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C-kit receptor (CD117) expression in acute leukemia

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Abstract The murine monoclonal antibody YB5.B8 (CD117) identifies a transmembrane tyrosine kinase receptor encoded by the human c-kit proto-oncogene. In this study we investigated the expression of c-kit on different types of acute leukemia to determine the degree of specificity and sensitivity of this marker for the myeloid and lymphoid lineages. C-kit was positive in over half of the 115 cases of acute leukemia studied. Overall, two thirds of AML cases expressed c-kit, whereas only one of 23 ALL patients was c-kit positive. C-kit was also positive in 16 of 19 cases of myeloid blast crisis of myeloproliferative disorders and negative in four with a lymphoid phenotype. There was no correlation between c-kit expression and the degree of myeloid differentiation by FAB subtypes or other markers. We conclude that c-kit is a specific marker for the myeloid lineage, which is expressed early during hematopoietic differentiation and can aid the diagnosis of AML in difficult cases. More patients need to be tested to establish whether the expression of c-kit may define AML subgroups of prognostic significance.

Key words C-kit · Acute leukemia · Immunophenotype

Introduction

The murine monoclonal antibody (MoAb) YB5.B8, clustered under CD117, identifies a transmembrane tyrosine kinase receptor encoded by the human c-kit proto-oncogene located in chromosome 4q21 [3, 17, 21, 32]. This receptor, designated stem cell factor, mast cell growth factor, multipotent growth factor, or steel factor [31], has been shown to play a fundamental role in hematopoiesis [17, 23, 32] as well as in enhancing B and T lymphopoiesis [13, 26, 27, 29].

YB5.B8 was obtained using as immunogen peripheral blood blast cells from a patient with acute myeloid leukemia (AML) [16]. C-kit is expressed in a small proportion (0.5–4%) of normal bone marrow mononuclear cells [2, 9, 19], preferentially in half the CD34-positive progenitor cells committed to the myeloid lineage [9, 19, 28]. Reactivity with c-kit has been detected in blast cells from AML [6, 12, 16, 18, 22, 30] and myeloid cell lines, including erythroleukemia lines [18, 20, 30]. There is no agreement on the expression of c-kit in acute lymphoblastic leukemia (ALL). Some studies have reported absence of c-kit expression in ALL [12, 18, 25, 30], while others have documented its expression, albeit weak, in 39% and 57% of cases of B- and T-lineage ALL, respectively [19], and in 33% of T-lineage ALL [24], although the number of cases investigated was small.

The clinical significance of c-kit expression is uncertain [19]; some reports have associated it with poor response to treatment and survival [1, 16], while others did not find any relationship between remission rate and percentage of c-kit-positive cells [25]

We have investigated the expression of c-kit in a variety of acute leukemias to determine: (a) the degree of specificity and sensitivity of this marker for the myeloid and lymphoid lineages and (b) whether there is any correlation between c-kit expression and AML FAB subtypes and the expression of other markers.

Material and methods

Patients and diagnoses

Peripheral blood (PB) and/or bone marrow (BM) from 115 patients with acute leukemia were investigated. The male/female ratio was 1.6, and 89 patients (77%) were adults (>14 years old). Ninety-nine patients were studied at presentation and the remaining 16 at relapse. The diagnosis was based on morphology and

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Table 1 Monoclonal antibodies used in this study

CD number	Antibody	Source
CD117	c-kit	Immunotech (Marseille)
CD34	Q-BEND	LRF Centre (Institute of Cancer Research)
	HLA-DR (GRB1)	Prof. Garrido Torres (Granada)
CD13	MY7-RD1	Coulter clone (Hialeah, Florida)
CD33	MY9-RD1	Coulter clone (Hialeah, Florida)
CD7	3A1	Harlan Sera-lab (Crawley Down, Sussex)
	anti-MPO	Dako (High Wycombe, Bucks)
	TDT	Harlan Sera-lab (Crawley Down, Sussex)
CD2	RFT11	Prof. G. Janossy (London)
CD10	J5-RD1	Coulter clone (Hialeah, Florida)
CD19	HD37	Harlan Sera-lab (Crawley Down, Sussex)
CD22	OKB22	Ortho Diagnostic Systems (Raritan, NJ)
CD14	FMC17	Harlan Sera-lab (Crawley Down, Sussex)
CD15	LeuM1	Becton & Dickinson (San Jose, CA)
CD56	Leu19	Becton & Dickinson (San Jose, CA)
CD79a	mB-1	Dr. D. Mason (Oxford)
	IgM	Dako (High Wycombe, Bucks)
CD41	IIb/IIIa	Dako (High Wycombe, Bucks)

cytochemistry according to FAB criteria [4, 5] and on immunophenotyping [10]. The series included 62 cases of AML classified as: M0 (10), M1 (17), M2 (7), M3 (10), M4 (3), M5 (7), M6 (5), M7 (3); 23 cases had acute lymphoblastic leukemia (ALL), of which 15 were B lineage (TdT+, CD19+, and/or CD79a+): one early B, ten common (CD10+), and four pre-B-lineage, (cyt μ+) and eight T-lineage ALL (TdT+, CD7+, cyt CD3+). Five cases had biphenotypic acute leukemia, four with cells coexpressing B and myeloid markers and one case with B, T, and myeloid involvement. Two cases had acute undifferentiated leukemia (AUL) with blasts which were negative for myeloperoxidase and nonspecific esterase and expressed only CD34, TdT, HLA-DR, and CD38. Twenty-three cases were blast transformation of chronic myeloid leukemia (CML), myelodysplastic syndrome (MDS), and polycythemia rubra vera (PRV). All samples had more than 30% of blasts, usually over 50%.

Cell markers

PB and/or BM mononuclear cells were isolated by density gradient centrifugation on Lymphoprep (Nycomed SA, Oslo, Norway) and washed twice with Hank's balanced salt solution (Gibco, Paisley, UK) prior to the immunological studies.

Immunophenotyping was performed by direct and indirect immunofluorescence [10] using a panel of monoclonal antibodies (MoAB) (Table 1) and fluorescein isothiocyanate-conjugated (FITC) goat anti-mouse immunoglobulin F(ab)2 fragment (Cappel, Durham, NC, USA) as second layer. Fc receptors were blocked with 2% pooled Ab serum in the buffer used in all incubations and washings. Negative controls were carried out by omission of the first layer and/or replacing the MoAB with a mouse Ig of matched isotype.

The MoAb YB5.B8 was used to assess the expression of c-kit in the leukemic cells. Terminal deoxynucleotidyl transferase (TdT) and cytoplasmic staining with CD3, CD22, CD79a, CD13, and anti-MPO were performed by flow cytometry after fixation and permeabilization of the cells [15] and/or by immunocytochemistry on cytocentrifuge-fixed slides using the APAAP technique [11].

The results were analyzed on a FACScan flow cytometer (BD, San Jose CA), using the LYSYS II software. In each sample, 5000 events were acquired and analysis was performed by gating the blast population in the FSC/SSC dot plot. A case was considered positive when the antibody was expressed on over 20% of blast cells above the control. The Mann-Whitney test was used when testing for a trend across the AML FAB subtypes.

Results

The proportion of cases positive with c-kit is shown in Fig. 1. Half of the acute leukemia cases tested were c-kit positive. The positive cases were mainly AML and myeloid blast crisis of myeloproliferative disorders, whereas ALL and lymphoid blast crisis were, as a rule, negative. Analysis of the ten cases of AML-M0 (Table 2) showed that in two anti-MPO-positive cases, CD33 and CD13 were negative while c-kit was positive. The two cases with AUL were c-kit positive, while the other myeloid markers were negative.

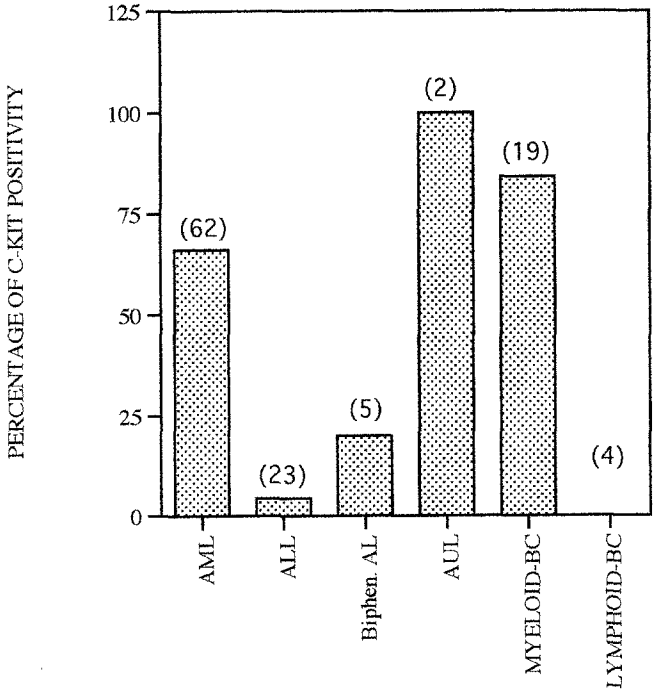

Fig. 1 Distribution of c-kit expression in cases of acute leukemia (numbers in parentheses indicate number of cases tested)

Table 2 Results of c-kit and myeloid markers in AML-M0 (cases 1–10) and acute undifferentiated leukemia (*ND* not done)

No.	MPO*	CD13	CD33	C-kit
1	+	+	−	−
2	+	+	+	+
3	+	+	+	+
4	+	+	−	+
5	+	−	−	+
6	+	−	−	+
7	+	+	+	+
8	+	+	+	+
9	ND	+	+	−
10	ND	+	−	−
11[a]	−	−	−	+
12[a]	−	−	−	+

[a] Acute undifferentiated leukemia; * anti-MPO

All the 17 B-lineage ALL, including three cases in which blasts expressed either CD33 or CD13, and all but one of the eight T-lineage ALL were c-kit negative. The lymphoblasts of the positive ALL expressed CD2, CD7, cyt CD3, and CD13.

C-kit retained myeloid specificity on cases of blast crisis of myeloproliferative disorders. The majority of the 19 cases with myeloid blast crises were c-kit positive, while the four cases with lymphoid blast crises were c-kit negative (Table 3).

Only one of the five biphenotypic leukemia cases was c-kit positive, and this corresponded to the patient with trilineage involvement whose cells were Philadelphia chromosome positive.

Two thirds of AML cases were c-kit positive (Fig. 2). There was no correlation between c-kit and FAB type (Fig. 3). C-kit expression was found in cells from all AML type categories regardless of the lineage involved and/or degree of differentiation. There were no statistically significant differences between c-kit expression and the other markers studied.

Discussion

This is the largest study examining the expression of c-kit in blast cells from patients with acute leukemia by flow cytometry. C-kit was expressed mostly on myeloid committed blasts in AML and those of blast crises of myeloproliferative disorders. The present results confirm and extend previous observations on the expression of c-kit in AML and CML in blast crisis [1, 9, 12, 19, 22, 25]. In agreement with published studies [6, 9, 12, 18, 30], we did not find any correlation between the expression of c-kit and the degree of myeloid differentiation or a particular FAB subtype. This contrasts with other data [19, 25] which related c-kit expression to undifferentiated myeloid cells and with M1 and M2 FAB categories [16].

Although there have been studies showing a correlation between the expression of c-kit and CD34 [9, 19], CD15 [22], CD13, and CD7 [24] we have been unable

Table 3 C-kit expression in blast crisis of myeloproliferative disorders[a]

Type	No.	C-kit + ve	C-kit − ve
Myeloid	19	16	3
Lymphoid	4	—	4

[a] CML, MDS, PRV.

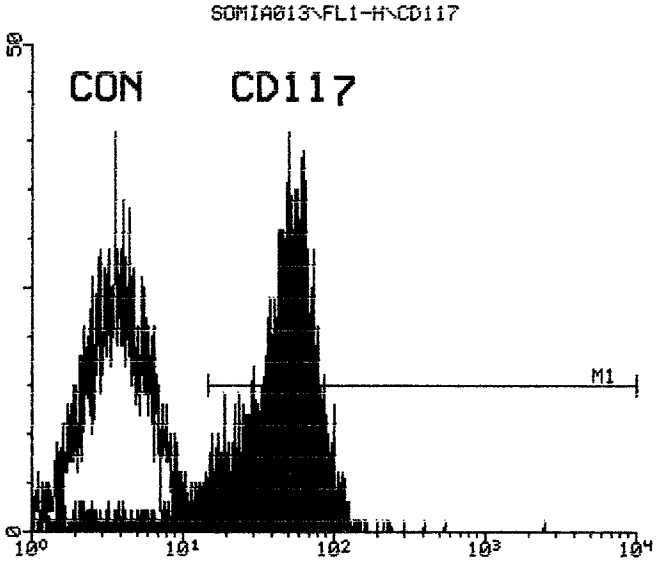

Fig. 2 Histogram showing a negative isotypic control and expression of CD117 in 91% of blasts in a case of AML

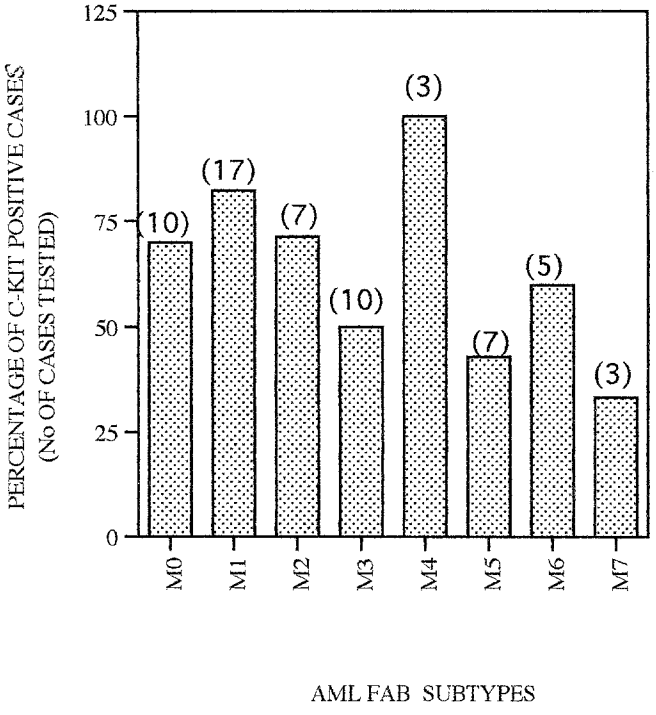

Fig. 3 Percentage of c-kit-positive cases in different FAB AML subtypes

to confirm this. This discrepancy could be related to differences in MoAbs identifying different epitopes or in the AML subtypes investigated.

Of the 23 cases of ALL, only one (4.3%) was c-kit positive (T-ALL, CD13 positive), while none of the lymphoid blast crisis expressed c-kit. This emphasizes the myeloid specificity of c-kit when compared with other myeloid markers such as CD13 and CD33, which were expressed in 23% of the ALL cases in our own data [8], while in a review article by Drexler et al. [14] it ranged from 5 to 46%.

Reactivity with c-kit in ALL has been found by others to be, as a rule, negative [9, 12, 16, 25]; exceptions are Knapp et al. [19], who reported a weak expression in nine of 23 B-lineage ALL and in four of seven T-ALL samples, and Nishii et al. [24] who reported reactivity in four of 12 T-ALL cases.

Our findings in M0 AML and AUL are of interest, as they suggest that c-kit is an early myeloid marker which may be useful for the diagnosis of immature forms of AML. In particular, c-kit was positive in two AML M0 cases where cells were CD13 and CD33 negative (Table 2). This is also supported by the positivity of c-kit in two rare cases of AUL. Whether c-kit expression indicates that these cases should be classified as myeloid can only be suggested but not confirmed at the present time. Although we consider that c-kit is a specific myeloid marker, in no case of AML defined by morphology, cytochemistry, and myeloid markers was its detection critical for the diagnosis. This suggests that the sensitivity of this c-kit is lower than that of anti-MPO, CD13, and CD33 [7], despite the fact that is specificity appears to be higher.

We conclude that c-kit is a useful and specific marker in cases difficult to diagnose. Because c-kit is expressed in a low percentage of normal bone marrow cells, it may also be useful in combination with other markers for detecting minimal residual disease, as suggested elsewhere [22]. More patients need to be evaluated to conclude whether c-kit may define leukemic subsets of prognostic significance.

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