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UNCOMMON LYMPHOCYTIC LEUKEMIAS IN PATIENTS FROM THE STATE OF ZULIA, MARACAIBO, VENEZUELA. I. THE COEXPRESSION OF LYMPHOCYTES B-CELL AND T-CELL MARKERS IN TWO PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Palabras Claves: cell membrane, chronic, human T-cell leukemia, human B-cell leukemia, leukemia lymphocytic, lymphocytes, monoclonal antibodies, case report, immunologic techniques.

ABSTRACT

In this report we describe the coexpression of T- and B-cell markers on lymphocytes from two patients with B-cell, chronic lymphocytic leukemia. The T-cell markers tested included the sheep red blood cell (SRBC) receptor and the T1 and T11 antigens; the B-cell markers included B4, B1, B2 and Dr (Ia); surface immunoglobulin IgM, IgD, IgG Kappa and lambda chains. Analysis of the results by independent multiplicative probability showed a minimum coexpression of T- and B-markers on 18.83% and 29.15% of the cells from patients 1 and 2, respectively. Two findings stand out from the results reported here. First, the report documents two additional cases of this rare form of CLL. Second, the cases were detected in patients from the state of Zulia, Venezuela, where the incidence of chronic lymphocytic leukemia is low.

INTRODUCTION

Chronic lymphoproliferative disorders, evolve from lymphoid cell clones that undergo malignant transformation (6, 17). Disorders emerging from B-cell clones include: B-cell chronic lymphocytic leukemia (B-CLL), B-cell cprolymphocytic leukemia, and leukemic reticuloendotheliosis or "hairy cell leukemia" (18).

In most cases of B-CLL, the predominant cell type is a small, mature B-lymphocyte that expresses low concentrations of surface immunoglobulin and forms rosettes with murine RBC (7, 8). Nevertheless, there is evidence for other types of CLL. These include the mixed-population type, in which a small percentage of the lymphocytes is of T-cell origin (19); a type in which the cells are of neither T nor B origin (5, 15);

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and the rare, T-cell type, in which the predominant pathologic cell is a lymphocyte that forms spontaneous rosettes with SRBC (1). Rarely, but with apparently increasing frequency, another type of B-CLL is being reported in which markers for both T- and B-cells are expressed concomitantly by the neoplastic lymphocyte (2, 6, 17, 20). In this communication we describe this last type of B-CLL in two patients from state of Zulia, Venezuela, an area in which the incidence of chronic lymphocytic leukemia is low (10).

CASE REPORTS

Patient number 1.

In January 1984, this patient, a 61-year old man, presented with a temperature of 39.2°C, chills and lumbar pain. Neither splenomegaly nor enlargement of the lympho nodes were noted. His leukocyte count was 29,000/ul with a differential cell count showing 77% mature lymphocytes, 10% of which were prolymphocytes. The urinalysis was normal so a urine culture was not performed. The patient was followed up without treatment (stage 0).

Approximately one year later, February 1985, the patient was found to have enlarged left axillary lymphonodes but no splenomegaly. The laboratory results were as follows; hemoglobin level, 14.6 g/dl; platelet count, 261,000/ul; leukocyte count, 23,250/ul. A differential cell count showed 70% mature lymphocytes, 10% of which were prolymphocytes. The only remarkable finding in a bone marrow smear was that 50% of the cells were mature lymphocytes. His serum uric acid was 6 mg/dl; aspartate aminotranferase (AST), 4.0 IU/ml; alanine aminotransferase (ALT), 7.0 UI/ml and total proteins, 6.7 g/dl of serum (4.4 g albumin and 2.3 g globulin/dl of serum). Serum immunoglobulin levels were: IgG 1,300mg/dl (normal range 800 mg to 1,800 mg/dl of serum), IgA 438 mg/dl (normal range 90 mg to 450 mg/dl of serum) and IgM 59 mg/dl (normal range 60 mg to 230 mg/dl of serum). The direct antiglobulin (Coombs') test was negative.

Based on these findings, a diagnosis of CLL was made. No medication was administrated.

Patient number 2.

This patient is a 69-year old woman who 4 years ago presented with a low grade fever (38°C) and enlargement of the cervical lympho nodes. All clinical and laboratory findings were compatible with a history of chronic, viral infections; therefore, she was released without treatment.

In May 1983, she presented with enlarged cervical, submaxillary and axillary lympho nodes but no splenomegaly. Her blood hemoglobin level was 13 g/dl; hema-

tocrit concentration, 42%; reticulocyte count, 1.2%, leukocyte count, 21,650/ul and platelet count, 394,000/ul. A differential cell showed 92% mature lymphocytes, 5% of which were prolymphocytes. The results of a direct Coombs'test were negative. Her immunoglobulin levels were: IgG, 720 mg; IgA, 272 mg and IgM 56 mg per dl of serum. Her serum alkaline phosphatase (AP) level was 65 U (normal range 19 U to 69 U). Based on these findings, a diagnosis of CLL was made; however, the patient was treated symptomatically for the recurring, upper-respiratory viral infection and released.

In November 1983, the patient still had the marked lympho node enlargement. Laboratory results showed a leukocyte count of 30,000/ul. She was treated with a dosage of 6 mg chlorambucil (Leukeran) per day for a total of 40 days. At the present time she is in remission and not receiving any medication.

MATERIALS AND METHODS

Blood specimens.

Venous blood was collected in disodium-ethylenediamine tetracetic acid (EDTA) (1.0 mg/ml blood) for the cytochemical analyses and in sodium heparin (0.1 to 0.2 mg/ml blood) for the immunocytochemistry and the SRBC-rosette formation assay.

Cytochemistry.

Blood smears were stained for peroxidase (benzidine and hydrogen peroxide), lipid (Sudan black B stain); esterase isoenzymes (naphthol ASD acetate with and without pre-exposure to sodium fluoride); acid phosphatase (Naphthol AS-BI phosphoric acid with and without the addition of tartrate) and for carbohydrate (periodic acid-Schiff, PAS, reaction). All cytochemical techniques were performed as described in detail elsewhere (13).

Immunocytochemistry.

The lymphocytes were isolated on a Ficoll-Hypaque gradient and phenotyped by indirect immunofluorescense as previously described (3, 4). In Wright-Giemmsa-stained smears 94% of the isolated cells identified as lymphocytes. The lymphocytes were stained for surface IgG, IgM, IgA, Ig Kappa and lambda chains and the antigens B1 (CD20); B2(CD21); B4(CD19); T1(CD5); T11(CD2); Dr; by murine monoclonal antibodies and goat anti-mouse IgG conjugated with fluorescein isothiocyanate (FITC). The antisera were obtained from Coulterclone (Hialeah, FL). The stained preparations were viewed in a Zeiss microscope (Zeiss, Oberkochen, West Germany) equipped with an epi-illuminator, a 540 nm excitation filter, and a 570 nm emission filter. Two-hundred cells were examined alternatively by phase contrast and fluorescence microscopy, and the percent cells effecting fluorescence calculated.

SRBC-rosette assay. The rosette-forming assay was performed as previously described (4, 14). The SRBC used were not treated with neuraminidase. Two hundred cells were viewed by phase contrast microscopy, and the percent rosettes (cells binding three or more SRBC) calculated.

RESULTS

Morphology and Cytochemistry.

The peripheral blood leukocytes from both patients were predominantly medium-sized mature lymphocytes with a high N:C ratio, some of the cells contained cytoplasmic azurophilic granules; the nuclear chromatin was clumped and nucleoli were visible in about 10% of the cells.

All cells examined failed to stain for peroxidase, lipid esterase isoenzymes or acid phosphatase. The PAS reaction was positive on 15% of the cells from patient 1.

Surface markers.

The percentage of lymphocyte expressing the individual markers can be judged from the data in Table 1. The Dr, B4 and B1, were the most frequently expressed; at least one B marker was detected on a minimun of 30% of the cells of either patient. The B2 antigen was detected only on 29% of cells from patient 1; patient 2 did not

TABLE I

EXPRESSION OF LYMPHOCYTE MARKERS*

LYMPHOCYTE	MARKER	PATIENT 1	PATIENT 2
B-cell	Dr	78	71
	B4	64	68
	B1	62	30
	B2	29	0
	IgM	0	0
	IgD	11	10
	IgG	4	1
	Kappa	15	5
	lambda	4	1
T-cell	SRBC	63	70
	T1	8	86
	T11	26	51

* Results are expressed as the percentage of cells expressing the marker of a total of 200 cells examined.

express this antigen. Immunoglobulin epitopes were displayed only by B cells; the most frequent, Ig kappa epitopes, were detected on only 15% of the cells from patient 1.

The SRBC receptor was detected on appoximately two-thirds of the cells from either patient. The T11 antigen was detected on 26% and 51% of the cells from patient 1 and 2, respectively. There was a pronounced discrepancy in the relative incidence of the T1 antigen; it was detected on 8% and 86% of the cells from patients 1 and 2 respectively.

The common acute lymphoblastic leukemia antigen (CALLA-CD10), J5 (16), and the (M01-CD11) monocyte antigen were manifested by 10% and 20% of patient 1 cells, respectively.

The degree of coexpression of two independent markers on a given cell was analyzed by multiplicative probability (9) (see Figs. 1 and 2).

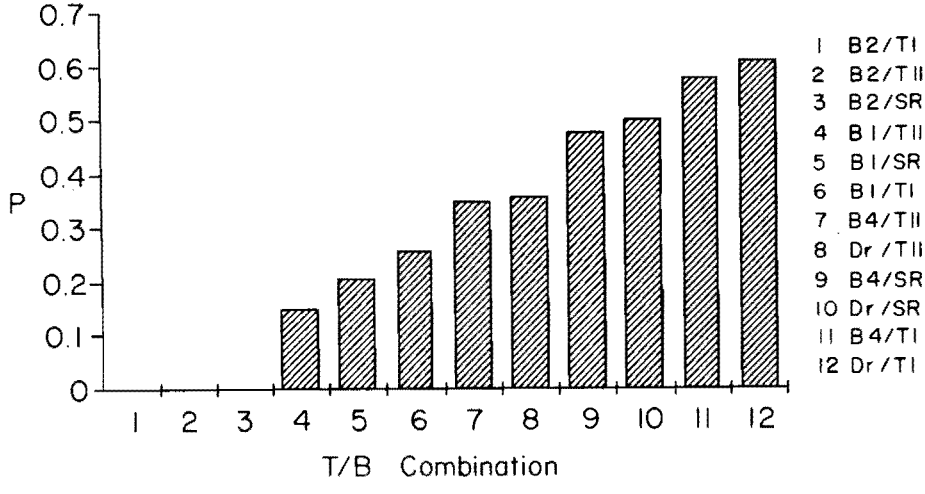

Figure 1.– Mean probability (P) of coexpression of B- and T-markers on the lymphocytes of patient number 1.

Using this method of analysis, the mean percentage of cells coexpressing a given B/T pair of markers was 18.83 ± 15.79 SD for patient number 1 and 29.15 ± SD 22.44 for patient number 2. The antigen combinations showing the highest probability of coexpression were Dr/SRBC-R (on 49.14% of patient number 1 cells) and Dr/T1 (on 61.06% of patient number 2 cells) (Fig 1 and 2). The least likely combination to show coexpression was B2/T1; this was true for the cells from either patient, and was attributable to the low incidence of the B2 antigen (Figs 1 and 2).

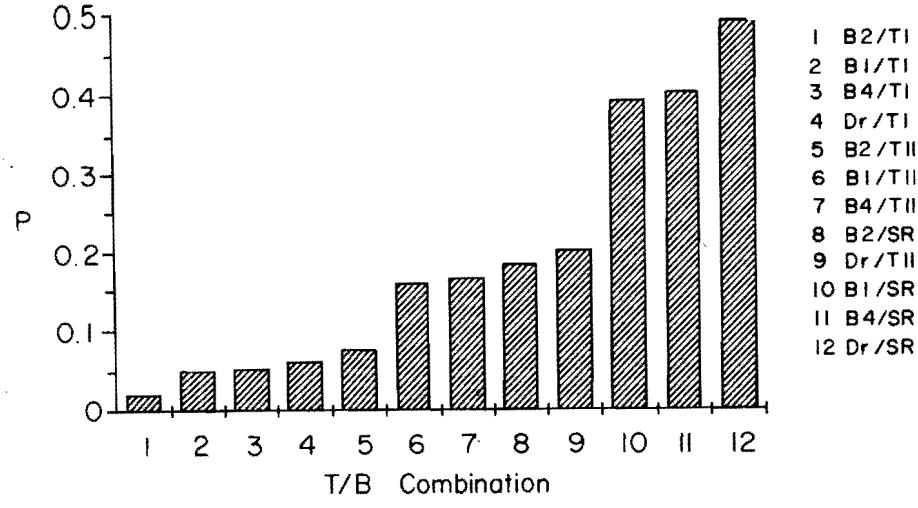

Figure 2.– Mean probability (P) of coexpression of B- and T-markers on the lymphocytes of patient number 2.

DISCUSSION

In rare cases of B-CLL, the lymphocytes coexpress B- and T-cell markers (1, 2, 6, 8, 10, 11, 12, 20, 21). Events involved in the concommitant expression are incompletely understood. Nevertheless, several mechanism can be evoked to explain the observations made in the laboratory (6). On one hand, some antigens may not be detected simply because many of the monoclonal antibodies available today define antigens expressed only by inmature cells. Additionaly, antigens in B-CLL cells express antigens weakly on the cell membrane, but strongly in the cytoplasms; hence, failure to examine the cytoplasms may result in a misclassification. It is also possible the laboratory worker attributes the presence of an antigen to a given B-cell, when indeed, the cell might be a lymphoid progenitors undergoing malignant transformations and endowed with multiple options for differentiation. Such a cell, would expresses unpredictable cell surface markers. Whether or not the phenomenon is attributable to the foregoing mechanisms or to frank, erratic manifestations of gene products are issues that remains to be resolved.

Presumably, these unusual types of B-CLL occur in geographic areas where the incidence of leukemia is high-a misconception that may have persisted because of the few reports from geographic areas where the incidence of leukemia is low. Here, we describe two cases of this unusual leukemia in two patients from such an area, Maracaibo in the state of Zulia, Venezuela.

The diagnosis of CLL in our patients was based primarily on clinical and laboratory findings, including morphologic characteristics of the neoplastic leukocytes and then, on the results of the phenotyping. The latter showed that the cells coexpressed B- (Dr, B4, B1, and B2) as well as T-(T1 and T11) markers (4).

We performed the SRBC rosette-forming assay because of its widespread success in identifying T-lymphocytes (14). Even though, cell-bound anti-SRBC immunoglobulins on some B-CLL cells can invalidate the results of the assay by effecting spurious rosette formation (17). Since our patients cells were not tested for the presence of anti-sheep agglutinins, the possibility of elusive rosettes was excluded by testing the cells for the T1 and T11 antigens which are impervious to anti-SRBC activity.

Analysis of the data showed that the probability of coexpression of given T- and B-marker combinations ranged from 18.03% ± 15.79 SD to 29.15% ± 22.44 SD of the cells. The probability of coexpression on lymphocytes from either patient was highest for the marker pair, Dr/T1 (61.06%), a combination that is independent of the rosette formation phenomenon.

Limited by the fact that the patients were no longer available for study some important assays were not performed. We were not able to prepare an SRBC+ populations of cells to test for the coexpression of B-cell antigens with double-labeled fluorescent antibodies. Also, we were not able to determine the presence of the antigens in the cytoplasm, or antigen re-expression after protease treatment of the cells. Limited by developmental costs and unavailability of technology, we could not implement assays to establish immunoglobulin and/or T cell receptor gene rearrangements.

The cells from patient 1 stained by antibodies to the acute leukemia markers J5; implying in acute leukemic state in this patient (16). The cells also stained by antibody to the Mol antigen, an antigen common to monocytes. The expression of these antigens in what is clearly a case of Chronic Lymphoid Leukemia (CLL) remains to be explained. It is possible that the expression of myeloid antigens represents an on-going transformation of a stem cell from which either B-cells of monocytes can differentiate (16). We were not able to locate patient 2 to test her cells for these two antigens.

The foregoing is clearly not a complete immunochemical documentation of coexpression of B- and T-cell antigen in CLL. That was not its intent. Our purpose was to show that the synchronous expression-for whatever reason-of T-and B-cell markers by the pathologic cells is a reality that represents problems in the immunological diagnosis of leukemia. Furthermore, the fact that the cases were patients from Venezuela reinforces the hypothesis that this aberrant form of leukemia is not restricted to areas where the incidence of chronic lymphocytic leukemia is high.

RESUMEN

Leucemias linfocíticas poco comunes del Estado Zulia. I. La coexpresión de marcadores B y T en linfocitos de dos pacientes con leucemia linfocítica crónica. *De Salvo-Cardullo L., (Instituto Hematológico de Occidente, Banco de Sangre, Maracaibo, Venezuela), Weir Medina, J.E., León M., Gómez O.* **Invest Clín** *29(1): 27-36, 1988.*– En este reporte describimos la coexpresión de marcadores T y B de linfocitos de dos pacientes con el diagnóstico de leucemia linfocítica crónica. Los marcadores T hechos incluyen: receptor para glóbulos rojos de carnero (SRBC) y los antígenos T1(CD5) y T11(CD2); los marcadores B incluyen los antígenos B4(CD19), B1(CD20); B2(CD21) y Dr(Ia), las inmunoglobulinas de superficie IgM, IgD, IgG y las cadenas livianas Kappa y Lambda. El análisis de los resultados por probabilidad multiplicativa independiente mostró una coexpresión mínima de marcadores T y B del 18.83% y 29.15%, sobre las células del paciente 1 y 2 respectivamente. Dos hallazgos se ponen en evidencia de los resultados reportados: Primero se documentan dos casos adicionales de esta forma rara de leucemia linfocítica crónica. Segundo, los casos fueron detectados en pacientes del Estado Zulia, Venezuela, donde la incidencia de Leucemia linfocítica crónica es baja.

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