Mem. Inst. Butantan
42/43:71-76, 1978/79

BLASTIC TRANSFORMATION AND CYTOGENETIC STUDIES OF PERIPHERAL BLOOD LYMPHOCYTES FROM LYMPHOSARCOMATUS PATIENTS, AFTER STIMULATION BY PHYTOHEMAGGLUTININ *

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ABSTRACT: Short term cultures of PBL, stimulated by PHA, were used for the study of the blastic transformation rate and the cytogenetics of two patients carrying non-Hodgkin's lymphomas. Immunologically, the two patients showed no repression of the T lymphocyte population. Cytogenetically, there were evidenced some chromosomal aberrations, although they were not patognomonic.

UNITERMS: Non-Hodgkin's lymphomas; blastic transformation; cytogenetics.

INTRODUCTION

Phytohemagglutinin (PHA), classified among the non specific mitogens, exerts its effect predominantly on the lymphocyte T cell population.

When human normal peripheral blood lymphocytes (PBL) are cultured in the presence of PHA, in a interval of 3 — 4 days, 60 to 80% of the lymphocytic population attain a blastic morphology. The remain of the population, even when maintained for several days under these conditions, did not show any morphological transformation and after 10 to 14 days, the growth curve of the culture starts to decline, occurring death of all cells on about the 17th day. In these cultures, the first mitotic figures appear between the 24 and 48th hours of culturing and are more numerous at about 60-72 hours [1, 3, 4, 6, 7, 12].

* This work was supported by the FEDIB, CNPq and DINECRODE.

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In the present paper, we study the blastic transformation rate and the cytogenetics of PBL from two patients carrying non-Hodgkin's malignant lymphomas, after a short-term culture stimulated by the PHA.

MATERIALS AND METHODS

Patient 1 — Female, 6 years old, caucasian.
Centroblastic-Centrocytic lymphoma.

Control 1 — Female, fraternal twin from patient 1.

Patient 2 — Female, 21 years old, caucasian.
Immunoblastic lymphoma.

Control 2 — Female, 37 years old, caucasian.

Venous blood was asseptically collected in a Liquemine Roche (5000 UI/ml) wetted syringe always before any radio or chimiotherapy has been started. After hemossedimentation, macrocultures were elaborated according to the slightly modified method of Moorhead et al [8].

After 72 hours, the culture was harvested, the cells fixed in methanol/acetic acid (3:1), the slides prepared and stained by the Giemsa.

For the evaluation of the blastic transformation rate, we estimated the relative percentage of activated and inactivated nuclei after stimulation by the PHA, both in cultures from the patients and in the control ones, in order to distinguish three main cell forms: a) Cells in mitosis (M) (any phase of the division), b) Blasts (B) (lymphocytes with a large nucleus, loose chromatin, frequently evidentiating a nucleolus) and c) Inactivated nuclei (IN) (small nucleus with dense chromatin and a narrow cytoplasmic band).

The likelihood ratio statistical test has been applied. For this, if we presume that when a lymphocyte enters mitosis it had necessarily supported a blastic transformation, we could then consider these two classes M and B) as a whole, grouping them in a class we will call "Blastic Transformation" (BlTr). This will be the biological class tested against the class of inactive nuclei (IN).

For the cytogenetic analysis, we adopted the criteria recommended by the Paris Conference (1971).

RESULTS

Table I shows the percentages and the statistical values of the morpho-physiological stages of PBL cultures from the patients under study and their controls.

TABLE I

PATIENT X CONTROL	Celular Stages (%) M	B	IN	Total	X^2_0
PATIENT 1	2,79	80,45	16,76	179	0,0932 (not significant)
CONTROL 1	13,77	70,66	15,57	167	
PATIENT 2	5,17	72,41	22,41	116	3,8078 (not significant)
CONTROL 2	11,71	75,68	12,61	111	

It is worthy to note that although the differences between the results obtained for the culture from patient 2 and its control are not significant, the X^2_0 (3,8078) is within the borderline of the X^2_c (3,841) considered in this analysis as 1 liberty degree and a significant level of 5%.

Table II summarizes the cytogenetic analysis and the statistical significance of these results, from the patients under study and their controls.

TABLE II

PATIENT X CONTROL	% of normal cells	% of abnormal cells	X^2_0
PATIENT 1	64,44	35,56	2,1260 (not significant)
CONTROL 1	78,13	21,87	
PATIENT 2	46,00	54,00	29,1776 (significant)
CONTROL 2	89,61	10,39	

The abnormality of the cells is based on both numerical and morphological changes of the chromosomes.

Although there were many disarrangements, it is interesting to note that no chromosomal change was consistent or patognomonic.

DISCUSSION

The PBL culture from Patient 1 presented quite a peculiar behavior in the presence of PHA. O significant interest ùas the high metabolism developed by these cells, reducing considerably the pH of the culture

medium during the first 24 hours. In spite of this high metabolic activity of the cells, of a diferent behavior than that of the control culture, such fact did not imply in the finding of a higher mitotic index; on the contrary, this value (2,79) has been much lower than the mean mitotic index of the control culture (13, 77).

Kowalewski & Rozynkowa [5], when studying the enzymatic activity during the blastic lymphocyte transformation in patients carrying lymphoproliferative syndromes observed that in chronic lymphatic leukemia, beside a delay in the blastic transformation, there was an increase in enzymatic activities preceeding the blastic transformation of the lymphocytes.

In the lymphocyte culture from Patient 1, however, the blastic cell percentage surpassed that of the control culture (80,45% and 70,66% respectively), and at a level similar and reverse to the difference found among the mitosis percentages. Consequently, if we analyse the BlTr class as a whole (B+M), the percentage get closer (83,24 and 84,43). Therefore, if the statistical test is applied to the results obtained for BlTr and IN classes, no rejection of equalty is found because the fusion of B and M classes conceal these discrepant values, normalyzing them. However, only in behalf of a commentary, if the BlTr class were again unfolded, and the statistical value analyzed in separate, such differences would become evident.

Such fact together with the observation of the abrupt decrease in the pH of the culture medium in the first 24 hours, could be explained by the hypothesis of a longer duration of the G1 cycle in these cells with a consequent delay in the beginning of the division. In the blastic phase, these cells could present a more intense rate of metabolism, what would contribute to the acidification of the medium due to the respiratory rate of these cells.

For the Patient 2, the results show (Table I) that the mitotic percentage in its culture was about a half of that found in the control culture, and that the percentage of the blastic cells in the culture from the patient has been slightly smaller than the percentage of the same cell class from the control culture. The application of the statistical test revealed no significance for these differences, although the X^2_0 (3,8078) were at the limit of the X^2_c (3,841), for 1 liberty degree and a significance level of 5%.

Based on these observations, we may conclude that, for the cases of lymphomas under study, there seems to have no deficiency, at least not marked in the response of PBL to PHA stimulus. For Patient 1, we may even say that it might exist an exacerbation of such a system.

Our experience on cytogenetic studies perfomed on patients carrying lymphoproliferative disorders including the patients under this study, shows that the chromosomal abnormalities of PBL are in evident processing, although they are neither consistent nor, in the most of cases, statistically significants.

These abnormalities included the occurrence of numerical alterations (at random losses and excess of chromosomic groups), due to divisional errors or to the result of a higher frailty of these cells during the

cytological preparations, or even to the presence of extra new elements (marker chromosomes) besides an apparent normal karyotype. The observation of these new elements could be understood in view of the other chromosomic alterations, consisting on chromosome breaks and rearrangements, appearence of quadriradial figures, suggesting the occurrence of chromaditic recombination and pseudodiploidy.

Our data agree with the results described by Wisniewski & Korsak [12], who found aneuploidies, pseudodiploidy and marker chromosomes in a PBL culture of a patient carrying a lymphosarcoma. On the other hand, Tjio et al [11], Baker & Atkin [2] and Reeves [9] did not find such abnormalities in PBL of patients showing these lymphoproliferative diseases.

These discrepancies could be explained by mading use of the speculation of Sandberg et al [10] who admitted that cells with chromosomal abnormalities, present at different sites, were metastatic cells of the neoplastic tissue, thus depending this fact on the degree of dissemination of the abnormal cells in the blood flow.

ACKNOWLEDGEMENT: The authors wish to thank Mrs. Sibylle Heller for the translation.

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RESUMO: Culturas temporárias de linfócitos do sangue periférico, estimuladas pela fitohemaglutinina, foram utilizadas para estudos da taxa de transformação blástica e de citogenética, de dois pacientes portadores de linfomas não-Hodgkin. Imunologicamente, os dois pacientes não mostraram repressão da população linfocitica T. Citogeneticamente, foram evidenciadas algumas aberrações cromossômicas, embora estas não fossem patognomônicas.

UNITERMOS: Linfomas não-Hodgkin; Transformação blástica; Citogenética.