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Expression of FMC7 Antigen and Tartrate-Resistant Acid Phosphatase Isoenzyme in Cases of B-Lymphoproliferative Diseases

H.G. DREXLER,*‡ M. MENON,* G. GAEDICKE† and J. MINOWADA*

**Department of Pathology, Loyola University Medical Center, Maywood, Illinois 60153 and Hines Veterans Administration Medical Center, Hines, IL 60141, U.S.A. and †Department of Pediatrics II, University of Ulm Medical School, 7900 Ulm, F.R.G.*

Abstract—*A panel of different B-cell malignancies representing various stages of B-cell differentiation were analyzed for the expression of an antigen labeled by the monoclonal antibody FMC7 and of tartrate-resistant acid phosphatase (TracP) activity. The FMC7 antigen and TracP were not found on early immature pre B-cell proliferations, appeared at early and intermediate B-cell stages, reached their peak of expression in terms of both incidence of positivity and staining intensity at the late B cell stage (as represented by hairy cell leukemia) and were lost at the B-cell/plasma cell transition. Although detected at similar stages of B-cell differentiation, FMC7 and TracP appear to be independently expressed and were not related to a particular Ig class. The simultaneous detection of FMC7 and TracP represents a distinguishing parameter for the identification of hairy cell leukemia.*

INTRODUCTION

A LARGE NUMBER of monoclonal antibodies (MoAbs) against B-cell antigens are now available. The MoAb FMC7, raised against a human B-lymphoblastoid cell line [1], became of particular interest as it appears to recognize distinct subsets of normal B-cells and only certain subtypes of chronic B-cell malignancies [2, 3].

We studied the immunological profiles of a series of B-cell malignancies. The cells were analyzed in a standardized routine leukemia-lymphoma phenotyping which combined clinical, morphological and cytochemical parameters with immunological data obtained by use of poly- and monoclonal antibodies [4]. We wish to report here the results regarding the reactivity of the malignant cells with the MoAb FMC7. The cells were further tested in the isoenzyme analysis of the enzyme acid phosphatase (E.C. 3.1.3.2) with special interest in the expression of the tartrate-resistant acid phosphatase (TracP) isoenzyme. This particular isoenzyme appeared at first to be specific for hairy cell leukemia (HCL) [5], but might be an enzymatic marker for certain subsets of normal and malignant B-cells [6].

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‡To whom correspondence should be addressed at his current address: The Royal Free Hospital, Department of Haematology, London NW3 2QG, U.K.

MATERIALS AND METHODS

Cells

Cells were obtained from patients with leukemia-lymphoma at Roswell Park Memorial Institute, Buffalo, New York, University of Ulm Medical School, Ulm, F.R.G., and Loyola University Medical Center, Maywood, Illinois. Peripheral blood, bone marrow or lymph nodes were analyzed. In all instances, the neoplastic cell populations contained greater than 90% monoclonal, monomorph cells by Wright–Giemsa morphology and surface marker analysis. All tissue specimens were immediately placed in physiological medium, finely minced with scissors and forceps, and made into single cell suspensions by passing through stainless steel mesh. The mononuclear cells from these processed populations, from peripheral blood and bone marrow samples were separated by Ficoll–Hypaque density gradient centrifugation. Mononuclear cell populations were used fresh or cryopreserved in liquid nitrogen and thawed as needed.

Immunological marker analysis

Diagnosis was established by standard Wright–Giemsa staining and by the presence or absence of definite immunological markers which are considered to be cell lineage- and stage-restricted or -associated.

The presence of surface membrane (SmIg) or intracytoplasmic (CyIg) immunoglobulins was determined by direct immunofluorescence using fluorescein-isothiocyanate (FITC)-conjugated goat anti-human immunoglobulin chain specific polyclonal antisera (Cappel Lab., Cochranville, PA). Reactivity with murine MoAbs against HLA-Dr/Ia-like antigen (Ia; MoAbs BA-4, OKI-1, B7/21), common ALL-antigen (cALLA; MoAbs J-5, BA-3, NU-N1), Pan-T antigen (T-Ag; MoAbs Leu-1, OKT-3, T28, Leu-4, T-101), myeloid antigen (M-Ag; MoAbs MCS-2, MY7, OKM1) was tested by indirect immunofluorescence. Characteristics of used MoAbs, references and commercial sources are summarized in refs. [4, 7].

Cells were incubated with the primary reagents at saturating concentrations for 30 min at room temperature. In case of indirect immunofluorescence, cells were washed and resuspended with FITC-conjugated goat anti-mouse IgG or IgM $F(ab')_2$ reagents (Cappel Lab., Cochranville, PA) for 30 min. After washing and centrifugation, the resulting cell pellets were suspended in 50% glycerol in phosplate-buffered saline, mounted on glass slides and analyzed at 1000 × magnification under an epi-immunofluorescence microscope. At least 200 viable cells were examined for positivity.

TdT-containing cells were identified by indirect immunofluorescence assay on methanol-fixed smears using rabbit anti-calf thymus TdT antiserum (PL Biochemical, Milwaukee, WI) and FITC-conjugated anti-rabbit IgG antibody (Cappel Lab., Cochranville, PA). CyIg were detected on methanol-fixed smears by direct immunofluorescence.

Erythrocyte rosette tests were performed by incubating the cells with sheep erythrocytes for 2 hr at 4° C (E-test for detection of T-cells) and with anti-bovine IgG-coated bovine erythrocytes for 1.5 hr at room temperature (EA-test for detection of cells with Fc-receptor) [4]. Sheep and bovine erythrocytes were obtained from Hazelton-Dutchland, Denver, PA.

Classification of cases

On the basis of morphological, clinical, histopathological data and immunological marker profiles (as shown in Table 1), cases were assigned to one of the following subtypes of B-cell malignancies: common acute lymphoblastic leukemia (cALL), pre B-ALL (cALL and pre B-ALL are here combined into one subgroup as testing for expression of intracytoplasmic μ was not performed on all cases), B-ALL, Burkitt-lymphoma, B-chronic lymphocytic leukemia (B-CLL), mature B-lymphoma (= non-Hodgkin's lymphoma), hairy cell leukemia (HCL) and multiple myeloma.

Isoelectric focusing

Harvested mononuclear cell populations were resuspended in a Tris–sucrose buffer of pH 7.4 at a concentration of 5×10^7 cells/ml. Cells were lysed by three cycles of freezing/thawing and enzymes were solubilized by addition of 1% Triton X 100. After centrifugation, the supernatant contained all enzymatic activities. Aliquots of this supernatant referring to equal numbers of cells were used for analytical isoelectric focusing on horizontal thin-layer polyacrylamide gels.

Gel matrix was composed of 4.8% (w/v) polyacrylamide, 12.5% (w/v) sucrose, 0.015% (w/v) ammoniumpersulfate/riboflavin and 0.1% (v/v) tetramethyl ethylenediamine; 2% (w/v) ampholyte of pH 2–11 (Serva, Heidelberg, F.R.G.) was added to establish a pH-gradient. Runs were performed for 1 hr at 5° C, 30W constant power, limitation of voltage to 1400 V using an LKB Multiphor/Power Supply unit (LKB, Bromma, Sweden).

Immediately after separation, isoenzymes were visualized directly on the gels using the following staining solution: 40 mg naphthol-AS-Bi-phospate

Table 1. Immunological marker profiles of various B-cell malignancies

Category	E	EA	CyIg	SmIg	Ia	cALLA	TdT	T-Ag	M-Ag
cALL	−	−	−	−	+	+	+	−	−
pre B-ALL	−	−	+*	−	+	+	±	−	−
B-ALL†	−	+	±	+	+	−	−	−	−
B-CLL‡	−	+	±	+	+	−	−	−§	−
HCL	−	+	±	+	+	−	−	−§	−
Multiple myeloma	−	+	+	−	−	−	−	−	−

*CyIg μ expression.
†Immunologically similar or identical to Burkitt-lymphoma.
‡Immunologically similar or identical to B-lymphoma.
§Positive for CD5 reagents (Leu-1, T-101, OKT1, etc.); negative for CD3 (OKT3, T28, UCHT1, etc.) and CD7 reagents (Leu-9, 3A1, etc.).

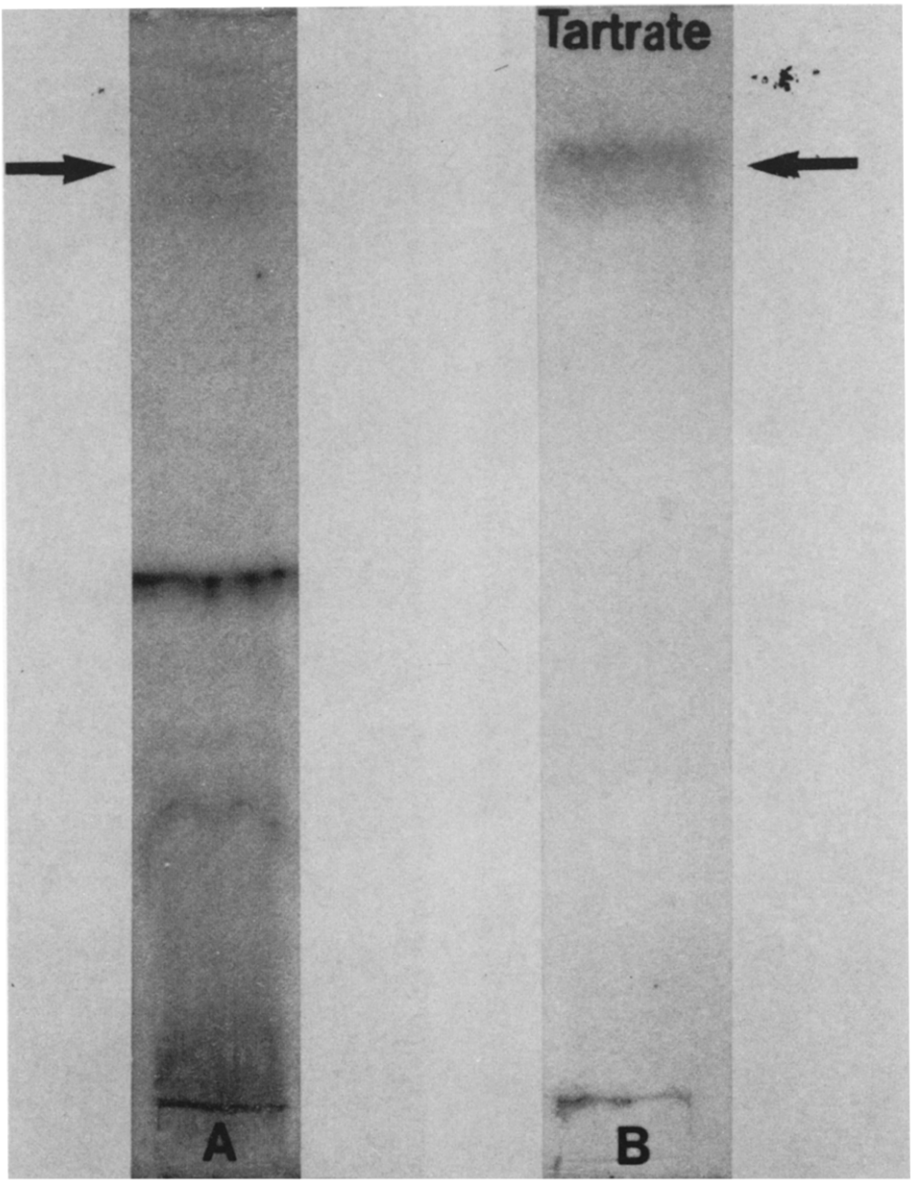

Fig. 1. *Original gelslabs of acid phosphatase isoenzymes after isoelectric focusing. Lane A: B-CLL; lane B: same case (inhibition with sodium tartrate). Note resistance of one band (arrow) to tartrate-inhibition.*

Table 2. Analysis of expression of FMC7 antigen and of TracP isoenzyme

Category	FMC7+	TracP+
	No. cases positive/cases studied (%)	
Common/pre B-ALL	0/8 (0%)	2/99 (2%)
B-ALL	1/2 (50%)	0/4 (0%)
Burkitt-lymphoma	1/2 (50%)	0/3 (0%)
B-CLL	51/99 (51%)	33/98 (34%)
B-lymphoma	31/41 (75%)	12/37 (32%)
HCL	15/16 (94%)	10/12 (83%)
Multiple myeloma	0/1 (0%)	1/3 (33%)

dissolved in 4 ml dimethylformamide, 1.2 ml hexazotized pararosaniline, 120 ml barbituric acid buffer of pH 5.0. Staining for 3–3.5 hr at room temperature. For the identification of the tartrate-resistant isoenzyme, 75 mM sodium tartrate was added to the staining bath [8].

RESULTS

Expression of FMC7 antigen

Expression of the antigen detected by the MoAb FMC7 was analyzed in 169 cases (Table 2). While cases of common/pre B-ALL and multiple myeloma were FMC7 negative, the determinant was found in B-CLL (51%), B-lymphoma (75%) and HCL (94%) (only two cases were tested in the categories B-ALL and Burkitt-lymphoma, each category had one positive case). In the majority of FMC7-positive cases, 50% or more cells of the monoclonal population were reactive. Positivity for FMC7 did not correlate with any particular Ig class. The strongest staining intensity was found in the HCL cases.

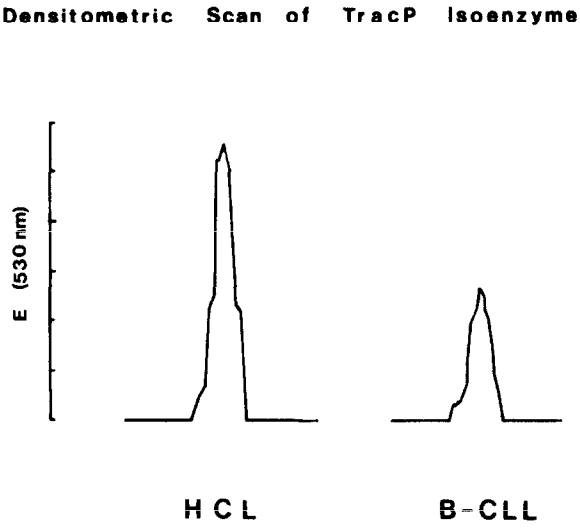

Fig. 2. Densitometric comparisons of the staining intensity of TracP isoenzyme in HCL and in B-CLL referring to equal numbers of cells per sample.

Expression of TracP

One or two bands in the isoenzyme profile of acid phosphatase, the tartrate-resistant isoenzyme, could be selectively demonstrated by inhibition experiments while all other bands were inhibited (Fig. 1). The expression of this isoenzyme marker was examined in 256 cases (Table 2). The highest percentages of incidences of TracP expression were found for the subtypes HCL and B-CLL. The staining intensity of the TracP isoenzyme was significantly stronger in 9 of the 10 TracP positive

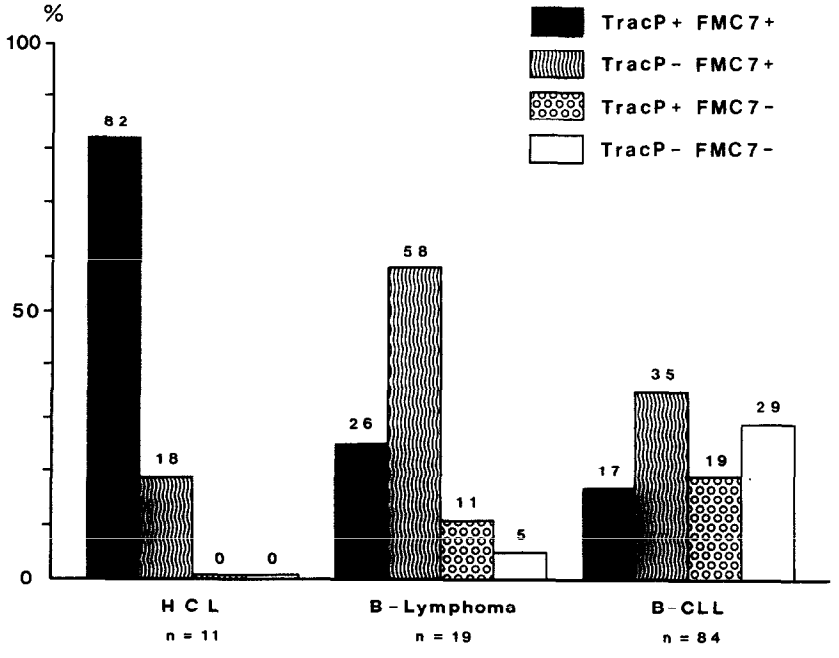

Fig. 3. Analysis of simultaneous expression of FMC7 and TracP (percentage of cases showing one of the four possible combinations).

Table 3. Comparison of FMC7-positivity in different studies of B-cell malignancies

Ref.	B-CLL	B-PLL	HCL	B-lymphoma
[2,11]	6/46 (13%)*	19/19 (100%)	10/11 (89%)	0/4 (0%)
[12]	34/162 (21%)†	27/28 (96%)	14/14 (100%)	37/55 (67%)
This study	51/99 (51%)‡		15/16 (94%)	31/41 (75%)

*Positive cases were described as "prolymphocytoid" [2].
†Includes 5/12 cases categorized as "prolymphocytoid variants" of B-CLL [12].
No differentiation was made between "typical" B-CLL and B-PLL.

HCL samples than in positive B-CLL and B-lymphoma cases (Fig. 2). Expression of TracP did not relate to an Ig idiotype.

Co-expression of FMC7 and TracP

In this analysis the co-expression of FMC7 and TracP was examined: none of the common/pre B-ALL, B-ALL, Burkitt-lymphoma and multiple myeloma cases were simultaneously positive for both markers; 17% of the B-CLL, 26% of the B-lymphoma, and 82% of the HCL cases were doubly positive (Fig. 3). Within the panel of 114 studied cases of B-CLL, B-lymphoma and HCL, 28 (25%) cases were positive for both markers and 25 (22%) samples were negative for both markers while 61 (53%) cases were positive for one marker and negative for the other indicating that these markers are expressed independently.

DISCUSSION

In the study presented here, we analyzed the phenotypic expression of two B-lineage associated markers in a large series of B-cell malignancies: common ALL (belonging to the B-cell lineage on the basis of heavy chain gene rearrangement studies [9]), pre B-ALL (common ALL and pre B-ALL were summarized as one category in this study), B-ALL, Burkitt-lymphoma, B-CLL, B-lymphoma, HCL and multiple myeloma.

The monoclonal antibody FMC7 bound 5.0 ± 2.8% of mononuclear cells in the peripheral blood [10] and reacted with approximately half the SmIg+ circulating B-cells [1]. Expression of the FMC7 antigen was found to be maturation-linked [3] and served to distinguish more mature B-cell leukemias from immature variants [11]. While the most immature and mature B-cells as evidenced in their malignant counterparts Non-T/Non-B ALL and multiple myeloma were negative for FMC7, intermediate to late stages of the B-cell pathway were described to be FMC7+ [2, 11, 12]. In our analysis, a large percentage of B-CLL, B-lymphoma and HCL cases were FMC7+ with the highest incidence of positivity in HCL; common/pre B-ALL and multiple myeloma cells did not react with FMC7. Caligaris-Cappio *et al.* showed that the expression of the FMC7 antigen could be induced by the phorbolester TPA in B-cells undergoing morphological, cytochemical and immunological differentiation to "hairy cell-like cells" [13].

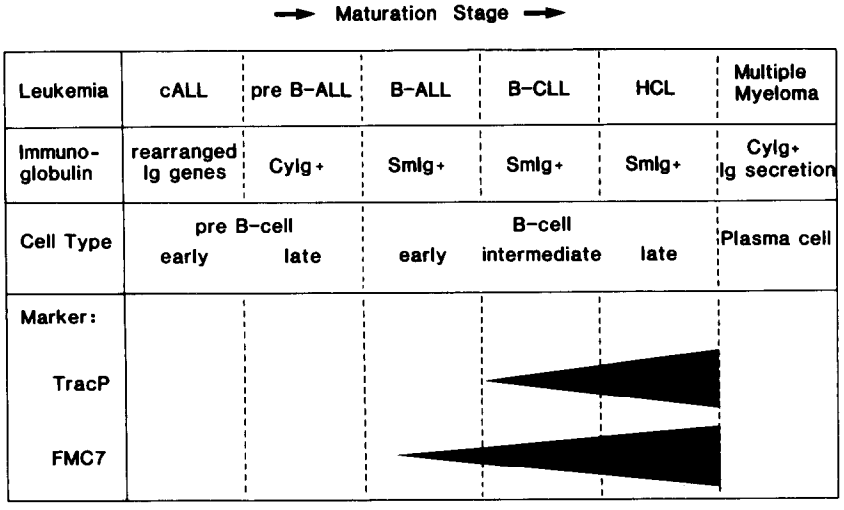

Fig. 4. Summary of TracP and FCM7 antigen expression during B-cell differentiation. The increased expression of the markers refers to an increase in the number of positive cases and to an increase in staining intensity.

Galton *et al.* [14] separated a special type of leukemia from typical B-CLL: the clinicopathological entity prolymphocytic leukemia (PLL). Whereas PLL can be distinguished from typical CLL by morphology, it is generally considered to be a "variant" of CLL [15]. As B-CLL cases might transform into the more aggressive B-PLL, a number of cases can be identified in this intermediate phase termed "prolymphocytoid transformation" [16]. Depending on the percentage of the larger PLL cells, these cases can be categorized as CLL, "prolymphocytoid" variants of CLL or PLL [12]. In the study presented here, all these subsets were included in the category B-CLL, and B-PLL was not differentiated from "typical" B-CLL. The staining of typical B-CLL with FMC7 as opposed to B-PLL has been studied in 2 series (Table 3). Nearly all cases of B-PLL and HCL were FMC7+ [2, 11, 12]. In contrast, FMC7 was negative in the majority of B-CLL. Several of the FMC7+ B-CLL cases were categorized as "prolymphocytoid" variants of B-CLL [2, 12].

Previous studies suggested that the characteristic cells in B-PLL and HCL have features placing these conditions in the late stages of the B-lymphocyte differentiation pathway [17]. Thus, it was concluded that the expression of FMC7 is related to the late maturation stages of B-cells [2].

The TracP isoenzyme separated by gel electrophoresis was first detected in malignant reticuloendotheliosis (= hairy cell leukemia) [18]. This enzyme activity was subsequently also identified by cytochemical means in activated macrophages [13], stimulated normal peripheral blood monocytes [19], monocytic leukemias [20], in cells of T- or B-CLL [6] and in reactive normal B-lymphocytes [6]. Although in the present study we found the highest incidence of TracP expression in HCL, a significant number of B-CLL and B-lymphoma cases were also TracP+, however, showing a lower degree of staining intensity. Using TPA, TracP-negative B-CLL cells could be induced to express TracP activity [13, 21].

The predominant reactivity of B-PLL and HCL with FMC7 and the possibility of inducing FMC7-negative B-CLL to differentiate to the stage of HCL and to express newly FMC7 suggest that this marker is acquired during B-cell differentiation at a stage corresponding to B-ALL, is most strongly expressed at a late stage corresponding to HCL and is finally lost at the terminal stage of plasma cells. Similarly, the expression of TracP is seen first in B-CLL, reaches its peak of expression in HCL and is subsequently lost in the transition from late SmIg+ B-cells to SmIg-negative plasma cells (Fig. 4). It appears (a) that FMC7 antigen and TracP isoenzyme expression are not correlated, and (b) that neither FMC7 positivity nor TracP activity are related to a particular Ig class.

The use of either FMC7 or TracP alone as markers of HCL is hampered by the expression of these parameters by a significant number of earlier malignant cells from B-ALL to B-CLL. However, the combined analysis of FMC7 and TracP offers the possibility of more accurately identifying HCL cells because immature and terminal B-cell stages are TracP- and FMC7-negative while only a relatively low percentage of B-CLL/B-lymphoma cells are TracP+ and FMC7+.

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