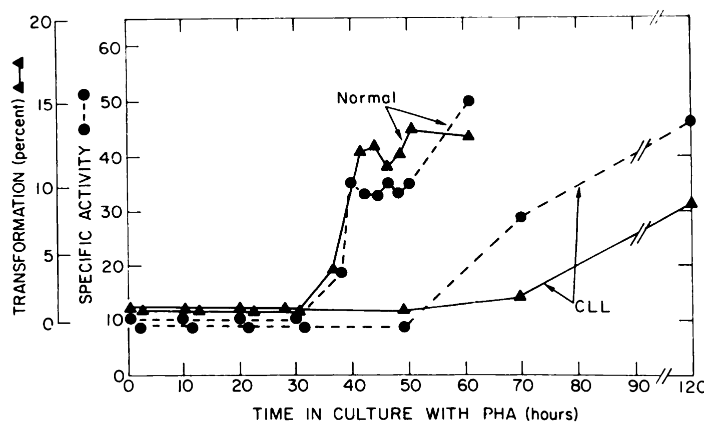


**Fig. 3.**—Schematic comparison of PHA induction of tRNA methylases in normal and CLL lymphocytes. Normal lymphocyte data have previously been reported.[2] CLL data represent an idealized composite of the three patients in Fig. 2.

Downloaded from http://ashpublications.net/blood/article-pdf/37/3/293/575601/293.pdf by guest on 29 May 2024

As illustrated in Fig. 3, the absolute values of both the initial and induced tRNA methylase activities of normal and CLL lymphocytes were similar (with the exception of Patient 3, noted above). How can these data be reconciled with previous reports demonstrating higher levels of tRNA methylase activity in tumors than in normal tissues?[4-8] We have previously presented evidence suggesting that the level of tRNA methylase activity, although not directly related to rapidity of cell division, may be related to the state of differentiation of the cell and not to neoplasia per se.[3] Both normal lymphocytes and CLL lymphocytes are mature cells not "in cycle," and activity is low in these cells. Conversely, PHA-transformed normal and CLL lymphocytes are blastic cells "in cycle" and activity is high. Thus, the present data lend further support to the concept that tRNA methylases are derepressed in both normal and neoplastic cells "in cycle."

## Discussion

Some of the known biochemical events that occur after the addition of PHA in normal lymphocytes have also been observed in CLL lymphocytes. However, some of these changes in CLL are known to be delayed.[1,9,10] PHA induces tRNA methylases between 40 and 45 hours of incubation in normal lymphocytes[2] and by 72 hours in CLL lymphocytes. The delayed induction in CLL lymphocytes is correlated with the delay in transformation. Since PHA-stimulated protein, RNA, and even DNA synthesis are already well under way prior to induction of tRNA methylases (in normal or CLL lymphocytes), the delayed induction clearly cannot be the primary reason for the defective response to PHA of CLL cells. However, we previously hypothesized that new tRNA methylases may be required to modify tRNA's specific for the translation of mRNA's for new late-appearing proteins in PHA treated lymphocytes.[2] If this is true, delayed or insufficient synthesis or abnormal types of tRNA methylases could render CLL lymphocytes incapable of synthesizing some proteins necessary for formation of the fully transformed PHA cell at the normal rate, thereby adding to the delayed response. The delayed tRNA methylase induction itself is apparently secondary to some as yet undiscovered primary defect in CLL lymphocytes. Of interest in this regard is the recent observation by Kornfeld that CLL lymphocyte cell membranes contain fewer PHA receptors than normal lymphocyte cell membranes.[11]

## ACKNOWLEDGMENT

The authors wish to thank Dr. Ralph Johnson, National Cancer Institute, for permission to study his patients. The authors also wish to thank Mrs. Elaine Ray and Mrs. Olga Collier for their aid in preparation of the manuscript.

## REFERENCES

1. Havemann, K., and Rubin, A. D.: The delayed response of chronic lymphocytic leukemia lymphocytes to PHA in vitro. Proc. Soc. Exp. Biol. Med. 127:668, 1968.

2. Riddick, D. H., and Gallo, R. C.: The transfer RNA methylases of human lymphocytes: I. Induction by PHA in normal lymphocytes. Blood 37:000.

3. Riddick, D. H., and Gallo, R. C.: Correlation of transfer RNA methylase activity with growth and differentiation in normal and neoplastic tissues. Cancer Res. 30:2484, 1970.

Downloaded from http://ashpublications.net/blood/article-pdf/37/3/293/575601/293.pdf by guest on 29 May 2024

4. Baguley, B. C., and Staehelin, M.: Substrate specificity of adenine-specific transfer RNA methylase in normal and leukemic tissues. Europ. J. Biochem. 6:1, 1968.

5. Gantt, R., and Evans, V. J.: Comparison of soluble RNA methylase capacity in paired neoplastic and nonneoplastic cell lines in vitro. Cancer Res. 29:536, 1969.

6. Hancock, R. L: Utilization of L-methionine and S-adenasyl-L-methionine for methylation of soluble RNA by mouse liver and hepatoma extracts. Cancer Res. 27:646, 1967.

7. Mittelman, A., Hall, R. H., Yohn, D. S., and Grace, J. T., Jr.: The in vitro soluble RNA methylase activity of SV40-induced hamster tumors. Cancer Res. 27: 1409, 1967.

8. Tsutsui, E., Srinivasan, P. R., and Borek, E.: tRNA methylases in tumors of animal and human origin. Proc. Nat. Acad. Sci. U.S.A. 56:1003, 1966.

9. Rabinowitz, Y., McCluskey, I. S., Wong, P., and Wilhite, B. A.: DNA polymerase activity of cultured normal and leukemic lymphocytes. Response to phytohemagglutinin. Exp. Cell Res. 57:257, 1969.

10. —, and Dietz, A. A.: Effect of phytohemagglutinin in cultures on the lactate dehydrogenases of lumphocytes from chronic lymphatic leukemia. Blood 31:166, 1968.

11. Kornfeld, S.: Decreased phytohemagglutinin receptor sites in chronic lymphocytic leukemia. Biochim. Biophys. Acta 192:542, 1969.

Downloaded from http://ashpublications.net/blood/article-pdf/37/3/293/575601/293.pdf by guest on 29 May 2024