A Monoclonal Antibody (WT1) for Detecting Leukemias of T-Cell Precursors (T-ALL)

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The selectivity of a monoclonal anti-T antibody, designated WT1, has been assessed in a series of 906 leukemias and lymphomas. In acute lymphoblastic leukemias, WT1 reacts comprehensively and selectively with thymic acute lymphoblastic leukemia (ALL) cells in untreated or relapsed patients, thus overriding the extensive antigenic diversity of this cancer and the immaturity of the cell type involved. All 80 cases of thymic ALL examined were WT1-positive. In addition, 18 cases of presumptive prethymic ALL were also WT1-positive, but were unreactive with other maturation-linked T-cell markers. The phenotype WT1+ HLA-DR+ TdT+ appears to be unique to T-ALL and can therefore be used systematically for the differential diagnosis of this subtype of ALL. Virtually all ALL cases can now be placed into one of two major subgroups representing transformed precursors of either the T- or B-cell lineage. WT1 identifies a single polypeptide of approximately 40,000 mol wt and is similar to two previously described monoclonal antibodies.

MONOCLONAL ANTIBODIES selectively reactive with different hematopoietic cell types are providing new insights into the heterogeneity of leukemic cells. The specificity and reliability of these reagents can be exploited for differential immunodiagnosis or immunotherapy of leukemia.

Paradoxically, the specificity of monoclonal antibodies can also be disadvantageous. In T-cell, or thymic, acute lymphoblastic leukemia (ALL), for example, there is extensive antigenic diversity between leukemic cells of different patients and also within the leukemic clone of an individual patient. No single monoclonal antibody analyzed so far in a large series of patients reacts efficiently and selectively with T-ALL (i.e., in all leukemic cells in all patients with T-ALL). This difficulty is compounded by the fact that T-ALL probably originates in very immature thymic precursor cells. The antigenic phenotype of these normal cells is currently unknown, and T-ALL corresponding to this cell type may therefore lack most T-lineage differentiation antigens and be difficult to classify.

The twin features of immaturity and diversity are likely to be common to many cancers and could in theory frustrate some desirable clinical applications of monoclonal antibodies. In view of the poor prognosis of T-ALL, accurate identification of this subtype of leukemia is of considerable importance. Also, the potential therapeutic application of monoclonal antibodies in T-ALL requires reagents that singly or in combination can effectively bind to and kill or eliminate all clonogenic leukemic cells, many of which, in T-ALL, can be expected to have immature cell surface phenotypes.

We describe an anti-T-cell monoclonal antibody designated WT1, which appears to be similar to two previously described monoclonal anti-T antibodies. These antibodies are identified as the reagents of choice in the routine diagnosis of T-ALL and are candidates for use in the treatment of this disease in the future.

MATERIALS AND METHODS

Patients

The study included 906 patients with confirmed acute or chronic leukemia/lymphoma at presentation or in relapse. Their diagnosis was based on the morphological FAB classification and on cytochemical criteria. The patients were admitted to the hospitals in Great Britain and Eire between January 1982 and October 1982. An additional 20 frozen bone marrow or peripheral blood samples from patients who were tested in 1981 and whose samples were stored in liquid nitrogen were included in the study. The only samples that were excluded from this study were: (1) inadequate (cells dead or sparse) or unlabeled samples; (2) those containing less than 30% blasts or mature malignant cells.

Cells

Heparinized samples of bone marrow and/or blood, lymph nodes, pleural effusion, and cerebrospinal fluid were examined. Mononuclear cells were isolated from the specimen using Ficoll/Hypaque (Pharmacia, Uppsala, Sweden). Frozen cells were thawed rapidly at 37°C and immediately diluted by dropwise addition of the Eagle's minimal essential medium (10% FCS). Dead cells were removed by Ficoll/Hypaque centrifugation and washed three times before testing for the immunologic markers.

Antibodies and Other Immunologic Markers

Monoclonal antibody WT1 was produced by immunization with normal pediatric thymocytes, as reported previously. Anti-T-cell...
lineage monoclonal antibodies UCHT-2,14 analogous to OKT1 or Leu-1,17 UCHT-1,14 analogous to OKT3 or Leu-4,18,19 and UCHT-4,20 analogous to OKT8 or Leu-2a,21 were provided by Dr. P. Beverley (U.C.H., London); OKT4 (Leu-3a);22 and OKT11A (Leu-5)23 were provided by Ortho Pharmaceutical Corp., Raritan, NJ. NA1/34,24 which was equivalent to OKT6,25 was a gift from Dr. A. McMichael (John Radcliffe Hospital, Oxford, U.K.). Anti-cALL antibody, J5,26 was donated by Dr. J. Ritz (Sidney Farber Cancer Institute, Boston, MA), and the anti-HLA-DR (polymorphic) antibody, DA-2,27 was donated by Dr. W. Bodmer (I.C.R.F., Cancer Institute, Boston, MA), and the anti-HLA-DR (polymorphic) antibody.  

Immunofluorescence  
The binding of monoclonal antibodies was assessed by using affinity-purified F(ab')2 goat anti-mouse Ig, which was cross-absorbed with insolubilized human Ig and labeled with fluorescein-isothiocyanate (FITC). Fluorescence was evaluated by microscopy using a Zeiss photomicroscope with incident illumination and, in some cases, also by the fluorescence activated cell sorter (FACS-1, Becton Dickinson, Mountain View, CA).

Isolation and Biochemical Analysis of the Antigen Recognized by WT1  
The structures recognized by the antibodies WT1 and OKT6 (control) were isolated from 125I-lactoperoxidase-labeled HSB-2 cell lysates. Immune complexes were analyzed by SDS-PAGE according to methods described previously in detail.29,30

RESULTS  
Leukemia Patients  
Monoclonal antibody WT1 was tested on 906 patients with diagnosed acute or chronic leukemia. Leukemic cells were simultaneously tested for other lymphoid markers, including HLA-DR antigen and, where appropriate, with T-cell-specific monoclonal antibodies (see Materials and Methods). In the ALL group, B-cell precursor lymphoblastic leukemias32 were comprised of 300 common ALL cases and 53 null ALL. Leukemic cells of these patients and of the additional 12 patients with a more mature B-cell leukemia, B-ALL, did not react with WT1 (Table 1, Fig. 1B). Seventy-two mature B-cell-derived chronic leukemias (B-CLL) and lymphomas were also WT1 negative (Table 1). In addition, WT1 did not react with cells of chronic myeloid leukemia (CML), chronic myelomonocytic leukemia (CMML), acute promyelocytic leukemia (APML), hairy cell leukemia (HCL), or with lymphoid blast cells of CML patients in blast crisis (see Table 1).  

WT1 reacted with lymphoblasts of all 80 patients with confirmed thymus-derived ALL at presentation and in relapse (Table 1, Fig. 1A). The T-ALL diagnosis was based on the clinical/hematologic features at presentation (thymic mass and/or polar acid phosphatase reaction), and the immunologic phenotype defined by E rosette/OKT11A positivity and by the absence of the HLA-DR antigen (E+ /OKT11A− DR−). Three cases of E-rosette-negative ALL were also included in this group due to their classical T-ALL-associated clinical picture at presentation and the reactivity of the majority of their blasts with some or most anti-T-cell monoclonal antibodies: OKT4, OKT6, and OKT8 (E+/OKT11A− DR− anti-T+).  

An additional group of 24 acute lymphoblastic leukemias were identified that were difficult to subclassify (Table 1). In this group, blast cells were nonreactive with anti-HLA-DR, anti-T monoclonal antibodies (excluding WT1), and did not form E-rosettes or react with anti-Ig. Eighteen patients in this category were highly reactive with WT1 (Table 2A), and 14 of these had at least one of the two typical T-ALL-associated features: mediastinal mass and acid phosphatase (see Table 2).  

Six additional cases diagnosed as ALL were non-
Fig. 1. Reactivity of WT1 with acute leukemia as assessed by indirect immunofluorescence and FACS analysis. Vertical axis: relative cell number. Horizontal axis: relative fluorescence intensity. All antibodies were added under saturating conditions.

Table 2. Reactivity of the Monoclonal Antibody WT1 With ALL of Uncertain Subtype

| Patient No. | Blast% | Medullary Mass | Focal Acid Phosphatase (x 10^5/Liter) | WBC | ER | SmIg | J5 | TdT | WT1 | UCHT-2 | UCHT-1 | OKT-4 | NA1/34 | UCHT-4 | OKT11A |
|------------|--------|----------------|---------------------------------|-----|----|------|----|-----|-----|--------|--------|--------|--------|--------|--------|--------|
| (A)        |        |                |                                 |     |    |      |    |     |     |        |        |        |        |        |        |        |
| 1          | 56     | -              | +                               | 8.2 | 0  | 0    | 30 | 0   | 40-50| 71†   | NT     | NT     | NT     | 0      | NT     | NT     |
| 2          | 85     | NT             | 10.0                           | 2   | 0  | 0    | 30-40| 73   | NT     | NT     | 0       | 0      | 0      | NT     |        |
| 3          | 94     | -              | 156.0                          | 3   | 4  | 2    | 80-90| 99   | 0      | 1      | 0       | 0      | 0      | NT     |        |
| 4          | 60     | +              | 23.0                           | 8   | 0  | 32   | 0    | 15-20| 79    | 0       | 0      | 13     | 1      | 0      | NT     |
| 5          | 95     | -              | 8.3                            | 0   | 0  | 1    | 50-60| 93   | 0      | 0       | 0      | 0      | 0      | 0      | 0      | 32     |
| 6          | 99     | -              | 50.1                           | 1   | 0  | 7    | 20   | 60-70| 93     | NT      | NT     | NT     | NT     | NT     | NT     |
| 7          | 84     | +              | 68.6                           | 1   | 0  | 0    | 0    | 0    | 95    | 0       | 0      | 0      | 0      | 0      | 15     |
| 8          | 77     | +              | 59.2                           | 1   | 0  | 0    | 0    | 57   | >90   | 95     | NT      | NT     | NT     | NT     | NT     |
| 9          | 95     | +              | 25.0                           | 0   | 0  | 1    | 59   | >90   | 95     | NT      | 0       | 2      | 0      | 0      | 3      |
| 10†        | 55     | -              | 3.8                            | 4   | 0  | 3    | 50-60| 65   | 1      | 0       | 0      | 16     | 8      | 3      |
| 11         | 86     | +              | 28.7                           | 0   | 0  | 0    | >90  | 73   | 0      | 0       | 6      | 1      | 0      | 3      |
| 12         | 98     | +              | 10.6                           | 0   | 2  | 3    | >90  | >95  | 30     | 2       | 0      | NT     | NT     | 2      |
| 13         | 66     | +              | 7.8                            | 0   | 0  | 2    | 80-90| 73   | 30     | 54     | 7       | 1      | 1      | 8      |
| 14         | 80     | -              | 140.0                          | 0   | 0  | 0    | 0    | 0    | 62     | 0       | 0      | 0      | 0      | 0      | 2      |
| 15         | +      | -              | 37.0                           | 0   | 0  | 0    | 70   | 97   | 23     | 0       | 0      | 0      | 0      | 0      |
| 16         | 95     | +              | 140.0                          | 0   | 0  | 0    | 52   | 50-60| 80     | 6       | 0      | 21     | 1      | 1      | 5      |
| 17         | 86     | +              | *                              | 0   | 0  | 2    | 0    | 10-15| 89     | 1       | 3      | 0      | 0      | 2      | 0      |
| 18         | 90     | +              | 53.5                           | 0   | 0  | 8    | 18   | 60-70| 37     | NT      | NT     | NT     | NT     | NT     | NT     |
| (B)        |        |                |                                 |     |    |      |    |     |     |        |        |        |        |        |        |        |
| 19         | -95    | -              | 245.0                          | 0   | 0  | 12   | 0    | 30-40| 1     | 0       | 0      | 0      | 0      | 0      | 0      |
| 20         | -95    | NT             | 200.0                          | 0   | NT | 18   | 0    | 80-90| 3     | NT      | NT     | NT     | NT     | NT     | NT     |
| 21         | 90     | -              | *                              | 1   | 0  | 0    | 0    | 0    | 3     | 0       | 0      | 0      | 0      | 0      | 0      |
| 22         | 90     | -              | NT                             | 1.8 | 2  | NT   | 2    | <1   | 80-90  | 5      | NT      | NT     | 1      | 0      | 3      |
| 23         | 40     | -              | NT                             | 2.3 | 0  | 4    | 13   | 10-15| 7     | NT      | NT     | NT     | NT     | NT     | NT     |
| 24†        | 90     | -              | NT                             | 2.0 | 3  | NT   | 5    | 0    | 50-60  | <1     | NT      | NT     | NT     | NT     | NT     |

NT, not tested.
*Data unavailable.
†Diagnosed initially as AUL.
‡Percent positive cells.
reactive with WT1. None of the cases in this subgroup was associated with the presence of mediastinal mass or focal acid-phosphatase-positive blasts.

WT1 also reacted with mature T-cell leukemias and lymphomas. Four of 6 patients with Sézary’s syndrome, defined by the mature T helper cell phenotype (OKT3⁺ OKT4⁺ OKT11A/E⁻ TdT⁺), were WT1-positive, albeit weakly. Two of 3 T-chronic lymphocytic leukemias (T-CLL), 8 of 10 mature T-cell non-Hodgkin lymphomas (T-NHL), and the single T-Prolymphocytic leukemia (T-PLL) were also positive with WT1. The intensity of staining on mature T cells found in chronic T-cell leukemias was lower than in T-ALL, but in most cases 30%–60% of leukemic cells were staining with WT1.

In the series of 245 AML cases, 16 patients had WT1⁺ blasts (Table 1, Fig. 1D). Of these 16 WT1⁺ AML, 10 had the common AML phenotype of DR⁺ TdT⁻ and 5 were DR⁻ TdT⁺. One case was DR⁺ TdT⁺, but none had the same phenotype as T-ALL, i.e., WT1⁺ TdT⁺ DR⁻. The intensity of the staining by WT1 on AML blasts was significantly lower than on T-ALL blasts; however, two AML patients contained brightly stained cells. Myeloblasts found in the blast crisis stage of CML also stained with WT1 in 13% of cases (6/45). In addition, 1/16 erythroleukemias and 2/7 megakaryoblastic leukemias were WT1-positive.

Immunoprecipitation of the WT1-Defined Antigen

Cell surface labeling of HSB-2 cells with ²⁵¹, followed by immunoprecipitation with WT1, identified a broad band of mol wt 40,000 under reduced conditions (Fig. 2, lane 1). Control antibody OKT6 identified a band of mol wt 49,000, as described previously (Fig. 2, lane 2).

Fig. 2. Autoradiograph of immunoprecipitates from HSB-2 cell lysates on SDS-PAGE (10%). (Lane A) WT1. (Lane B) OKT6. Both samples were run under reducing conditions.

DISCUSSION

Monoclonal WT1 was raised against normal human thymocytes. It binds selectively to most blood T cells and to thymocytes, including, significantly, the large thymic blasts that are considered to be immature prothymocytes residing in the subcapsular, outer cortical regions of the thymus and poorly reactive with other T-lineage markers, e.g., OKT11A/E rosettes.

Two previously described antibodies have essentially similar serologic and biochemical characteristics. Monoclonal 3A1, described by Haynes and colleagues, identifies a 40,000 mol wt T-cell surface protein. We reported elsewhere that WT1 and 3A1 react with the same protein (Sutherland et al., in preparation). Studies with T-cells from nonhuman primates indicate, however, that WT1 and 3A1 recognize different epitopes. In six T-ALL patients reported, 3A1 was superior to other anti-T monoclonals in reacting efficiently with thymic leukemia blasts, but did not react with cutaneous T-cell lymphomas. Our data with WT1 support this distinction; Sézary’s syndrome leukemic cells were either unreactive (2 of 6) or weakly reactive (4 of 6) (see Table 1). Recently, Morishima et al. reported a monoclonal antibody, 4A, which also identifies the same 40,000 mol wt protein as 3A1 and appears to be expressed at quantitatively different levels on different functional subsets of T cells.

As shown in the study here, WT1 has a pronounced selectivity for T-ALL when applied to a large series of leukemic patients’ samples. In addition to staining all 80 ALL considered to be T-ALL by standard markers (and other anti-T monoclonals), WT1 reacted with another 18 cases that were identified as putative pre-T-leukemias (ALL) on the basis of the phenotype DR⁻ E rosette/OKT11A TdT⁺, but lack of reactivity with other anti-T monoclonal antibodies.

A small proportion of AML (~6%) reacted with WT1, usually weakly, indicating that the WT1-defined antigen (gp40) may not be unique to the T lineage. Dual staining for WT1 and TdT should distinguish T-ALL from AML, although a few (~2%–5%) of AML are TdT⁺. Very rare cases of TdT⁻ WT1⁻ DR⁻ AML may exist (although none of 245 were found in the present AML series) but could be identified as AML by cytochemistry or other markers (e.g., antimonyeloid monoclonal antibodies). We have no definitive proof that E DR⁻ WT1⁻ ALL are indeed thymic precursors. This will require induction of further differentiation in these cells.

Several of these putative pre-T-ALL did, however, have other features suggestive of thymic leukemia, i.e., thymic, mediastinal mass, and/or focal acid phospho-
tase staining. Whether this subgroup of thymic leukemia shares the poor prognosis of most T-ALL remains to be established, although previous studies with xenonantisera to T cells suggest that this will be the case.

These and previous data therefore indicate that ALL can be divided into two major subsets representing transformed precursors of the T- or B-cell lineages in maturation arrest, i.e., WT1\textsuperscript{+}DR\textsuperscript{+}TdT\textsuperscript{+}: early T; WT1\textsuperscript{+}DR\textsuperscript{+}TdT\textsuperscript{+}: early B. The latter have rearranged Ig genes and may synthesize \(\mu\)-chains.

WT1 reacts with essentially all TdT\textsuperscript{+} thymoblasts (cf., Fig. 1A) in T-ALL, both at diagnosis and in relapse. Normal TdT\textsuperscript{+} cells in bone marrow are WT1\textsuperscript{+}. This consistency and selectivity of expression suggests that identification and quantitation of extrathymic cells with the WT1\textsuperscript{+} TdT\textsuperscript{+} phenotype will be useful for monitoring the efficacy of treatment and for detecting residual or re-emerging leukemia. Also, since WT1 does not react with the majority of BFU-E, CFU-GM, and multipotential progenitors (C. Myers, P. Thorpe, W. Ross, and M. F. Greaves, manuscript in preparation), it has a therapeutic potential, especially perhaps in the context of autologous marrow transplants. A combination of WT1 with other monoclonals with broad (though incomplete) T-ALL reactivity with T-ALL (e.g., T11/gp45 E-rosette receptor or T1/gp69 reactive) may prove especially effective.

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T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA


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