Immunologic and Cytogenetic Studies of Chronic Lymphocytic Leukemic Cells

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PATIENTS with chronic lymphocytic leukemia (CLL) frequently have hypogammaglobulinemia, depressed circulating antibody response to antigenic challenge,[1,2] and decreased ability to reject homografts.[3] The decreased antibody production is presumably the major cause of the frequent infections which occur in these patients.[1,2] These immunologic defects occur despite the increase in the number of small, morphologically mature lymphocytes in this disease.

It has been demonstrated that in short-term tissue culture the majority of lymphocytes from normal human blood will, in the presence of phytohemagglutinin (PHA), transform to large "blast-like" cells.[4,5] It also increases the mitotic index, thus affording ideal circumstances for chromosome analysis of cultured peripheral blood lymphocytes.[6] Investigators have variously reported the transformation of CLL lymphocytes to PHA stimulation to be either largely absent,[4,7] markedly decreased,[8,9] or normal.[10]

Recently it has been demonstrated that lymphocytes obtained from a donor who has previously been stimulated with a given antigen will, when exposed to the same antigen in the in vitro short-term culture also transform to "blast-like" cells and divide.[11] This transformation with specific antigens is therefore a function of the immunologic competence of lymphocytes, whereas the mechanism of action by which PHA stimulates lymphocytes is still unknown.

PHA-M° and PPD† were employed to evaluate the lymphocytes from 17 patients with CLL, using normal subjects' cells as controls. In addition, cytogenetic analyses of the cultured peripheral white blood cells (WBC) were performed.

EXPERIMENTAL PLAN

Seventeen consecutive patients with CLL visiting the Outpatient Clinic of the Clinical Center, National Institutes of Health, were studied (table 1). Their disease duration from diagnosis ranged from 1 month to 24 years (median 7 months). Four were tuberculin (PPD†) negative, and 13 PPD positive, i.e., they responded with an 8 mm. diameter or

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Table 1.—*Comparison of Chronic Lymphocytic Leukemia and Normal Peripheral Lymphocyte Transformation and Mitotic Response to Tuberculin and Phytohemagglutinin in Vitro*

		Chronic Lymphocytic Leukemia		Normal Subjects	
		PPD Positive	PPD Negative	PPD Positive	PPD Negative
	No. of patients	13	4	14	2
	Age	64(47-78)*	68(62-72)	41(29-64)	39(23-55)
	White cell count x 10³	29(12-560)	124(12-330)	6(5-8)	7(6-8)
	% Lymphocytes	92(53-100)	82(53-98)	31(20-51)	30(23-38)
PPD Stimulated	No. of experiments	36	5	23	2
	% Transformation	12(0-27)	3.9(0-7)	22(12-44)	2.8(1.6-4.0)
	Increase over unstimulated response	7.3(0-23.6)	0.4(0-3.0)	12.3(0-33)	2.3(1.6-3.0)
	Mitotic index	1(0-6)	0(0-0)	2(0-8)	0(0-0)
PHA Stimulated	No. of experiments	19		14	
	% Transformation	21(4.4-59)		82(70-91)	
	Increase over unstimulated response	17(0.6-55.3)		75(33.6-90)	
	Mitotic index	2.5(0-7.5)		10(4-16)	
Unstimulated Cultures	No. of experiments	27		17	
	% Transformation	3(0-11.8)		8(0.5-37)	
	Mitotic index	0(0)		0(0)	

*Median and range.

greater area of induration at 48–72 hours. Of the latter group, one was positive to first strength PPD, 7 to intermediate strength PPD, and 5 only to second strength PPD. The age and peripheral WBC counts for both patients and normal subjects are given in table 1.

One of the PPD positive patients was receiving 5 mg. of prednisone daily at the time of the study. None of the remaining patients or controls had received any immunosuppressive therapy within the month prior to the study. Four of the patients had had significant radiation exposure 1 to 15 years before testing. None of the subjects had a history of penicillin or streptomycin sensitivity. None had a family history of CLL or related diseases.

By serum protein electrophoresis, 8 of the patients had hypogammaglobulinemia (0.2–0.7 Gm. per cent) and 3, hypergammaglobulinemia (1.4–2.0 Gm. per cent).

Thirty to 50 ml. of heparinized blood was obtained by venipuncture and allowed to sediment at a 15–20° angle for 1–2 hours at room temperature. The WBC rich plasma was gently withdrawn, and sufficient volume of it added to 199 tissue culture medium (containing 100 μ penicillin and 100 μg. of streptomycin/cc.) to obtain a final cell suspension with 1000–4000 cells/mm.³ Additional supplements of autologous cell-free plasma were added to obtain a final plasma concentration of 25–30 per cent. Cell suspensions were cultured in separate 12 cc. aliquots. To the unstimulated controls were added isotonic saline and occasionally 200 μg./cc. dextran in saline, or 200 μg./cc. polyvinylpyrrolidone (PVP).* To the experimental cell suspensions 0.2 cc. of PHA-M or PPD in concentrations from 0.5 to 20 μg./cc. were added. These concentrations of PPD had been found optimally effective over this broad range in preceding pilot studies. All the cultures were incubated at 36.5–37.5 C for 5 days. This was chosen as the optimal duration for incubation because lymphocyte transformation increased progressively up to then, and became obscured by increased cell death thereafter (table 2). WBC counts

°Plasdone C (PVP) by Antara Chemicals Division of General Aniline and Film

Table 2.—*The Effect of Increasing Duration of Culture Incubation on the Mitotic Index, Percentage of Lymphocyte Transformation and Proportions of Transformed Lymphocytes Labeling with Thymidine H^3*

PPD Positive Duration of Incubation in Days	CLL Patients		Normal Controls	
	3	5	3	5
PPD Stimulated Cultures				
Mitotic index	0(0-1)*	2(0-6)	0(0-1)	2(1-8)
% Lymphocyte transformation	3(2.5-6.5)	12(3-27)	7(5-8)	37(22-44)
Proportions of transformed cells labeled with H³TdR	—	50(43-75)	—	45(35-70)
PHA Stimulated Cultures				
Mitotic index	6(2-14)	2.5(0.5-6)	30(14-75)†	10(4-16)
% Lymphocyte transformation	8(5-12)	15(8-24)	86(80-93)	82(70-91)
Proportions of transformed cells labelled with H³TdR	39(20-45)	20(10-50)	43(35-50)	17(5-26)
Unstimulated Cultures				
Mitotic index	—	<1	—	<1
% Lymphocyte transformation	—	3(0-11.8)	—	8(0.5-37)
Proportions of transformed cells labeled with H³TdR	—	0(0-17)	—	0(0-15)

*Numbers indicate median and range.

†0.5 μg. Colcemide (CIBA, Summit, New Jersey) added 2 hours prior to harvesting.

were performed daily on half of the cell cultures. Cell viability was determined by the trypan blue dye exclusion method.[12]

The cell suspension was harvested by centrifugation at 500 rpm (International Centrifuge with IEC No. 240 head) for 5 minutes, discarding the supernatant, fixing for 10 minutes in modified Carnoy's solution (3:1 absolute ethyl alcohol to glacial acetic acid), recentrifugation, resuspending in fixative and then dropping the cell suspension onto clean slides for air drying and staining with Giemsa (Harleco).

The mitotic index was defined as the number of mitoses in a 1000 mononuclear cell count. The proportion of small lymphocytes, larger monocytic and "transformed blast" cells were also determined from the 1000 cell count. Unrecognizable cells and polymorphonuclear leukocytes were excluded. The omission of granulocytes diminished the difference between the heterogeneous normal and the lymphocytic leukemia WBC populations.

Small lymphocytes were identified by the usual criteria of size, scanty cytoplasm, and darkly staining nuclei. The larger monocytes often had considerable amounts of phagocytized intracytoplasmic eosinophilic granular debris, copious cytoplasm, and an ill-defined cytoplasmic membrane. In contrast the "transformed blasts" usually had a well-defined cytoplasmic membrane, moderate amounts of basophilic, nongranular cytoplasm, rounded nuclei with prominent nucleoli, and were never phagocytic. There was no problem distinguishing between the small lymphocytes and the larger mononuclear cells. However, sometimes it was difficult to distinguish monocytes from "blasts" using solely morphological criteria. Thus they were combined, and the "per cent transformation" in tables 1 and 2, and figure 1 is defined as "blast cells" plus large monocytes over the total mononuclear cell count. Comparison of the control and experimental cultures was used to correct for this inclusion of monocytes with the "transformed blasts."

Often multiple and repeated determinations were done on a given subject in which case his median response was used for comparison to others. Excluded from the tabulations were about 5–10 per cent of the cultures in which all the cells died or no cellular response was evident. The plasma of a normal and a CLL patient was exchanged on two occasions, and the resultant cell growth compared to appropriate controls.
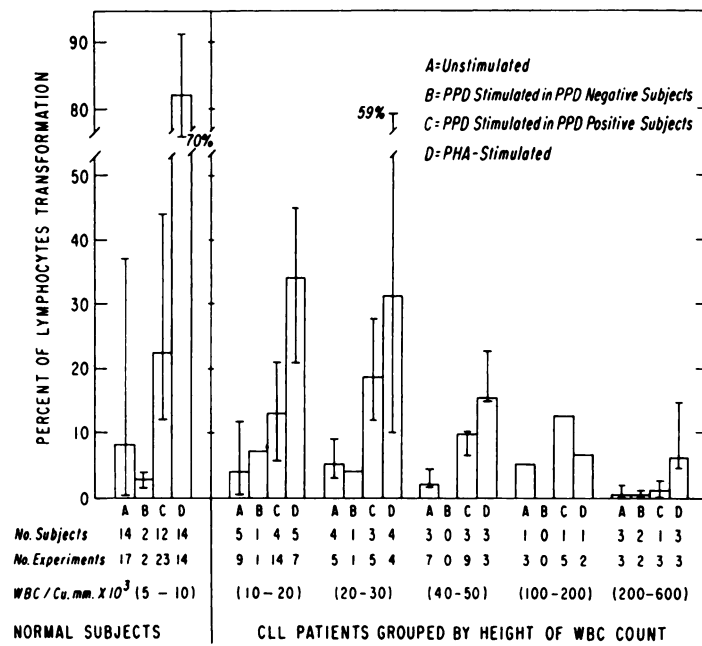
Fig. 1.—The relationship of peripheral white blood cell count to the proportions of transforming lymphocytes present in normal subjects and chronic lymphocytic leukemia patients.

Tritiated cytidine* (2.5 μc./cc.) and tritiated thymidine† (2.0 μc./cc.) were added to different cultures of 7 CLL patients and 5 normal subjects 4 hours prior to harvesting. Radioautographs were prepared in the usual manner.[13]

Chromosome preparations of the CLL cells were made using the peripheral WBC culture technic,[6] modified by adding 0/2 cc. PHA-M directly to the 12 cc. cell suspensions and harvesting at 72 hours after 2 hours incubation with colcemide‡ (0.5 μg./cc.). Several preparations using the direct bone marrow technic[14] also were attempted.

RESULTS

The preincubation cell suspensions contained 2–10 per cent monocytes in the normal subjects and 0–6 per cent in the CLL patients. After 5 days incubation, the unstimulated normal cultures contained 8 per cent, and the CLL cultures 3 per cent "transformed cells" (table 1). Morphologically most of these large mononuclear cells still looked like monocytes, but an occasional typical transformed lymphocyte (<1 per cent) was seen in the unstimulated cultures. The moderate increase in the proportions of monocytes during incubation is probably due to the selective survival of this hardier cell type.[15] No mitoses were observed in the unstimulated cultures.

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*Cytidine H³ (CR³H) sp. act. 1.5 c/mM, New England Nuclear Corporation, Boston, Massachusetts.

†Thymidine H³ (TdR³H) sp. act. 0.36 c/mM, Schwarz Bioresearch, Inc., Orangeburg, New York.

‡Colcemide, CIBA, Summit, New Jersey.

There was no difference between the unstimulated cultures and those cultured with PPD in the 4 PPD-negative patients and the 2 PPD-negative normal subjects.

Eleven of the 13 PPD positive patients responded to PPD with a median increase of 7.3 per cent in the proportion of transformed lymphocytes over the response in their unstimulated cultures (p = 0.01 by the Sign test). Thirteen of the 14 PPD positive normal subjects had a median increase of 12.3 per cent of their lymphocyte transformation with PPD compared with their unstimulated cultures (p = .0009). The ability of the normal lymphocytes to transform with PPD was significantly higher than that of the CLL lymphocytes (p = 0.02 by the Chi^2 method).

The lymphocyte transformation with PHA was significantly higher than with PPD in all but one of the CLL cultures (p = 0.002) and in all of the normal cultures. The median increase of 75 per cent in the normal lymphocyte transformation with PHA over the unstimulated controls was significantly higher than the median increase of 17 per cent in the CLL cultures (p = >0.001) (table 2).

The changes in mitotic index, in general, correlated well with the change in cell transformation (table 1).

There was no inhibition of the ability of normal lymphocytes to transform when grown in the presence of plasma of a CLL patient. Conversely, the CLL cells did not respond any better when cultured with normal homologous plasma than with autologous plasma.

The proportions of cells that failed to exclude trypan blue in the 5 day old cultures generally correlated well with the proportions that appeared morphologically damaged on the slides. Judging by these criteria, the degree of cell death varied widely in the cultures. It was usually most prominent in the unstimulated and least evident in the PHA stimulated cultures.

Daily WBC counts generally revealed a drop to less than half the original count by 120 hours in both the unstimulated and PPD stimulated cultures of patients and normal subjects. However, in the PHA stimulated cultures of the normal subjects and about 60 per cent of the patients the WBC count decreased during the initial 2 days, and then returned to the original level, or even higher, by the fifth day. Differential cell counts revealed that the decreases in WBC counts were predominantly due to small lymphocyte death in the CLL patients, whereas granulocytic cell death accounted for the decrease in the normal subjects.

The tritiated cytidine labeled almost all large mononuclear cells. Moderate numbers of small lymphocytes often were also lightly labeled by it in cultures of normal as well as of CLL cells.

The tritiated thymidine labeled <1 per cent of all the mononuclear cells in the unstimulated cultures (table 2). It labeled 20–50 per cent of the PHA transformed lymphocytes at 72 hours in both normal and CLL cell cultures. After an additional 48 hours of incubation the PHA stimulated cell labeling decreased to 17 per cent in the normal cultures and to 20 per cent in the CLL cultures. This decrease parallels a decrease in mitotic index during

that time. With prolongation of incubation from 3–5 days the proportions of normal PHA transformed lymphocytes remained stable but continued to increase in the patient's cultures. In contrast, the PPD stimulated normal as well as CLL lymphocyte transformation, mitotic index, and proportions of large mononuclear cells labeling with tritiated thymidine all continued to increase from day 3 to day 5 (table 2). There were no significant differences in the proportions of CLL versus normal labeled transformed lymphocytes. Both patients' and normal subjects' cells generally labeled equally heavily.

The level of the patient's peripheral WBC count was inversely related to the proportion of their lymphocytes transforming in response to both PHA and PPD (fig. 1). This finding is corroborated by the twentyfold decrease in the proportion of transforming lymphocytes that occurred in a patient whose WBC were recultured after an eightfold rise in his peripheral count. The reverse occurred in another patient whose lymphocyte transformation approached normal after treatment had drastically reduced his WBC count to the normal range.

Although the proportions of responding cells was markedly decreased in the patients, the median of the total number of responding lymphocytes present in the peripheral blood of the patients was 6300/mm.[3] (1200–34,000/mm.[3]) which was much higher than the 1000–2500/mm.[3] responsive lymphocytes present in the normal subjects. These figures are the product of the per cent transformation multiplied by the absolute lymphocyte count.

Those CLL patients who were positive only to second strength PPD also responded less effectively to it in vitro than the more sensitive patients but they also tended to have higher WBC counts. No such correlation of in vivo sensitivity and in vitro responsiveness to PPD was found in the normal PPD positive subjects.

The degree of in vitro cellular response was not enhanced by increasing concentrations of PPD from 0.5–20 μg./cc. of cell suspension. Concentrations of 20 μg./cc. of PPD became less effective due to the development of varying degrees of cytotoxicity, and usually all the cells died at 40 μg./cc. This cytotoxicity could not be related to the degree of in vivo PPD sensitivity of the PPD positive patients.

Neither the patients' gamma globulin level nor their disease duration could be correlated with their in vitro cell responsiveness. Although the CLL patients were significantly older than the controls, in neither was the response effected significantly by age. The patient on prednisone therapy responded just as well as the untreated group.

Direct bone marrow chromosome preparations in the CLL patients were inadequate for analysis because of their extremely low mitotic indices. This was not improved by the in vivo administration of vincristine therapy 12 hours prior to marrow aspiration. Peripheral WBC cultures in the 17 patients provided from 2–100 (median 28) metaphases for counting. The modal number was 46 in each patient, and the chromosome count distribution was within normal limits. Whereas most of the patients had an incidence of less than 4 per cent chromosome breaks, 5 of them had 5–20 per cent morphologic

aberrations of their metaphases which included gaps, breaks, fragments, dicentrics and rings. Only 3 of these patients had a history of radiation exposure, but the other 2 patient's cultures had been exposed to tritiated thymidine. Aside from the above, no cytogenetic abnormalities were observed

DISCUSSION

Lymphocytes in vitro have been found to respond to a variety of antigens, provided the cell donor has been previously exposed to the antigen. This has been found to be true for PPD,[11] pertussis vaccine, tetanus toxoid, typhoid-paratyphoid vaccine, diptheria toxoid, polio virus vaccine, small pox vaccine,[16] grass pollen,[17] penicillin,[18] and those endotoxins derived from microorganisms commonly found in man.[19] These responses are qualitatively similar to the PHA response but are considerably less intense and occur at a much slower rate. A rabbit antihuman WBC serum,[20] staphylococcal filtrate,[21] and Streptolysin S[22] have been reported to be quantitatively as effective as PHA. It has been suggested that PHA acts as an ubiquitous antigen,[11] or nonspecific growth stimulator,[23A] and that it stimulates the transformed lymphocytes to produce gamma globulin.[18,23B] Our findings suggest that the diminished response of CLL cells to antigenic stimulation in short-term culture is a reflection of their immunologic incompetence, and that their markedly diminished response to PHA may be due to the same cellular defect.

Attempts to immunize CLL patients with antigens such as typhoid, influenza, mumps and diptheria vaccines have revealed them to be poor producers of circulating antibodies.[2] This leaves PPD as the most applicable antigen to test on sensitive patients and PPD-negative controls.

The in vivo delayed tuberculin reaction consists predominantly of a mononuclear inflammatory response.[24] If one assumes that the same cells are responsible for both the in vivo and in vitro reactions, then to be able to manifest a positive skin test a certain quantity of responsive lymphocytes must be available. Thus the in vitro culture does not provide any more information about a patient's delayed response to PPD than does the in vivo test, but it does provide a means of evaluating his immunologic competence on a cellular level. However, the in vitro response of lymphocytes has been helpful in the diagnostic evaluation of several patients with lymphocytic leukemia.

The transformed lymphocyte labels with thymidine,[5] and does not phagocytize.[25,26] That most of the large mononuclear cells in the unstimulated cultures are monocytes rather than blasts is shown by their failure to label with tritiated thymidine. Furthermore, the addition of polystyrene latex particles* (1.3 μ diameter) 5 hours prior to harvesting has shown that almost all the large mononuclear cells in the unstimulated cultures, a similar per cent in the PPD stimulated, and generally only a few cells in the PHA stimulated cultures phagocytize these particles. In contrast, even acid fast

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*Polystyrene latex particles, Dow Chemical Corporation, Midland, Michigan.

bacilli (M. battei) are not phagocytized by PPD transformed lymphocytes. Nor are they found in an intracellular location when they are used as the antigen to transform the lymphocytes from a PPD-positive donor. The number of cells that do phagocytize the acid fast bacilli are roughly proportional to those ingesting the polystyrene particles and are thus presumably non-proliferating monocytes.[27] These findings confirmed our impression obtained from the less reliable morphological studies that almost all the large cells in the PHA cultures are "transformed lymphocytes," but that moderate numbers of monocytes survive in the PPD cultures.

Both the normal and leukemic lymphocytes responded relatively well to a fairly wide range of PPD concentrations. The quantities of PPD used were considerably above the minimal threshold for effective in vitro stimulation. This is evident from the studies of others who used considerably less PPD and obtained a higher transformation and mitotic response.[18] These higher responses achieved by other workers are probably due to washing cells with hypotonic medium which, in our experience, eliminates many nonviable cells and small mononuclears,[27] and are also due to the use of a mitotic arrestor which allows mitoses to accumulate and be recognized more easily.

Cell death which resulted in cell disappearance or prevented cell identification could not be corrected for. Cell counts were not accurate enough to do this because of the leukoagglutination which invariably accompanied any PPD as well as PHA response. It was our impression from the greater degree of cell damage seen with the leukemic than with the normal cells that the former generally did not survive as well in the cultures as did the normal cells. This introduces an error that makes the CLL responses somewhat falsely high.

Recent studies by us indicate that despite their limitations, morphologic studies are a fairly good indicator of lymphocyte proliferation, and in general correlate well with the degree of cellular DNA production as determined from tritiated thymidine radioautographs.[27] The tritiated thymidine labeled only the decreased numbers of transformed lymphocytes in the CLL cultures. The actual proportions of *large* mononuclears that labeled were not significantly different in patients and controls, nor was there a significant difference in the degree of cell labeling. However, our studies do not rule out the possibility that CLL cells may label less heavily than normal.

The increase in PHA lymphocyte transformation from day 3 to day 5 in the CLL cultures, and slightly higher than normal thymidine uptake on the fifth day suggests that perhaps some CLL cells respond at a slower rate than normal cells. This is consistent with the in vivo mitotic index and prolonged generation time of CLL lymphocytes.[28] The kinetics of the thymidine labeling indicate that the rate of transformation of all lymphocytes was slower in response to PPD than to PHA in all cultures. This, and the low mitotic index suggest that many of the transformed lymphocytes seen on the fifth day in the PPD cultures may not be the products of cell division prior to the fifth day.

The CLL patients' lymphocytes are able to respond specifically to PPD when the cell donors were PPD positive. In all but one an even greater proportions of lymphocytes was transformed by the PHA. Normal lymphocytes

respond significantly better to PPD and their PHA response surpasses by far that of the leukemic lymphocytes. That this CLL cell hyporesponsiveness is primarily due to a cellular defect rather than serum factor is indicated by the failure of CLL plasma to inhibit the normal cell response and the inability of normal plasma to enhance the CLL cell response. The proportions of CLL cells that transform generally decreased with increased peripheral WBC counts. These findings indicate that, in large part, the neoplastic lymphocytes are refractory to immunologic and perhaps to other environmental stimuli. This may be one aspect of their autonomous behavior and independence from the usual homeostatic control mechanisms.

It has been suggested that two distinct populations of lymphocytes, normal and neoplastic coexist in patients with CLL and that as the disease progresses the WBC count increases due to an absolute increase in the neoplastic cells.[8,9] This interpretation is consistent with our data which indicates that as the WBC count increases, the proportion of immunoresponsive (normal) lymphocytes decreases. However, our data also show that the absolute number of immuno-responsive cells in CLL is significantly higher than the number in the normal peripheral blood. These are, however, maximum values since considerable cell death occurs in the CLL cultures and a proportion of the transformed cells are products of divisions. The high incidence of hypogammaglobulinemia in CLL suggests that the normal cell population present is often markedly decreased. The cases of hypergammaglobulinemia and reported finding of dysgamma-globulinemia in CLL[29] indicate that some of the abnormal cells must be capable of producing globulins. Thus the alternative hypothesis that lympho-cytes in patients with CLL can manifest a wide spectrum of capabilities ranging from none to partial or completely intact immunologic responsiveness cannot be ruled out.

Cytogenetic studies have revealed the consistent findings of the Ph' abnor-mality of chronic myelogenous leukemia[30] and other inconsistent aberrations in the acute leukemias.[31] The abnormal immature leukemic cells have been found to grow well in unstimulated tissue cultures, and grow independently of PHA stimulation.[4,10] The neoplastic lymphocyte of CLL is most often similarly unresponsive to PHA but unlike the other leukemic cells fails to grow inde-pendently. The mitotic rate in bone marrow and peripheral blood cultures of CLL is very low so that any metaphases in the cultures are probably derived from those relatively normal lymphocytes present that are still capable of responding to PHA. The increased numbers of chromosome breaks and rearrangements present in 5 of the patients metaphases are probably due to radiation therapy or the addition of tritiated thymidine to some of the cultures.[32] The failure to find any significant chromosomal aberrations in the CLL cell cultures also suggests that the more malignant cells are least re-sponsive and that another way of stimulating these cells to divide will have to be found before they can be studied cytogenetically. The finding of a Ch' chromosome abnormality in a family with high incidence of CLL[33] is probably a coincidental autosomal defect which may predispose to the development of CLL.

SUMMARY

The lymphocyte transformation response of 17 chronic lymphocytic leukemia patients when tested in the short-term tissue culture with PHA-M, and PPD was found to be significantly decreased when compared to normal subjects. Serum factors were not found to be responsible for this cellular hyporesponsiveness. The proportions of immunoresponsive lymphocytes found in the patients' peripheral circulation decreased as their white blood cell count increased. The transformation response to PHA-M was generally better than to PPD. Neither the PPD negative patients nor the normal PPD negative subjects' cells responded to PPD stimulation in vitro.

Monocytes usually would phagocytize particles added to the cultures and could thus be distinguished from the nonphagocytic proliferating lymphocytes which were the only cells that incorporated thymidine H^3. Radioautographs of tritiated thymidine also revealed the rate of PPD lymphocyte transformation to be slower than with PHA-M. There were no significant differences in the proportions or the degree of leukemic and normal transformed lymphocyte labeling with tritiated thymidine.

Cytogenetic studies revealed that the patients' mitotic indices both in vivo and in vitro were markedly depressed. The modal chromosome number was 46 in each patient, and no cytogenetic abnormalities other than those due to exposure to radiation were found.

SUMMARIO IN INTERLINGUA

Esseva trovate que le responsa transformatori del lymphocytos de 17 patientes con chronic leucemia lymphocytic, quando testate durante breve periodos in histoculturation con phytohemagglutinina M e purificate derivato de proteina, esseva reducite significativemente in comparation con subjectos normal. Esseva trovate in plus que factores seral non es responsabile pro iste hyporesponsivitate cellular. Le proportion de lymphocytos immunoresponsive in le circulation peripheric del patientes declinava in tanto que lor numeration leucocytic montava. In general, le responsa transformatori a phytohemagglutinina M esseva melior que illo a purificate derivato de proteina. Ni le cellulas del patientes con negativitate pro purificate derivato de proteina ni le cellulas de subjectos normal con negativitate pro purificate derivato de proteina respondeva in vitro a stimulation con purificate derivato de proteina.

Le monocytos usualmente phagocytava particulas addite al culturas, lo que rendeva possibile distinguer los ab le nonphagocytic lymphocytos proliferante le quales esseva le sol cellulas in que thymidina a tritium esseva incorporate. Radioautographias de thymidina a tritium revelava etiam que le transformation del lymphocytos sub le effecto de purificate derivato de proteina esseva plus lente que ille transformation sub le effecto de phytohemagglutinina M. Ni le proportion ni le grado del marcation del thymidina a tritium differeva inter leucemic e normal lymphocytos transformate.

Studios cytogenetic revelava que le indices mitotic del patientes esseva deprimite marcatemente tanto in vivo como etiam in vitro. Le numero-

modello del chromosomas esseva 46 in omne le patientes, e nulle anormalitates cytogenetic esseva trovate a parte tales attribuibile al exposition irradiatori.

ACKNOWLEDGMENTS

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