Monoclonal Antibodies with Specificity for Hairy Cell Leukemia Cells

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ABSTRACT Hairy cell leukemia is a well described clinical entity, but the cell of origin for this leukemic cell and its function are still unknown. There are no totally specific markers for this cell, although tartrate-resistant acid phosphatase staining has been used extensively as a diagnostic test. This study describes three monoclonal murine antibodies with variable specificity for hairy cells. Antibody 1 was highly specific for hairy cells and was not found to react with normal or leukemic cells in this limited study. It did not react with the cells of all patients. It also did not react with all of the hairy cells of some of the positive cases. Antibodies 2 and 3 reacted with virtually all hairy cells but not with normal peripheral blood cells. However, reactions were obtained with certain leukemic myelomonoblasts and some activated B cells. The most obvious use for these three antibodies is for diagnostic purposes. They should also be helpful reagents to investigate the origin of the leukemic hairy cell. The possibility that antibody 1 detects a tumor-specific antigen is discussed.

INTRODUCTION

Hairy cell leukemia (HCL, leukemic reticuloendotheliosis)[1] is a clinically well defined entity (1). However, the origin of the hairy cell itself is still controversial, and no normal equivalent of this leukemic cell has been found. The cell is clearly related to the B cell because it possesses the highly specific property of having membrane immunoglobulin M (IgM) and IgD (2–6); it occasionally, however, shows properties of the T cell (7). Apparently T and B cell phenotypes may coexist or alternate on hairy cells (8–12). The T cell phenotype may appear after mitogen stimulation (12). These observations suggest the presence of subgroups of HCL or the capacity of hairy cells to differentiate into cells as widely divergent as B and T cells. Such studies, however, suffer from the absence of a specific diagnostic test for hairy cells. So far these cells have been identified by morphology and cytochemical characteristics (1). Demonstration of tartrate-resistant acid phosphatase (TRAP) has been used extensively as a marker for hairy cells in both clinical (13) and experimental situations (7, 14). This test, however, has been shown not to be totally specific for hairy cells (15), especially when results show intermediate positivity. HCL with a negative TRAP test has also been described (15).

The diagnostic usefulness of a hairy cell marker is apparent. Such a marker might also help uncover the true identity and origin of the hairy cell. We have, therefore, used the hybridoma technique to make monoclonal murine antibodies to hairy cell leukemia cells. Three such antibodies with varying degrees of specificity are described in this paper.

METHODS

Patients

Cells from 15 consecutive patients with HCL were studied. Control patients had various leukemias or lymphomas. Patient material was obtained from several medical centers. In each case the diagnosis was made according to standard clinical criteria. HCL was diagnosed on the basis of the morphology of the cells found in peripheral blood, bone marrow or spleen, the presence of TRAP in hairy cells, and a compatible clinical picture. Certain patients (Ya, Ne) had electron microscopic studies confirming the presence of hairy cells. Patient Ne has been reported (16).

Techniques

Cell separation. The cells used for immunization were obtained from patient Ya, who underwent leukapheresis in July 1980. The cells obtained from this procedure were aliquoted and frozen in a medium of RPMI 1640, 10% dimethylsulfoxide, 20% fetal calf serum. A programmable freezer (Cryo Med., Mt. Clemens, MI) with liquid nitrogen was used to freeze cells at a rate of 1°C/min. When thawed

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[1] *Abbreviations used in this paper*: HCL, hairy cell leukemia; PBMNC, peripheral blood mononuclear cells; TRAP, tartrate-resistant acid phosphatase.

for immunizations or immunofluorescence, the cells remained viable (usually >95% viability by trypan blue exclusion) and contained 80% typical hairy cells by phase microscopy. Other patients' cells were obtained from heparinized blood, bone marrow aspirates, or from teased organs. Mononuclear cell preparations containing the hairy cells were obtained using a standard separation method with Ficoll-Hypaque (d = 1.077). After washing three times, the cells were used for immunofluorescent staining and frozen in liquid nitrogen for future use.

E rosettes. Spontaneous rosette formation between lymphocytes and neuraminidase-treated sheep erythrocytes was performed as described elsewhere (17).

Reagents

Monoclonal antihairy cell antibodies. The hybridoma technique used is described elsewhere (18). Briefly, BALB/c mice were immunized with 20×10^6 hairy cells on three occasions over a 7-mo period. 3 d after the last immunization, the spleens of these mice were harvested, and the splenocytes were fused to the murine lymphoma cell line SP 2/0-Ag 14 (19) using polyethylene glycol 1000. Hybrid cells were selected in a hypoxanthine, aminopterin, and thymidine (HAT) medium and supernatants from growing clones were screened 12–18 d after fusion. 200 supernatants were screened by indirect immunofluorescence for the presence of antibodies reacting with peripheral blood mononuclear cells (PBMNC) from a normal donor and with cells used as the immunogen (Ya). A tetramethylrhodamine isothiocyanate (TRITC)-conjugated, affinity absorbed, $F(ab')_2$, rabbit anti-mouse immunoglobulin antibody was used as the second antibody for indirect immunofluorescence. Only supernatants staining the hairy cells but not the normal PBMNC were selected. The corresponding hybridoma cell lines were expanded and cloned on soft agar. Three supernatants from cloned hybridomas were used extensively.

Other antibodies. Murine anti-kappa monoclonal antibody was obtained from an immunization with a purified human monoclonal IgM kappa. Murine anti-lambda monoclonal antibody was obtained commercially from Bethesda Research Laboratories (Bethesda, MD). The antimonocyte monoclonal antibodies (61D3, 63D3) were a gracious gift from Dr. D. Capra (20).

RESULTS

Three monoclonal antibodies, αHC1, αHC2, and αHC3, reacted with the immunizing cells, the hairy cells from patient Ya, but not with normal PBMNC. These were selected for further study. Antibodies αHC2 and αHC3 gave weak granular staining with some variation of intensity when used for indirect immunofluorescence on hairy cells. Antibody αHC1 gave brighter and more speckled staining also with variation of intensity (Fig. 1). Whereas the former two antibodies appeared to stain all hairy cells in the specimen from patient Ya, the latter antibody was clearly negative with some hairy cells (Fig. 1).

The reactivity of these antibodies was also tested on hairy cells from patient Ya with a cytofluorograf (Fig. 2). The tracings show positive fluorescence with hairy cells but not with normal PBMNC. The fluorescence intensity observed with αHC2 and αHC3 was less than the intensity with αHC1. All three antibodies showed somewhat less fluorescence intensity than that observed with monoclonal anti-kappa light chain antibody. The fluorescence curves of αHC2 and αHC3 follow a Gaussian distribution and suggest that all cells are positive with these antibodies. On the contrary, the curve observed with αHC1 indicates the presence of both a negative and a positive cell population. These findings confirm the above visual description of the staining characteristics of these antibodies.

To define the specificity of these anti-hairy cell antibodies, various normal and pathological cell types were used for immunofluorescent staining. As can be seen in Table I, normal lymphocytes and monocytes contained within the PBMNC and normal granulocytes contained within the buffy coat cell preparation did not react with the αHC antibodies. Likewise cells from teased tonsils, mesenteric lymph nodes, spleen, and thymus were mostly negative when stained with the αHC reagents. However, a few cells in normal uninvolved spleen, thymus, and bone marrow did stain with αHC2 and αHC3. αHC1, the most specific of the three antibodies, did not react with any cells from these organs. αHC1 did not react with any of the cell lines tested, including a promyelocytic cell line, HL 60 (22), a histiocytic lymphoma cell line, U 937 (23), and a leukemic cell line from a patient with chronic granulocytic leukemia in blastic crisis, K562 (24). αHC2 and αHC3 did not react with T cell lines or the nonlymphoid cell lines. Some B cell lines were entirely negative as in the case of RPMI 8866 P (Table I). Others were positive and the percentage of cells staining with αHC2 and αHC3 ranged widely (from 5 to 68% of the cells) from cell line to cell line. Further studies of several B cell lines indicated that the percentage of positive cells changed with the phase of cell growth. In addition, although purified preparations of normal resting B cells were usually negative with all three antibodies, positive cells were obtained after pokeweed mitogen stimulation with αHC2 and αHC3. These positive cells appeared before the development of maximal numbers of plasma cells and disappeared at the plasma cell stage. These findings are currently the topic of a separate study.

In Table II individual data on 15 different HCL patients are summarized. The percentage of cells staining with the αHC antibodies showed a general correlation with the percentage of hairy cells seen by phase microscopy on the same sample. αHC1 stained less cells than the two other αHC antibodies in most cases and probably identifies a fraction of the hairy cells in these patients. Supporting this contention was the finding of some morphologically typical hairy cells that did not stain with αHC1 (Fig. 1). Patients in a leukemic phase of their illness, such as patients Ya and

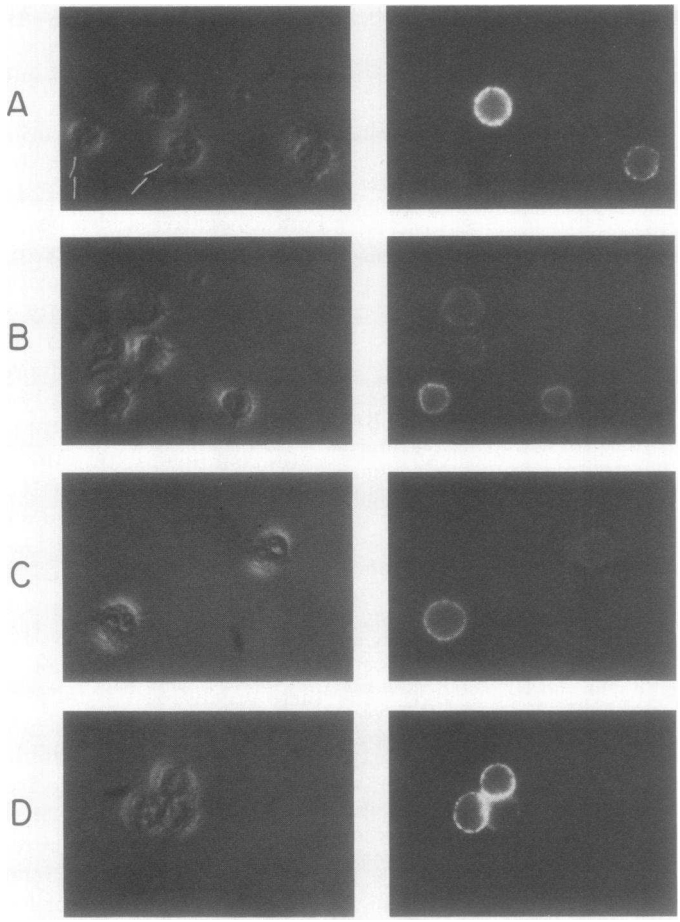

FIGURE 1 Corresponding phase microscopy and immunofluorescence on hairy cells from patient Ya: monoclonal antibodies were used for indirect immunofluorescence, αHC1 (A), αHC2 (B), αHC3 (C), and anti-kappa light chain (D). The two arrows indicate hairy cells that do not stain with αHC1.

St had many cells staining with αHC1. Others such as Pe had no cells staining with this antibody in spite of the presence of many hairy cells in the sample. This difference in positivity with the αHC1 antibody was also seen in two spleen samples (Ho and So). There seemed to be no common feature among the patients with hairy cells lacking reactivity with αHC1 (patients Pe, So, Co, and possibly Ne). In several patients only few hairy cells were found in the peripheral blood and percentages of cells staining with the αHC reagents were small, although each cell that stained positively also had the morphology of a hairy cell. In such a patient a sample from a different source, such as spleen (patient By), can be helpful to confirm the staining results.

In two cases follow-up samples of blood obtained at a later date were tested (Table II). In patient Ne clinical deterioration had occurred, manifested by an increasing degree of pancytopenia and an enlarging spleen. This correlated with the presence of more hairy cells in the peripheral blood, both by phase microscopy and by immunofluorescence using the αHC reagents. Staining results and the percentage of hairy cells showed little change in patient St over a 3-mo period during which the patient seemed to be deteriorating.

In Table III cells from nine patients with typical B cell chronic lymphocytic leukemia were totally nonreactive with all three αHC antibodies. Two lymphocytic lymphomas and three patients with acute lymphoblastic leukemia had no reactive cells except for patient Cn, where a few cells reactive with αHC2 were found; their significance remains unclear. Similarly only a few cells from a patient with plasma cell leukemia (60% plasma cells in the specimen) stained with αHC2 and αHC3.

αHC1 did not react with any cells from 12 different

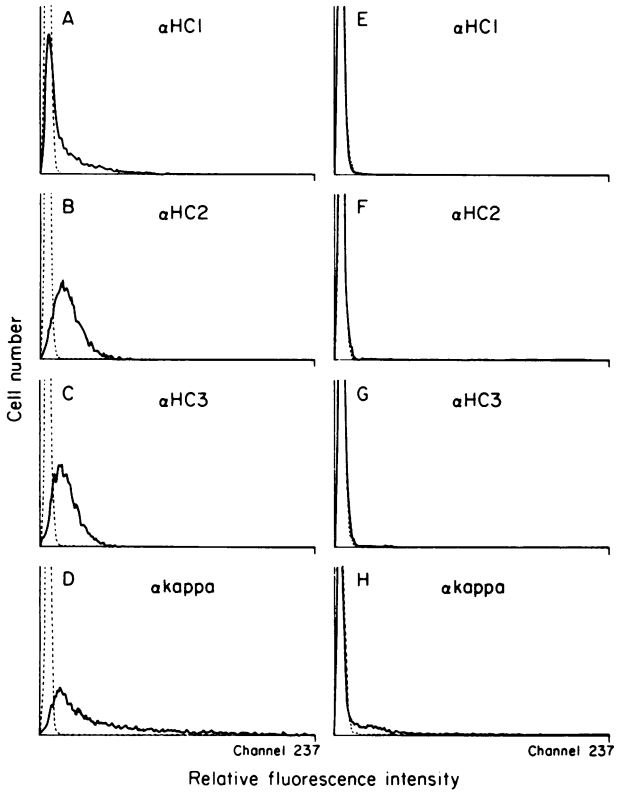

FIGURE 2 Fluorescence intensity distribution obtained with a cytofluorograf. In panels A–D hairy cells from patient Ya were used and in panels E–H normal PBMNC were used for immunofluorescence. The dotted line describes the histogram of cells stained with control medium rather than the hybridoma supernatant and indicates the location of fluorescence negative cells. Whereas most hairy cells stained positively with αHC2 and αHC3, there was a definite negative subpopulation with αHC1. Most hairy cells also stained with the anti-kappa reagent.

patients with various myelomonocytic disorders (Table IV). However, αHC2 and αHC3 stained cells, especially in samples containing early myeloid precursors. The highest counts of staining cells occurred in samples from acute myelo- or monoblastic leukemias containing mainly blasts. These results indicate the presence of cell surface antigens shared by leukemic myelomonoblasts and hairy cells and recognized by αHC2 and αHC3.

Hairy cells are known to possess avid Fc-receptors. The αHC antibodies we describe do not react with other cells known to have strong Fc-receptors such as monocytes and granulocytes. Aggregated human IgG did not inhibit the staining by the three αHC antibodies (data not shown). Therefore, nonspecific attachment of these antibodies via an Fc-receptor can be excluded.

By Ouchterlony precipitation αHC1 is an IgG2a kappa mouse immunoglobulin. αHC2 and αHC3 are both IgG1 kappa immunoglobulins. Mouse hybridoma antibodies of the same subclasses, but with different specificities did not stain hairy cells. The α-lambda light chain reagent used is an IgG1 kappa mouse immunoglobulin and constitutes an additional control for nonspecific Fc binding.

In most of the HCL cases, staining with anti-kappa and anti-lambda light chain antibodies demonstrates a monoclonal cell membrane immunoglobulin (Table II) as described previously from this laboratory (2) and others (4, 5). This was less readily apparent when only a few hairy cells were found (for example in patient Na). In some other cases where lambda light chain was found on the hairy cells (Co and Gi), another population of smaller lymphocytes staining with anti-kappa was seen, demonstrating the coexistence of the leukemic hairy cells and the nonleukemic B cells.

Because hairy cells do have phagocytic capacity and have been thought to be related to monocytes, it is of interest to note the negative results of staining with

TABLE I
Reactivity of Normal Cells and Cell Lines with αHC Antibodies by Indirect Immunofluorescence

Cell source	No. of specimens	Cells staining with αHC1	αHC2	αHC3
			%	
Normal (Nl) PBMNC	8	0	0*	0*
Nl buffy coat	2	0	0	0
Nl tonsil	1	0	0	0
Nl mesenteric lymph node	1	0	0	0
Nl spleen‡	6	0	1.4	1.3
Nl thymus	2	0	0.4‖	1.6‖
Nl bone marrow§	3	0	0.1	0.1
T cell lines¶		0	0	0
U 266 (IgEλ plasma cell line)§§		0	0	0
2132 (IgGλ plasma cell line)‡‡		0	0	0
3638 (B-cell line derived from ALL)‡‡		0	52	68
RPMI-8866 P (B-cell line)		0	0	0
Nonlymphoid cell lines**		0	0	0

* A few individuals did show low percentages of positive cells with αHC2 and αHC3 but not with αHC1.
‡ Uninvolved spleens from patients with hemolytic anemia and Hodgkin's disease.
§ Uninvolved bone marrow from patients with systemic lupus erythematosus and hemolytic anemia.
‖ The cells staining positive were large.
¶ Cell lines 1301, Ke 37, Molt 4, Cem-T.
** Cell lines HL 60, U 937, K 562, fibroblast cell lines.
‡‡ Cell lines obtained from the Human Genetic Mutant Cell Repository.
§§ See reference 21.

TABLE II

HCL Patients—Cell Surface Characteristics

Patients*	WBC 10^9 cells/l‡	Splenectomy	Hairy Cells§	N-SRBC‖ Rosettes	Cells staining with						
					αKappa	αLambda	61 D3	63 D3	αHC1	αHC2¶	αHC3¶
			%	%			%				
Ya—lph	50.0	+	80	6	85	1	0	0	53	73	85
Ho—spl			83	8	73	0	0	0	27	83	77
St 6-18-81	34.0	−	83	10	78	0	0		67	78	67
8-6-81		−	71	11	76	0	0		58	77	74
9-23-81	40.0	−	73	7	85	0	0		54	67	68
Pe			79	11	1	75	0	0	0	38	40
Ma	9.1	+	45	19	43	0			44	68	72
So—spl			30		3	75			1	85	86
He	6.0	−	27	27	1	78	0	0	14	28	42
Co	7.2	+	21	50	13	14‡‡			0	18	19
Hu	7.1	+	13						4**	20	7
An	3.3		6	76	13	1	0	2	7**	7**	7**
Ne 11-27-80	2.5	−	6	50	27	1	0	1	2**	7**	8**
6-17-81	2.0	−	33	51	57	1	0	0	1	45	60
By	1.1	−	5	82	14	2	2	1	3**	6**	6**
By—spl			39	9	58	0	0	1	17	37	25
Kl	4.5	+	5	68	10	0	1	0	4**	4**	3**
Gi	3.6	+	4	93	20	13‡‡	0	2	2**	0	1**
Na	2.1	−	3	68	1	1	1	0	2**	0	1**

* PBMNC were used in each case, except where indicated by: lph = leukapheresis, spl = spleen. In patient By both samples were obtained simultaneously.

‡ The leukocyte count was routine clinical data.

§ Assessed by phase microscopy at ×1,000 magnification.

‖ Neuraminidase-treated sheep erythrocytes.

¶ Usually the αHC antibodies stained cells that were hairy cells by morphology; however occasionally they stained additional cells that had a less typical hairy appearance, as in patients He, Ma, and So.

** Each cell that stained positively also had the morphology of hairy cells.

‡‡ All hairy cells seen stained with this reagent and the kappa-bearing cells were not hairy cells by morphology.

two monoclonal antimonocyte antibodies described previously (20) in all patients with HCL tested (Table II). These antibodies stained ~20% of normal PBMNC. The results are consistent with reports of monocytopenia in HCL (25).

DISCUSSION

We have described three unique anti-hairy cell monoclonal antibodies, one of which (αHC1) demonstrates specificity for hairy cells and does not react with any other cells tested. Thus αHC1 reacted only with hairy cells, but not all hairy cells in a given patient reacted with this antibody. Also, several HCL patients showed only few or no cells reacting with αHC1, even when the percentage of morphologically identifiable hairy cells was quite high. Therefore, this antibody may identify a subset of hairy cells or only react with hairy cells at a specific stage of differentiation. Subsets of HCL have previously been suggested on the basis of (*a*) the clinical picture, i.e., pancytopenic vs. leukemic form of the disease, remission vs. progression after splenectomy, (*b*) the varied results of cell marker and cell functional studies from patient to patient (5), and (*c*) differences in the occurrence of two hairy cell specific membrane proteins from patient to patient (26). Some evidence was obtained indicating that αHC1 may identify hairy cells found more frequently during clinically progressive disease such as the leukemic phase of HCL.

Both αHC2 and αHC3 reacted with the great majority of hairy cells in all patients, but they also reacted with certain other cell types. This was most apparent for the myeloblasts or myelomonoblasts from patients

TABLE III

Lymphoid Leukemias and Lymphomas: Reactivity with αHC Antibodies

Patient	WBC 10^9 cells/l*	Lymphocytosis*	Cells staining with α-kappa	α-kappa & α-lambda	αHC1	αHC2	αHC3
		%			%		
Ol, CCL‡	220.0	94		79	0	0	0
Bi, CLL			97	100	0	0	0
Gr, CLL	73.0	92	90		0	0	0
To, CLL	26.0	69	1	94	0	0	0
Se, CLL	207.0	94		93	0	0	0
Me, CLL	43.5	97	30	51	0	0	0
Si, CLL	39.0	89	0		0	0	0
Ro, CLL	64.0	87	86		0	0	0
Cp, CLL	17.0	95	68		0	0	0
Mi, B-Cell ALL‡	45.4	51§	79		0	0	0
Ri, Null-cell ALL	5.0	68§			0	0	0
Cn, T cell ALL	45.2	97§	3		0	2	0
Ma, WDLL‡	8.0	96		0	0	0	0
Go, PDLL‡	16.0	64		1	0	0	0
Sv, PCL‡			0	36	0	1	1

* Leukocyte count and percentage of lymphocytosis by peripheral smear were routine clinical data.

‡ All specimens were PBMNC. Abbreviations are: CLL, chronic lymphocytic leukemia; ALL, acute lymphoblastic leukemia; WDLL, well differentiated lymphocytic lymphoma; PDLL, poorly differentiated lymphocytic lymphoma; PCL, plasma cell leukemia.

§ Percentage of lymphoblasts.

TABLE IV

Myeloid Leukemias: Reactivity with αHC Antibodies

Patient	WBC 10^9 cells/l*	Myeloblasts*	Cells staining with αHC1	αHC2	αHC3
		%		%	
Da, CGL‡	69.2	0	0	0	2
Li, CGL	88.7	2	0	0	1
Mo, CGL	165.0	0	0	0	1
Gb, CGL	19.0	0	0	4	4
Ch, CMOL‡	60.7	14	0	0	8
Mk, CGLBC‡, lph		90	0	50	67
Vi, CGLBC‡	186.0	99	0	94	47
Do, AML‡	9.8	60	0	6	68
Ke, AMML‡	45.7	85	0	15	40
Se, AMML, bm	56.7	72	0	78	77
Wi, AMML, lph	200.0		0	0	27
As, monocytosis§	8.9		0	0	14

* Lymphocyte count and percentage of myeloblasts on peripheral smear were routine clinical data.

‡ All specimens were PBMNC except for lph = leukapheresis and bm = bone marrow aspirate. Abbreviations are: CGL, chronic granulocytic leukemia; CMOL, chronic monocytic leukemia with 26% early monocytes; CGLBC, chronic granulocytic leukemia in blastic crisis; AML, acute myelogenous leukemia; AMML, acute myelomonoblastic leukemia.

§ Monocytosis of unclear etiology, 35% monocytes on a peripheral smear.

with acute nonlymphocytic leukemia. In addition some positive cells were found in some lymphoblastoid B cell lines and among mitogen-activated B cells. The latter finding, which is the subject of a separate study, suggests that these antibodies detect B cell differentiation antigens that appear transitorily during B cell maturation between the lymphocyte and plasma cell stages. This also probably explains the positive cells observed in some normal peripheral blood specimens. Despite these findings these two monoclonal antibodies proved of value in the study of hairy cell leukemia. For example they clearly differentiated hairy cells from those of chronic lymphocytic leukemia.

The two antibodies αHC2 and αHC3 gave very similar results, but clear differences were noted in a few patients, especially those with myeloid precursor cells (for example patients Do and Wi, Table IV). The studies on the cells of patient Wi were repeated four times and in each experiment only αHC3 gave positive results.

Others have described antibodies reacting with hairy cells. Espinouse et al. (27) describe a rabbit antiserum retaining specificity for hairy cells after absorptions with erythrocytes, T cells, monoblastic leukemic cells, and CLL B cells. Brooks et al. (28, 29) describe a monoclonal murine antibody (FMC-7) made by immunization with a common B cell lymphoblastoid line that reacts with a subset of normal B cells, with B cells from ~16% of various chronic lymphocytic leukemia patients, and B cells from most prolymphocytic and hairy cell leukemias tested. Neither of these antibodies appear to be identical to the three monoclonal antibodies described in this paper.

The most specific monoclonal antibody of the three described in this paper, αHC1, could not be shown to definitely react with any cells in the normal tissues examined. Thus by this criterion no normal cell equivalent of the hairy cell could be detected, which was one of the objectives of this investigation. However, it may be that the αHC1 antibodies are directed against a specific tumor antigen common to different hairy cell leukemias and would not react with the analogous hypothetical normal cell. If this is indeed the case, this monoclonal antibody is of added interest because of the rarity of human tumor specific antigens. The potential use of this antibody in the therapy of hairy cell leukemia, possibly covalently linked to cytotoxic compounds, deserves consideration. At the moment the most obvious use of these monoclonal antibodies is for diagnostic purposes.

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