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CD117 (c-kit) is a restricted antigen of acute myeloid leukemia and characterizes early differentiative levels of M5 FAB subtype

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Abstract

Background and Objective. **The CD117 molecule is an antigen more frequently found on early normal and leukemic hematopoietic cells, but its correlation with the FAB subtypes and with other lineage and stage associated antigens is still not well established. In this study we investigated the surface expression of CD117 antigen in 135 patients with acute leukemia in relationship to *de novo* or *secondary* origin of AML, subtypes of FAB classification, expression of other antigens such as CD34, HLA-DR, CD15, CD14, CD45RA, CD45RO, CD11b, CD11c, CD4, CD7, mixed antigen co-expression (LyAg$^+$AML and MyAg$^+$ALL) and features of leukemic mass.**

Design and Methods. **The CD117 antigen expression (clone 95C3) was determined by flow cytometry in a series of 135 patients with acute leukemia at diagnosis consecutively observed during the years 1995-1997: 82 AML (including 51 cases of *de novo* AML, 22 cases of AML following myelodysplastic syndromes (MDS), 9 cases of myeloid blastic crisis of chronic myeloid leukemia (BC-CML) and 53 ALL. All cases were stratified in CD117$^+$ and CD117$^-$ groups and the differences were analyzed by using appropriate statistical analyses.**

Results. **CD117 antigen was found over 10% in 74% of AML without significant differences of positivity between AML after MDS or BC-CML and *de novo* AML. We did not note a significant correlation between FAB classification and CD117 which was expressed in 100% of M1 and M7 cases, in 80% of M0 cases, in 75% of M2 cases, in 70% of M3 cases and in 82% of M4 cases. Instead, in M5 subtype CD117 was strictly restricted to earlier stages: ten of the eleven M5b (91%) cases completely lacked CD117 antigen expression, whereas 100% of M5a cases were positive. The results of Pearson's coefficient showed: 1) a significant inverse relationship between CD117 and CD15, CD4 and CD14 (only in M5 subtypes) and CD11b, CD11c and CD45RO (in all cases); 2) a significant direct correlation between CD117 and CD34 and CD45RA (in all cases); and 3) an independent expression between CD117 and CD15 associated with a low correlation between CD117 and HLA-DR antigen (only in non-monocytic cases). In ALL, whether of B or T lineages, surface expression of CD117 was never observed.**

Interpretation and Conclusions. **We conclude that the CD117 antigen shows a high specificity for AML, independently upon FAB classification, and represents a reliable marker in characterizing the differentiative degree of the monocytic blasts.**

Key words: CD117, AML, ALL

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The significance of the CD117 antigen, a transmembrane tyrosine kinase receptor better known as stem cell factor receptor (SCFR) or c-kit receptor,[1] has been previously investigated on normal and leukemic marrow cells. Numerous studies reported that this antigen and its ligand or stem cell factor (SCF) play an important role in the proliferation, differentiation and survival of normal and leukemic hematopoietic tissues.[2-4] The CD117 antigen is more frequently found on progenitor cells of normal bone marrow and in earlier FAB subtypes of *de novo* acute myeloid leukemia (AML), in blastic crisis of chronic myeloid leukemia (BC-CML) and in AML following myelodysplastic syndromes (MDS), where it correlates well with the expression of other *early* antigens, such as CD34 and CD7.[5-14] Regarding its clinical usefulness, CD117 antigen can sometimes be useful in detecting minimal residual disease (MRD) by recognizing unusual immunophenotypes on CD117 positive blast cells,[15] but it does not seem to have a real value as prognostic factor for response to chemotherapy and survival.[16-18] This receptor is generally absent on blast cells of the B-lineage acute lymphoblastic leukemias (ALL), whereas on those of the T-lineage ALL it has been found only in some more immature cases co-expressing myeloid antigens.[8,9,12,17,19]

On the basis of these data and with the aim of investigating the correlation between CD117 antigen and the differentiative level of leukemic blasts, we evaluated, in 135 cases of acute leukemia, the pattern of CD117 antigen surface expression in relationship with the immunophenotype, the FAB classification and some features of leukemic mass.

Materials and Methods

Patients

We analyzed 82 patients with AML (median age

52 yrs, range 11- 87; 47 males and 35 females; 5 M0, 14 M1, 20 M2, 10 M3, 11 M4, 20 M5, 2 M7 FAB) and 53 patients with ALL (37 adults and 16 children, 29 males and 24 females, 19 L1, 30 L2, 4 L3 FAB) consecutively observed during the years 1995 and 1997. Diagnosis of AML and ALL was made according to French-American-British (FAB) Group criteria.[20]

Immunological analysis

Monoparametric, and when necessary, biparametric immunophenotypic analyses, on bone marrow cells, were performed using the following a) fluorescein isothiocyanate (FITC) conjugated monoclonal antibodies (MoAbs): CD7, CD2, CD20, CD22, CD14, CD15, CD41, CD45RA (Becton Dickinson), CD1a, CD3, CD8, CD10, HLA-DR (Ortho Diagnostic Systems) and CD61 (Immunotech); b) phycoerythrin (PE) conjugated MoAbs: CD5, CD13, CD33, CD11b, CD11c, CD45R0 (Becton Dickinson); c) FITC and PE conjugated MoAbs: CD34, CD4 and CD19 (Becton Dickinson). The surface expression of CD117 antigen was assessed by c-kit (PE conjugated) MoAb (clone 95C3) (Immunotech). All samples were prepared as previously reported[21] and analyzed by a flow cytometer (FACSort, Becton Dickinson) with 15 mW argon laser emitting at 488 nM and equipped with the software Lysis II. At least 10,000 ungated list mode events were collected and analyzed by selecting an appropriate gate on the basis of side and forward scattering properties of blast cells. The blast cell percentage detected for every sample was always greater than 50% (range 53-97%). Negative controls with isotype matched irrelevant IgG1, IgG2a and IgM monoclonal antibodies were used in all cases. In general, the reaction was considered positive when antigenic expression was present in 20% or more of the gated blast cells. For CD34 and CD117, however, we used a threshold of 10% for two reasons: a) these antigens have a lower expression on the normal marrow cells; and b) in the present series only two cases for CD34 and two cases for CD117 (Figure 1) had a pattern of expression between 10% and 20%. On the basis of the antigenic analysis B lineage ALL were classified into four groups: early pre-B ALL ($CD19^+/CD10^-$), common ALL ($CD19^+/CD10^+/CD20^\pm$), pre-B ALL ($Cy\text{-}IgM^+$) and B ALL ($Sm\text{-}Ig^+$).[22] According to personal experience,[23] T lineage ALL were divided instead into two groups: early T ALL ($CyCD3^+/CD7^+/CD5^\pm$/ $CD2^\pm$) and late T ALL ($CD7^+/CD5^+/CD2^+CD1^\pm$/ $CD3^\pm$). The definition of myeloid antigen (MyAg) ALL was carried out by the co-expression greater than 20% of CD33 and/or CD13 and/or CD15 and/or CD14.

Statistical analysis

All patients with AML were stratified for CD117 expression in two groups ($CD117^+$ and $CD117^-$). In each group sex, age, FAB cytotype, *de novo* or secondary type of leukemia, serum lactate dehydrogenase (LDH) level, splenomegaly, WBC count, CD34, HLA-DR CD45RA, CD45RO, CD15, CD11c, CD11b, CD7, CD4 and lymphoid antigens co-expression (Ly-Ag) were evaluated. The differences between $CD117^+$ and $CD117^-$ groups were tested using Chi square tests or Fisher's exact tests.[24] Concerning the serum LDH and peripheral WBC count the statistical analyses were performed by Student's t-test. This test was also used for a statistical evaluation of the CD117 expression in *de novo* and *secondary* AML.

We also tested the correlation between CD117 with CD34, CD45RA, CD45RO, HLA-DR, CD15, CD14, CD11b, CD11c and CD4 by calculating Pearson's correlation coefficient. Since no case of ALL showed a significant surface expression of CD117, no statistical evaluation was carried out in these patients.

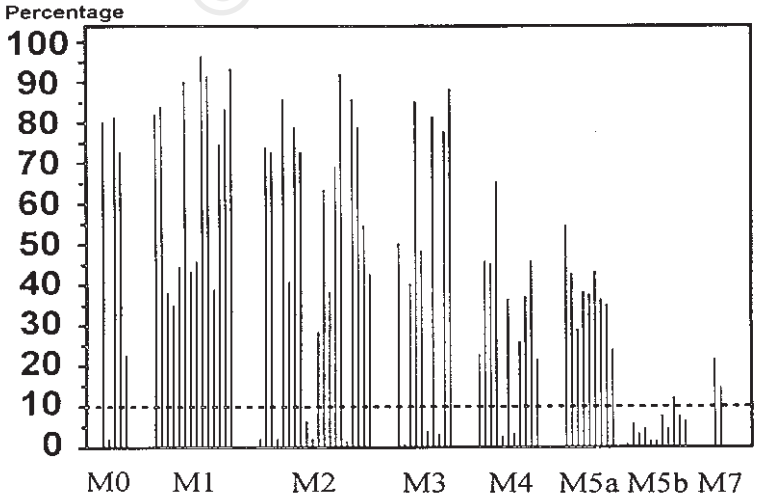

Figure 1. Expression of CD117 antigen in single AML patients according to FAB classification.

Table 1. Stratification of the evaluated parameters on the basis of CD117 antigen expression.

	$CD117^+$ No.	AML %	$CD117^-$ No.	AML %	p
Total	61	74%	21	26%	
Males/females	35/26		12/9		ns
Mean age (range)	52.3	(11-87)	52.9	(12-79)	ns
Splenomegaly	17	28%	9	43%	ns
LDH mean (range)	1438	(216-5500)	1653	(170-6360)	ns
WBC mean (range)	37.5	(1-251)	51.2	(1-211)	ns
De novo	40	65%	11	53%	ns
After MDS	15	25%	7	33%	ns
BC-CML	6	10%	3	14%	ns
M0	4	6%	1	4%	ns
M1	14	23%	-	-	0.011
M2	15	25%	5	24%	ns
M3	7	11%	3	14%	ns
M4	9	15%	2	10%	ns
M5a	9	15%	-	-	0.036
M5b	1	2%	10	48%	<0.0001
M7	2	3%	-	-	ns
$CD34^+$	44	74%	7	33%	
$CD34^-$	17	26%	14	67%	0.0007
$CD45RA^+$	39	78%	7	44%	
$CD45RA^-$	11	22%	9	56%	0.008
$CD45RO^+$	10	20%	8	50%	
$CD45RO^-$	40	80%	8	50%	0.017
$CD15^+$	25	41%	11	52%	
$CD15^-$	36	59%	10	48%	ns
$CD11c^+$	34	57%	15	71%	
$CD11c^-$	26	43%	6	29%	ns
$CD11b^+$	12	31%	6	46%	
$CD11b^-$	27	69%	7	54%	ns
$CD7^{+}$^	12	20%	1	5%	
$CD7^-$	49	80%	20	95%	0.044
$CD4^{+}$*	12	20%	11	52%	
$CD4^-$	49	80%	10	48%	0.004
$LyAg^{+\#}$	11	18%	2	9%	
$LyAg^-$	50	82%	19	91%	ns

WBC: $x10^9$/L; LDH: u/L.
*^Single co-expression of CD7; *single co-expression of CD4; #positivity for CD2 and/or CD5 and/or CD19 and/or CD10 (with or without CD7 or CD4 co-expression).*

Results

All myeloid leukemias

The expression of CD117 antigen greater than 10% occurred in the majority (74%) of the AML. The percentages of positivity in single cases of AML are depicted in Figure 1. In Table 1 clinical and biological characteristics of $CD117^+$ and $CD117^-$ AML are reported. Notably, in AML following MDS and in BC-CML we observed neither a higher incidence of CD117 expression, nor a greater mean percentage of positivity with respect to *de novo* AML: in fact, in our series *de novo* AML, AML after MDS and BC-CML had a mean expression±SD of CD117 antigen respectively equal to 44±30, 36±27, 31±26 (p NS). The indexes of *leukemic mass* such as LDH, splenomegaly and WBC count were not significantly different between $CD117^+$ and $CD117^-$ groups.

As shown in Table 2, based on the Pearson's coefficient value, CD117 shows a significant direct correlation with CD34 and CD45RA and an inverse correlation with CD45RO, CD11b and CD11c. Indeed, within the sixty-eight AML positive for CD117 or CD34 these antigens were co-expressed 44 times (65% of the cases) and the mean percentage of expression was 46% for both, 4% for CD117 alone and 14% for CD34 alone.

Table 2. Pearson's correlation coefficient between CD117 and the other antigenic markers in AML.

	No.	r	p
CD117-CD34	82	.486	< 0.0001
CD117-CD45RA	66	.448	< 0.0001
CD117-CD45RO	66	.515	< 0.0001
CD117-CD11b	52	.447	0.001
CD117-CD11c	81	.440	< 0.0001
CD117-CD15*	49	.014	0.927
CD117-HLA-DR*	49	.317	0.030
CD117-CD15°	20	.465	0.043
CD117-CD14°	20	.584	< 0.007
CD117-CD4°	20	.619	< 0.004

**In MO, M1, M2 and M3 FAB subgroups; °only in M5 (M5a and M5b) FAB subgroup.*

Granulocytic leukemias

CD117 antigen was only partially restricted to more undifferentiated granulocytic leukemias based on the FAB criteria: 80% of M0 AML, 100% of M1 AML and 75% of M2 AML resulted CD117 positive (Figure 1). In addition, seven of the ten M3 AML [eight *standard*, $CD2^-/CD34^-$/HLA-DR^-, PML-RARα^+ with BCR-2 (four cases), BCR-3 (three cases) and BCR-1 (one case); and two *microgranular variant*, both $CD2^+/CD34^-$/HLA-DR^-, PML-RARα^+ with BCR-1 and BCR-3] also expressed CD117. Moreover, in pure acute granulocytic leukemias (MO-M3 AML) we didn't observe any inverse correlation between CD117 and CD15, whereas a low direct correlation between CD117 and HLA-DR antigen was also found (Table 2).

Table 3. Some clinical and immunological findings of M5a (from #1 to 9) and M5b (from #10 to 20) AML. In no case central nervous system involvement has been observed.

No.	*Sex*	*Age*	*Type of leukemia*	*Splenomegaly*	*LDH*	*WBC*	*CD117 (%)*	*CD34 (%)*	*CD4 (%)*	*Other*	*ANAE*
1	M	65	after MDS	no	450	14	54	49	3		±
2	F	62	BC CML	yes	1350	12	43	50	2	CD7+	±
3	M	66	de novo	no	1907	30	29	2	65		+
4	F	47	de novo	no	1186	13	38	0	28		±
5	M	55	de novo	no	468	2	37	71	2	CD7+	+
6	M	42	de novo	no	277	2	44	60	4		±
7	F	31	de novo	yes	5304	82	36	5	5	CD7+	+
8	F	45	after MDS	no	1474	26	35	25	1		+
9	M	66	after MDS	yes	2133	52	24	32	31		+
10	M	12	de novo	yes	170	2	6	0	94		+
11	M	71	After MDS	no	483	11	4	3	59		+
12	M	54	BC CML	yes	247	15	5	0	69		+
13	F	74	after MDS	no	5358	53	1	0	62		+
14	M	64	de novo	no	3674	39	1	2	47	CD10+	±
15	M	48	de novo	yes	645	24	7	8	61		+
16	F	79	de novo	yes	5060	129	5	8	83		+
17	F	39	de novo	no	3380	65	12	0	27		+
18	F	51	after MDS	no	2011	28	7	28	36		+
19	F	67	after MDS	yes	3467	61	6	17	5		+
20	F	64	de novo	no	6360	211	0	1	1		+

WBC: x10^9/L; LDH u/L. ANAE (α-naphtyl acetate esterase): no fewer than 500 cells were counted to evaluate the reaction. +: > 50%; ±: > 30% < 50%. In all cases the cells were sensitive to the sodium fluoride (NaF).

Monocytic leukemias

Of the twenty cases with pure monocytic leukemia (Table 3), all nine M5a AML always expressed CD117 antigen, whereas ten (eight of which CD34 negative and CD4 positive) of the eleven M5b AML did not. Furthermore, in the M5 FAB AML we observed a very high inverse correlation between CD117 and CD14 (detected by LeuM3 MoAb), CD4 and CD15 (Table 2). In M5a and M5b AML, due to the absence of CD117 in more mature cases, its frequency was lower than in MO-M3 AML and M4 AML (50% vs 83% and 82%, respectively, p 0.030).

Lymphoblastic leukemias

Of the 53 ALL, 46 cases (87%) belonged to B lineage (5 early Pre-B, 32 common, 5 pre-B and 4 B) and 7 cases (13%) to T lineage (5 early T and 2 late T). Ph' chromosome was present in 7 cases (13%), CD34 antigen in 37 cases (70%) and MyAg co-expression in 22 cases (41%). Surface expression of CD117 antigen was never observed. Not even in the 5 early T ALL (expressing CD34 in all cases, HLA-DR and CD33 in four cases and CD13 in two cases) did we observe a positivity of CD117 antigen over 2%.

Discussion

Our results did not support the reliability of the CD117 in detecting leukemic myeloid lineages with earlier differentiative level. In fact, the frequent positivity of CD117 antigen in M3 AML, the lack of any correlation between CD117 and CD15 and the low direct correlation between CD117 and HLA-DR antigen, in pure acute granulocytic leukemias demonstrate that, differently from CD34 antigen expression,[25,26] CD117 antigen is irrespective of FAB classification and may be retained in more advanced maturational levels of the granulocytic lineage. In agreement with our observations, Di Noto *et al.*[8] observed that the expression of CD117 antigen on promyelocytic blasts was rather frequent and that it was also regulated by *in vitro* treatment with all-trans retinoic acid. Furthermore, we didn't find significant differences in CD117 positivity among the FAB subtypes for the high heterogeneity of CD117 antigen expression (Figure 1).

Interestingly, only in pure acute monocytic leukemias was CD117 antigen strictly restricted to earlier stages. Of note, some reports, by using YB5.B8 clone[8,17] as well as 17F11 clone,[11] have shown less

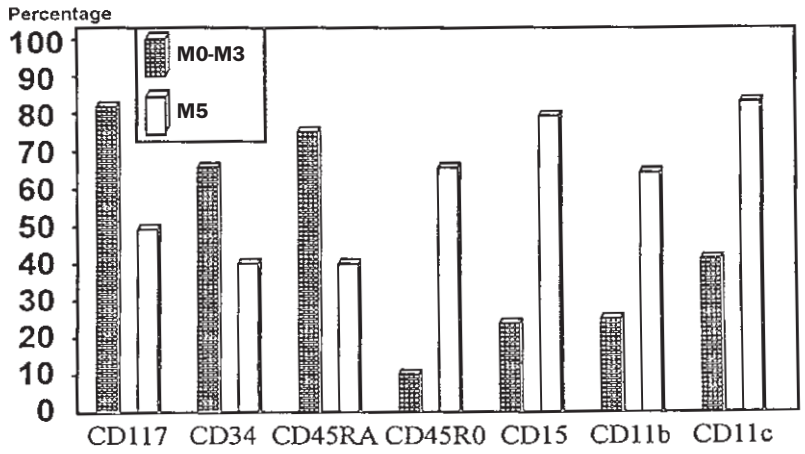

Figure 2. Percentage of positive cases in pure granulocytic and monocytic lineage AML.

frequent and lower expression of CD117 antigen in monocytic subtypes confirming that blast cells from this group express a phenotype which corresponds more closely to a late differentiative stage.

Although CD4 molecule may be expressed in various AML FAB subtypes,[27] this antigen showed an inverse association with CD117 in our monocytic leukemias. In a previous study[16] CD4 was expressed in four out of six CD117 negative and in three out of five $CD117^{+}/CD34^{-}$ M4-M5 AML; this suggests a possible association with precursors differentiating towards the myelomonocytic lineage. The results achieved by the Pearson's coefficient value between CD117 and the other antigens showed that CD34 and CD45RA, which directly correlated with CD117, had a greater percentage of positivity in granulocytic leukemias than in monocytic ones. The opposite was true for the antigens showing an inverse correlation with CD117, such as CD11b, CD11c, CD15 and CD45RO (Figure 2). In the M5b FAB subtype the similarities or the differences between these antigens and CD117 were even more clear-cut: for CD34 and CD45RA we observed a percentage of positive cases equal respectively to 9% and 25%, whereas for both CD11b and CD45RO the percentage of positive cases was 75% and for both CD15 and CD11c the percentage was 82%.

Thus, our study indicates that the positivity of CD117 antigen does not depend on *de novo* or *secondary* type of leukemia. We think that, although the leukemic blasts of MDS and CML originate from multipotent progenitor cells,[28,29] the expression of CD117 antigen is above all influenced by the developmental granulocytic or monocytic lineage of these cases.

Although other series[7,16,30] also showed the complete absence of CD117 receptor on B and T lineage lymphoblasts, from our study it is not possible to draw a definitive conclusion on this topic due to follwing reasons: 1) the few cases of T lineage ALL investigated; 2) a probable lower ability of the clone used to detect CD117 antigen in the lymphoblastic leukemias; and 3) the particular immunophenotyping technique not including multiple staining for the specific identification of the leukemic blast cells. Indeed, the expression of CD117 antigen on early thymocytes[31-33] and on some published cases of immature T lineage ALL[8,9,17,19] does not seem to suggest a myeloid specificity of CD117. All studies agree, however, that, with respect to CD33 and CD13, expressed on cells from almost all AML cases and also in a substantial subgroup of B and T lineage ALL, CD117 could have more relevant myeloid specificity. Thus, in the debate stimulated by the EGIL proposals,[34,35] our data justify a greater pointing score of CD117 in the immunological classification of AML.

In conclusion, our data support the view that CD117 may be of help in excluding a diagnosis of ALL in positive cases and well characterizes the differentiative degree of monocytic blasts.

Contributions and Acknowledgments

NC was the principal investigator, designed the study and performed the statistical analyses. PM contributed to the analysis and writing of the paper. GDA contributed to the cytofluorimetric assays. LM and ANC were involved in clinical management of the patients. MPP and GS collaborated in data handling. MC is director of the department and contributed to the conception of the study. The authors are listed by degree of importance, except for the last name (MC) who is the senior author.

Disclosures

Conflict of interest: none

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

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