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WT1: A Monoclonal Antibody Reactive with T-ALL but not with Other Leukemias

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A. Introduction

There is ample evidence that the different leukemias represent clonal derivatives of cells "frozen" in a specific state of differentiation or maturation [1]. The hybridoma technology introduced by Köhler and Milstein [2] has permitted the production of monoclonal antibodies directed against differentiation antigens. Such antibodies can contribute considerably to our insight into hemopoietic differentiation and malignancy.

Acute lymphoblastic leukemias (ALL) can be subdivided into four subgroups according to membrane markers [3]. In a majority of cases, leukemic cells carry is the common ALL antigen (CALLA), which can be demonstrated with conventional antiserum or with the monoclonal antibody J-5 [4]. The cells from T-ALL patients are reactive with conventional antithymocyte antisera. The rare variant of B-ALL is characterized by the presence of surface immunoglobulin. When the leukemic cells have lymphoid morphology but lack these three markers, the classification "null ALL" has been suggested [3]. The T-ALL subgroup is heterogeneous. Most, but not all, cases are positive for E-rosetting, and sometimes both CALLA and thymocyte antigens are expressed [5, 6]. It would be useful if a monoclonal antibody were available which reacts with all T cells and is T-lineage specific. The antibodies from the OKT series are either not T-cell specific (OKT9, OKT10) or not reactive with the immature T phenotype which is found in the majority of T-ALL cases [7]. Even OKT11A, reactive with the E-rosette receptor [8], is not ideal since it fails to label those T-ALL which are E-rosette negative.

We have produced a monoclonal antibody, termed WT1, which is specific for human thymocytes and T-lymphocytes [9]. This antibody reacts with all thymocytes including the large, terminal deoxynucleotidyl transferase (TdT) positive blasts (Tax, Janossy et al., manuscript in preparation). The reactivity of WT1 with this putative prothymocyte population raised the possibility that this antibody might be useful for the diagnosis of T-ALL, especially the immature (E-rosette negative) cases. WT1 was therefore tested on a panel of human cell lines of different phenotypes, and on a broad panel of leukemic cells. The results obtained indicate that WT1 is specific for cells of the T lineage and is useful for the diagnosis of T-ALL.

B. Materials and Methods

The characteristics of all cell lines used in this study (except Jurkat) have been summarized by Minowada [10]. Jurkat is an E-rosette positive leukemic T-cell line [11]. Cells were cultured with 5% CO_2 in RPMI-1640 medium (Dutch modification, containing both HEPES and sodium bicarbonate) supplemented with 7.5%–15% heat-inactivated fetal calf serum, 2 m*M* glutamine, 1 m*M* sodium pyruvate, and gentamicin (50 μg/ml). Blood and marrow samples from leukemic patients were sent to I.C.R.F., London, from hospitals throughout the United Kingdom. Preliminary data on the production and specificity

of monoclonal antibody WT1 have been reported previously [9]. Hybridoma cells were injected into pristane-treated Balb/C mice and antibody was purified from the ascites by ammonium sulfate precipitation and protein A-Sepharose chromatography. Reactivity of cells with antibody WT1 was evaluated by indirect immunofluorescence using $F(ab')_2$ goat antimouse IgG antibodies labeled with fluorescein. E rosettes were formed by incubating lymphoid cells for 1 h at 4 °C with aminoethylisothiouronium-treated sheep erythrocytes [12].

C. Results and Discussion

When monoclonal antibody WT1 is tested on human cell lines, only cells with T phenotype are reactive (Table 1). Importantly, the antibody also binds to cells with an immature T phenotype like HSB-2 and CEM, which do not form E rosettes.

WT1 appears to be T-lineage specific when tested on leukemic cells (Table 2). All T-ALL, including several cases which are E-rosette negative, react with WT1, but the other types of leukemia do not react. However, in some cases of myeloid leukemias and one case of erythroleukemia, a weak staining was observed. The optimal diagnostic approach for T-ALL would probably be to combine the membrane staining by WT1 with staining of the nuclei by antiserum against TdT [13].

In conclusion, WT1 appears to be a useful monoclonal antibody for diagnosis and monitoring of T-ALL. Furthermore, the antibody is cytotoxic (IgG2a), and incubation of bone marrow cells with WT1 and rabbit complement did not affect the outgrowth of myeloid or erythroid committed progenitor cells (CFU-GM and BFU-E, respectively: 9 and Th. de Witte, personal communication). WT1 might therefore also be useful for the in vitro elimination of T-ALL blasts from bone marrow. This would render autologous bone marrow transplantation feasible as treatment for T-ALL.

Table 1. Reactivity of monoclonal antibody WT1 with human cell lines

Cell line	Origin[a]	Reactivity
CCRF-CEM	T-ALL	+
CCRF-HSB-2	T-ALL	+
JURKAT	T-ALL	+
CCRF-SB	Normal B[b]	–
RPMI 1788	Normal B[b]	–
DAUDI	BL	–
REH	CALL	–
NALM-1	CML-BC (Ph^1)	–

[a] BL, Burkitt's lymphoma; CML-BC (Ph^1), chronic myeloid leukemia in blast crisis, positive for Philadelphia chromosome

[b] Transformed in vitro with Epstein-Barr virus

Table 2. Reactivity of monoclonal antibody WT1 with leukemic cells

Leukemia/diagnosis	WT1 binding	
	+	–
Acute lymphoblastic leukemia		
Common ALL	0	43
Null ALL	0	5
B-ALL	0	1
T-ALL	8	0
Possible T-ALL[a]	7	0
Mature lymphoid leukemias		
T-Sezary	0	1
T-LCL[b]	0	2
B-CLL, PLL, lymphoma	0	20
Myeloid leukemias		
CML	0	2
CMML	0	1
AML	2[d]	17
CML-BC (M)[c]	1[d]	8
E-L	1[d]	3

[a] Possible T-ALL (all diagnosed as ALL): Four cases: DR^-, TdT^+, E^-, Ig^-, $cALL^-$, other T antigens$^-$; One case: DR^-, TdT^+, E^-, Ig^-, $cALL^-$, other T antigens$^+$; One case: DR^-, TdT^-, E^-, IG^-, $cALL^-$, other T antigens$^-$; One case: DR^+, TdT^+, $cALL^-$, $E/T11^+$, other T antigens$^-$,

[b] T-lymphosarcoma cell leukemia ("helper" phenotype)

[c] TdT^- "myeloid" blast crisis

[d] Weak staining

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