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Simultaneous expression of CD13, CD22 and CD25 is related to the expression of FcεR1 in non-lymphoid leukemia

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Abbreviations: MPO, myeloperoxidase; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; MBP, major basic protein; EP, eosinophil peroxidase; ALL, acute lymphoblastic leukemia; ET, essential thrombocythemia; PMF, primary myelofibrosis; MDS, myelodysplastic syndrome; ABL, acute basophilic leukemia; EM, Electron microscopic.

Abstract Like CD19 or CD56, CD22 in non-lymphoid leukemia has been considered an aberrantly expressed antigen. CD22 had been believed to be restricted to B lymphoid, however, it was also found to be expressed in human basophils. Mature basophils are unique granulocytes expressing CD13, CD22 and CD25. To estimate whether the expression of CD22 in non-lymphoid leukemia is aberrant or related to basophilic character, we analyzed 108 patients with primary and secondary leukemia. Among non-lymphoid leukemias, surface CD22 expression was more frequently observed in peroxidase-negative AML and secondary leukemia, and was usually observed simultaneously with CD13 and CD25. Samples obtained from 17 cases were further analyzed, and all the FcεR1-positive cases were observed in peroxidase-negative AML and secondary non-lymphoid leukemia, and mostly expressed CD13, CD22 and CD25. Therefore, surface CD22 expression in non-lymphoid leukemia was thought to relate to basophilic phenotype in some cases. Like CD22, some of the so-called aberrantly expressed antigens may not actually be aberrant, but are expressed in a stage of differentiation and maturation of progenitors. Moreover, CD22 may be one of target molecules for treatment of CD22-positive, poorly prognostic leukemia.

Introduction

Diagnosis of acute leukemia has been made by the characterization of blasts in patients in comparison with normal hematopoietic cells including morphology, function-related molecules such as myeloperoxidase and esterase, surface and cytoplasmic antigen expression, and gene rearrangement of T and B cell receptors. Non-myeloid phenotype, such as CD19 (one of B cell markers) and CD56 (NK) expression in M2-subtype of AML, is usually considered aberrant expression. However, normal counterparts of leukemia cells should be searched for progenitor and precursor cells but not in mature cells, and some of the so-called aberrantly expressed antigens might be expressed in a stage of differentiation and maturation of normal hematopoietic cells.

Since basophils are the lowest constituent of blood cells, and acute basophilic leukemia is very rare, the phenotype of mature and immature basophils, such as surface-expressed CD antigens and even peroxidase molecule, was not established for a long time. KU812 (1), a reference cell line for basophilic leukemia established in our laboratory, is not really a basophil-restricted precursor since it also differentiates to myelocytic, monocytic, erythrocytic and megakaryocytic lineages. We also reported a t(15;17) cell line, HT93, that had the capacity to differentiate into neutrophils, eosinophils, and possibly basophils (2), however, the differentiation potential to basophils was uncertain.

A patient with CML in myeloid blast crisis in our hospital expressed unusual basophilia (Table

1), and the pathological cells showed a sequential change in morphology from blasts to mature basophils (Figure 1). The blasts expressed CD13, CD22 (Leu14), CD25, CD33, CD34, CD38 and FcεR1 on the surface, and eosinophil MBP, EP and histamine in the cytoplasm to some degree. We were interested in the expression of CD22 and CD25 in the cells, because CD22 had been believed to be restricted to B lineage, and CD25 is IL-2Rα. Our question at that time was whether this phenotype was aberrant. If not, CD22 may negatively regulate the activation signal in basophils stimulated by the antigen-IgE complex through FcεR1α/β/γ2 just like CD22 regulates BCR/CD79-mediated B cell activation. We then studied the phenotype of mature and immature basophils, and started a collection of basophil-related phenotypes in patients with primary and secondary leukemia paying particular attention to peroxidase-negative myeloid leukemia that was possibly related to basophil-lineage to some degree.

We reported on the expression of mRNA and antigen of EP in the immature basophil cell line, KU812 (3). We then developed a technique to directly analyze the phenotype of normal human basophils without purification since a purification procedure may activate the cells and alter the phenotype (4). Human basophils expressed CD13, CD25, CD33, CD38 and FcεR1 on the surface, and MBP, EP and histamine in the cytoplasm. Clone BL-B3C4 (IgG2a) was used to detect CD22 antigen, and CD22 was absent in basophils in the study, since a multi-color indirect method was utilized with anti-MBP (IgG1), histamine (IgG1) and FcεR1 (IgG2b), and therefore Leu14 (CD22, IgG2b) was not used. We further studied CD22 expression in purified basophils using several anti-CD22 clones, and found that the CD22 molecules expressed in human basophils reacted with clones Leu14 and 5.8HK, but not with 4KB128 and B3. The mechanism of the different antigenicity of CD22 molecules expressed in human B cells and basophils involved the different disulfide bonds between Ig-like domains 1 and 2 and the resulting 3D conformation (conformational forms), but not the primary structural difference in amino acids by alternative splicing, or antigen modification by glycosylation (5).

To understand the expression of basophil-related antigens in immature cells, we analyzed 25 human acute leukemia cell lines, and found that FcεR1 and CD22 mRNA were expressed in early B-lineage and myeloid leukemia cell lines (6). Therefore, CD22 expression in the basophil lineage possibly begins from a quite undifferentiated stage of common B and myeloid progenitors. In this study, we analyzed the relationship between FcεR1 and simultaneous expression of CD13, CD22 and CD25 in blasts in patients with primary and secondary leukemia, to estimate whether the simultaneous expression of CD13, CD22 and CD25 was related to basophil character like in mature basophils.

Patients and Methods

Patients

Between September 1995 and March 2002, 108 patients, 46 male and 62 female, age 0 to 93 with primary and secondary leukemia were analyzed. The primary diseases of these individuals were as follows: 60 patients with AML, 28 with ALL, 10 with CML, 5 with ET, 1 with PMF, and 4 with MDS (Table 2). Bone marrow aspirates from all 108 patients were analyzed for two-color surface phenotyping using FITC- or PE-conjugated monoclonal antibodies against 27 lineage markers. Among the cases, samples from 17 cases were analyzed for FcεR1 expression by an indirect staining method (Table 3). Peroxidase-negative myeloid cases were mainly analyzed for FcεR1 early in the series of this study, and peroxidase-positive and lymphoid cases were added later as controls. Cytoplasmic MBP, EP and histamine were also analyzed by flow cytometry in a few cases with peroxidase-negative myeloid leukemia.

Antibodies and controls.

FITC-conjugated anti-LeuM1 (CD15), CALLA (CD10), anti-HLe1 (CD45), Leu4 (CD3), Leu5b (CD2), Leu9 (CD7), Leu3a (CD4), HPCA2 (CD34), control IgG1, phycoerythrin (PE)-conjugated anti-HPCA2 (CD34), LeuM9 (CD33), IL2R (CD25), Leu1 (CD5), Leu2a (CD8), Leu14 (CD22), Leu12 (CD19), LeuM3 (CD14), Leu16 (CD20), Leu19 (CD56), HLA-DR and control IgG1 were purchased from Becton Dickinson Immunocytometry Systems (B.D.), Mountain View, CA. FITC-conjugated anti-MCS2 (CD13), c-kit (CD117), Mik-β1 (CD122), unconjugated anti-EP (eosinophil peroxidase, IgG2a) and BMK-13 (MBP, IgG1) were purchased from Nichirei, Tokyo, Japan. FITC-conjugated anti-CD36 (FA6.152), CD38

(T16), CD41 (P2), and PE-conjugated anti-glycophorin A (KC16) were purchased from Immunotech, Marseille, France. FITC-conjugated anti-OKT6 (CD1) and PE-conjugated anti-OKB7 (CD21) were purchased from Ortho Diagnostic Systems, Raritan, NJ. Unconjugated anti-CRA1 (FcεR1, IgG2b) was purchased from Kyokuto Pharmaceutical, Ibaraki, Japan. Unconjugated anti-D2 (Hist, IgG1) was purchased from Biogenesis, Poole, England. FITC-conjugated goat anti-mouse IgG2a, and PE-conjugated goat anti-mouse IgG1 and IgG2b were purchased from Southern Biotechnology, Birmingham, AL. Mouse myeloma proteins IgG1 (MOPC21), IgG2a (UPC10) and IgG2b (MOPC141) were purchased from Sigma Immuno Chemicals, St. Louis, MO, and used as controls of monoclonal antibodies in an indirect staining method. FITC-conjugated anti-MPO (IgG1, clone H-43-5) and control IgG1, and PE-conjugated anti-cytoplasmic CD3ε (cCD3, clone UCHT1), CD22 (cCD22, RFB4) and control IgG1 were purchased from Caltag Laboratories, Burlingame, CA. Leu14 reacts both with CD22 expressed in B cells and basophils, while RFB4 reacts only with B cells, but not with basophils.

Flow cytometry

For the surface marker analysis, aliquots of bone marrow samples were stained simultaneously with FITC- and PE-conjugated lineage markers. Labeled cells were treated with FACS Lysing Solution™ (B.D.) for 10 min, washed once with CellWASH™ (B.D.), and analyzed. For the analysis of FcεR1 expression, samples were serially stained with anti-CRA1 and PE-conjugated second antibody. A blast-gate was set in a FSC/SSC dot plotgram to maximize CD45-dull/CD14-negative population using a CD45-FITC/CD14-PE sample. For the cytoplasmic marker analysis, aliquots of the samples were stained with FITC- and PE-conjugated antibodies against cytoplasmic antigens (MPO, cCD3 and cCD22), or serially stained with unconjugated antibodies (EP, MBP and histamine) and FITC- and PE-conjugated second antibodies for the indirect method. Fix & Perm™ cell permeabilization kits (Caltag Laboratories, Burlingame, CA) were used as described previously (4). The cell fluorescence was analyzed with a FACScan™ flow cytometer using Lysis II™ and CELLQuest™ software (Becton Dickinson).

Results

Among mature myeloid blood cells, both CD22 (Leu14) and CD25 are expressed only in basophils, and simultaneous expression of CD13, CD22 and CD25 is the feature of this unique granulocyte. The 108 cases included 76 cases of non-lymphoid, 24 with B-lymphoid and 8 with T-lymphoid blasts (Table 2). There were 7 cases with peroxidase-negative (M0) and 53 with peroxidase-positive AML (M1 to 6). Surface CD22 (Leu14) was positive in 3 of 7 cases with peroxidase-negative AML (43%), 7 of 53 with peroxidase-positive AML (13%), 5 of 16 with non-lymphoid secondary leukemia (31%), 22 of 24 with primary and secondary B-lymphoid leukemia (92%), and 0 of 8 with T-lymphoid leukemia (0%). Therefore, among non-lymphoid leukemias, surface CD22 expression was more frequently observed in peroxidase-negative AML and secondary leukemia than peroxidase-positive AML. Simultaneous surface expression of CD13, CD22 and CD25 was observed in 11 of 15 CD22-positive non-lymphoid cases (73%), and 6 of 22 CD22-positive B-lymphoid cases (27%). Most of the CD22-positive cases simultaneously expressed CD13 and CD25 in peroxidase-negative AML and secondary non-lymphoid patients (7 of 8 cases, 88%).

The expression of FcεR1 was further examined in 17 cases (Table 3 and Figure 2). Although this series of 17 cases was mainly peroxidase-negative AML and secondary myeloid leukemia, and was therefore biased, all the FcεR1-positive blasts were observed in such cases, but not in peroxidase-positive or lymphoid cases, as expected. All the FcεR1-positive cases simultaneously expressed surface CD13, CD22 and early hematopoietic markers, CD34 and CD117, and 6 of 7 FcεR1-positive cases also expressed CD25. Erythroid or megakaryocyte markers, glycophorin A, CD36 or CD41, were often observed in FcεR1-positive cases. The blasts in case #04 expressed cytoplasmic basophil-related antigens, histamine, MBP, and EP, to some degree (Figure 3), however the expression of FcεR1 was low. In Contrast, blasts in case #05 expressed FcεR1 to a high degree, however, the expression of cytoplasmic basophil-related antigens was scarcely observed.

Discussion

The major question in this study was whether CD22 expression in myeloid leukemia was

aberrant. CD22 had been believed to be restricted to B lineage, however, it was also expressed in mature basophils in a conformational form (5). This molecule was expressed in myeloid leukemia blasts simultaneously with CD13, CD25 and FcεR1, therefore, this was thought to be a basophil-related expression, but not aberrant, although this analysis was somewhat biased. If so, then what is the physiological role of CD22 expressed in immature basophils? CD22 regulates the activation signal in B cells stimulated by an antigen through the BCR/CD79 complex (7), and the ligand for CD22 is CD45R expressed in T cells (8). CD22 is also expressed in mature basophils presumably in a different conformational form in which the 3D structure surrounding the ligand-binding domain is believed to be different from that expressed in B cells, and the amino acid sequence including ITAM and ITIM motifs in the cytoplasm is the same (5). Therefore, CD22 expressed in basophils may also regulate the activation signal in cells stimulated by the antigen/IgE complex through FcεR1, and the ligand for CD22 may be another subclass of CD45R. FcεR1 is also expressed in immature basophils, and its role may be in the induction of cell growth to respond to IgE. If so, CD22 in immature basophils may also regulate growth. Further examination of this hypothesis is necessary.

There have been 3 major models of basophil hematopoiesis (9): 1) basophils arise from hybrid progenitors with basophil/eosinophil-differentiation potential (CFU-Eo/Baso), supported by clonogenic assays *in vitro* (10), 2) a common ancestor shared by basophils and megakaryocytes (CFU-Baso/Mega), suggested by studies on leukemia cell lines (11), and 3) basophil/mast cell progenitors, supported by the description of cells sharing phenotypic features of both lineages in human pathology (12). Our previous analysis of 25 human leukemia cell lines revealed that 4 of 4 peroxidase-positive myeloid cell lines showed hybrid features of neutrophils/eosinophils, and 9 of 9 peroxidase-negative lines expressed basophil markers (FcεR1 $\pm$ CD203c) simultaneously with erythroid and/or megakaryocyte phenotype (glycophorin A, CD36 and/or CD41). The present study also supports this model. In fact, eosinophilia is sometimes observed in patients with *de novo* peroxidase-positive AML, namely CFU-GM-derived AML, and eosinophils are observed in CFU-GM *in vitro* within 2 weeks of culture. On the other hand, basophils are observed after 3 weeks in the presence of IL-3.

There have been reports of rare cases with primary acute basophilic leukemia (ABL). Another question in the present study pertains to the relationship between CD22+/FcεR1+ leukemia and ABL. ABL blasts present coarse basophilic granules with metachromasia lacking an peroxidase reaction. EM study is often necessary for diagnosis in cases with blasts showing specific immature basophilic or theta granules (13). However, EM is not routinely studied in most institutions for leukemia diagnosis, and reports of ABL have furthermore decreased in the last decade. We also disregarded whether blast cells were consistent with the criteria of ABL in the present study.

There have been several types of basophil-related hematopoietic malignancies. Remarkable basophilia is sometimes associated with chronic myeloproliferative disorders and MDS, especially in cases with CML (14) and MDS with myelofibrosis (15, 16). Like case #03 in our series, some CML cases show basophil leukemia crisis (17). A few cases with peroxidase-positive *de novo* AML are also accompanied by basophilia, and the blasts themselves sometimes express immature basophilic features, such as in cases with inv (16) (18), t(6;9) (19-21), and t(15;17) (22, 23). Typical ABL is peroxidase-negative non-lymphocytic, and could be diagnosed as M0 type AML, or even misclassified as ALL (24). *De novo* ABL is sometimes associated with chromosomal abnormality like t(9;22) (13, 25), t(X;6) (13, 26), t(3;6) (27), or without abnormality (24). Among the ABL cases reported, some cases expressed coarse basophilic granules with metachromasia in the blasts, and some cases with immature granules did not show metachromasia. Some cases were accompanied by basophilia, and others were not. In the present study, CD22+/FcεR1+ cases also consisted of well-differentiated and undifferentiated types of basophil-related leukemias. As shown in Table 2 and 3, FcεR1+ cases were often found in secondary myeloid leukemia transformed from CMPD and MDS. Although the reason is not clear, the cases with CMPD and MDS are sometimes accompanied with basophilia in blood before transformation, and the blast cells after transformation often show erythoid and/or megakaryocyte-related phenotypes, i.e., absence of

peroxidase-activity, presence of plateletperoxidase, surface expressions of CD41, CD36 or glycophorin A. Among them, CD36 is likely expressed in the differentiation stage of virtual erythromegakaryocytic common precursors (28).

Interestingly, a few cases with *de novo* ABL reported showed morphologically and phenotypically B-lineage ALL (25, 29). These cases usually express CD10-positive biphenotypic features, and are accompanied by minor bcr/abl. Granular ALL also expresses azurophilic granules and common ALL phenotype (30), therefore, this disease entity may include ABL in a part. These findings may suggest a relationship between immature B cells and basophils. In our previous study of leukemia cell lines, both immature B-lineage and peroxidase-negative myeloid lines expressed FcεR1 and CD22 mRNA (6). In fact, case #03 (CML in basophil blast crisis, Figure 1) expressed CD13, CD22, CD25, CD34, CD38 and FcεR1, and the phenotype gradually altered during the 4 months of the acute phase: the degree of expression of CD13 and CD33 decreased and that of CD34, CD19 and HLA-DR increased, and finally the patient died of B-lymphoid blast crisis (Figure 4). However, further study including *in vitro* culture of normal and abnormal precursor cells is necessary to support the relationship between B cells and basophils.

Recently, anti-CD20 (31) and anti-CD22 (32) monoclonal antibodies have been utilized for treatment of B cell malignancies. Interestingly, the CD22-positive leukemias in the present study consisted of chemotherapy-resistant and/or poorly prognostic diseases as peroxidase-negative non-lymphoid leukemia, secondary leukemia, and Ph-positive leukemia. Therefore, CD22 may be one of target molecules for treatment of such leukemias.

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Table 1 Clinical course of a patient with CML (case #03 in Table 3)

Date	Phase	Tx	WBC (/ml)	Hb (g/dl)	Plt ($x10^3$/ml)	Blast %	Neutro %	Baso %	Eosino %
1985.2.28	Before onset		3,800	14.4	179		50	0	1
1986.2.24	Onset	HU	75,400	10.3	387	<1	86	5	2
1989.4.4		HU + aIFN	3,200	12.5	115	0	41.5	3.5	0.5
1997.1.16	Accelerated	HU	91,500	7.3	67	6	39	21	3
1997.5.6	Crisis	HU + Etp	63,000	9.1	82	26.5	54	12	1.5
1997.7.18		HU + Etp	7,550	7.1	173	3	32	55	1
1997.9.9		HU + Etp	23,800	7.5	86	21	12	39	3
1997.9.18		HU + Etp	107,800	7	90	62	20	10	2
1997.10.3	Dead	VCR + PSL	19,700	10.9	58	76	11	1	0

HU, hydroxyurea; aIFN, natural interferon-alpha; Etp, etoposide; VCR, vincristine; PSL, prednisolone; Tx, treatment

Table 2 Mature basophil-related phenotype (CD13, CD22 and CD25). Expression in blasts of 108 cases with primary and secondary leukemias.

Diagnosis	total cases	CD22+ cases	CD13+/CD22+/CD25+ cases
AML	60	10	6
M0	7	3	2
M1	17	2	1
M2	13	1	0
M3	7	1	0
M4	9	1	1
M5	3	1	1
M6	4	1	1
ALL	28	18	4
B-lin	20	18	4
T-lin	8	0	0
CMPD	16	8	6
CML-MTF	6	2	2
CML-LTF	4	4	2
ET - MTF	5	2	2
PMF-MTF	1	0	0
MDS-MTF	4	1	1
total	108	37	17

AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; CMPD, chronic myeloproliferative disorders; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; ET, essential thrombocythemia; PMF, primary myelofibrosis; B-lin, B cell-lineage; T-lin, T cell-lineage; MTF, myeloid transformation; and LTF, B cell-lineage lymphocytic transformation.

Table 3 Phenotype of blasts in 17 cases in which FcεR1 was analyzed.*

case #	Diagnosis	Baso% in PB	FcεR1	Baso markers	Early markers	T-cell markers	B-cell markers	Myeloid markers	Other markers
1	AML/M0	0	+	CD13/CD22/CD25	CD117/CD34			CD33/CD15	DR
2	AML/M0	0	+	CD13/CD22	CD117/CD34	CD7	CD38	CD33/GPA	DR
3	CML/MTF	39	+	CD13/CD22/CD25	CD117/CD34		CD38	CD33/CD36	DR/CD56
4	CML/MTF	9	+	CD13/CD22/CD25	CD117/CD34	CD4	CD38	CD33/CD36/CD41	DR/CD56
5	ET/MTF	9	+	CD13/CD22/CD25	CD117/CD34	CD4	CD38	CD33/CD14/CD41/GPA	DR
6	MDS/MTF	2	+	CD13/CD22/CD25	CD117/CD34	CD4	CD38	CD33	DR/CD56
7	MDS/MTF	0	+	CD13/CD22/CD25	CD117/CD34	CD4	CD38	CD33/CD36	DR
8	AML/M1	0	-	CD13/CD22/CD25	CD117/CD34		CD38	CD33/CD15/CD41	DR/CD56
9	AML/M2	0	-	CD13		CD4	CD38	CD33	DR
10	AML/M2	0.5	-	CD13	CD117	CD4	CD38	CD33/CD15	DR
11	AML/M3	2	-	CD13	CD117			CD33/CD15	
12	AML/M3v	1	-	CD13	CD117/CD34		CD38/CD19	CD33/CD15	DR
13	ALL/B	0	-	CD13/CD22	CD117/CD34		CD38/CD19	CD33/GPA	DR
14	ALL/B	0	-	CD22/CD25	CD117/CD34		CD38/CD10/CD19/CD20	CD33	DR
15	ALL/B	0	-	CD13/CD22/CD25	CD34		CD38/CD10/CD19/CD20/CD21	CD33/CD36	DR
16	ALL/T	0	-	CD13	CD34	CD5/CD7	CD38/CD19/CD21	CD33	DR
17	CML/LTF	0	-	CD13/CD22/CD25	CD34		CD38/CD10/CD19/CD21	CD33/GPA	DR

*Positive markers among 27 lineage markers are presented.

AML, acute myelogenous leukemia; M3v, M3 variant; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia;

ET, essential thrombocythemia; MDS; myelodysplastic syndrome; MTF, myeloid transformation; and LTF, B cell lineage lymphocytic transformation.

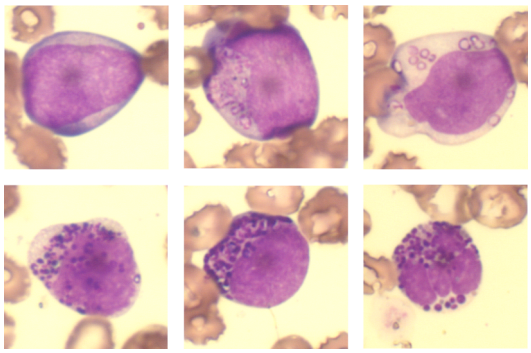

Figure 1 The morphology of peripheral blood cells in case #03 (May-Giemsa stain, Original magnification x1,000). A sequential change in the morphology from blasts to mature basophils was observed.

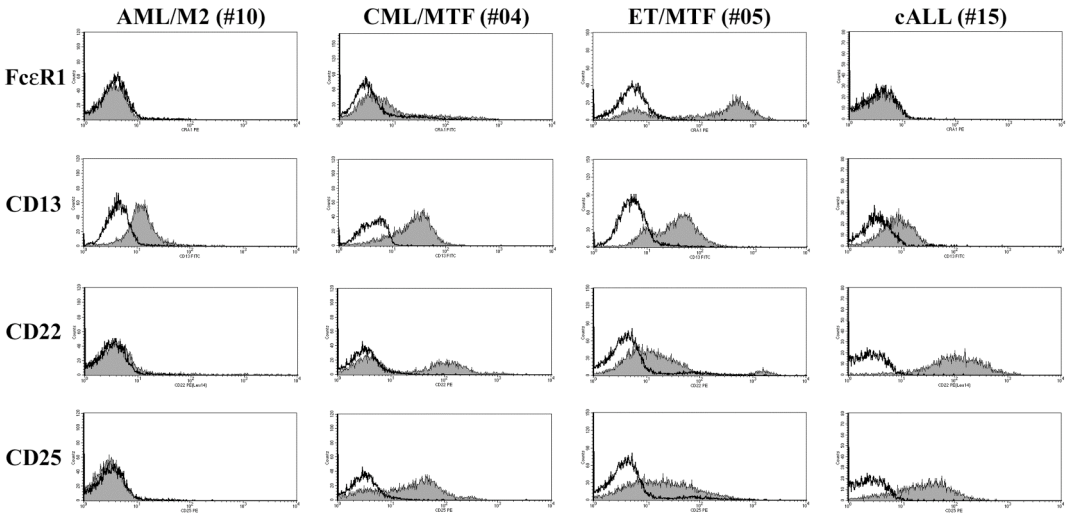

Figure 2 Expression of mature basophil-related antigens in blasts. Antigen expressions of FcεR1, CD13, CD22 (Leu14) and CD25 are shown in 4 cases (see Table 3). A blast-gate was set on a FSC/SSC dot plotgram to maximize CD45 dull-positive/CD14-negative immature cells.

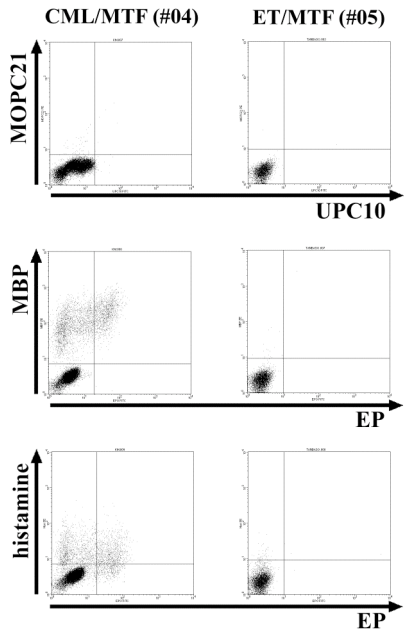

Figure 3 Cytoplasmic expression of basophil-related antigens: MBP, eosinophil major basic protein; EP, eosinophil peroxidase; and histamine. The cells in case #04 showed basophil-related antigens in the cytoplasm in part, while the cells in case #05 did not. MOPC21 and UPC10 are control mouse myeloma proteins IgG1 and IgG2a, respectively. A blast-gate was set on a FSC/SSC dot plotgram.

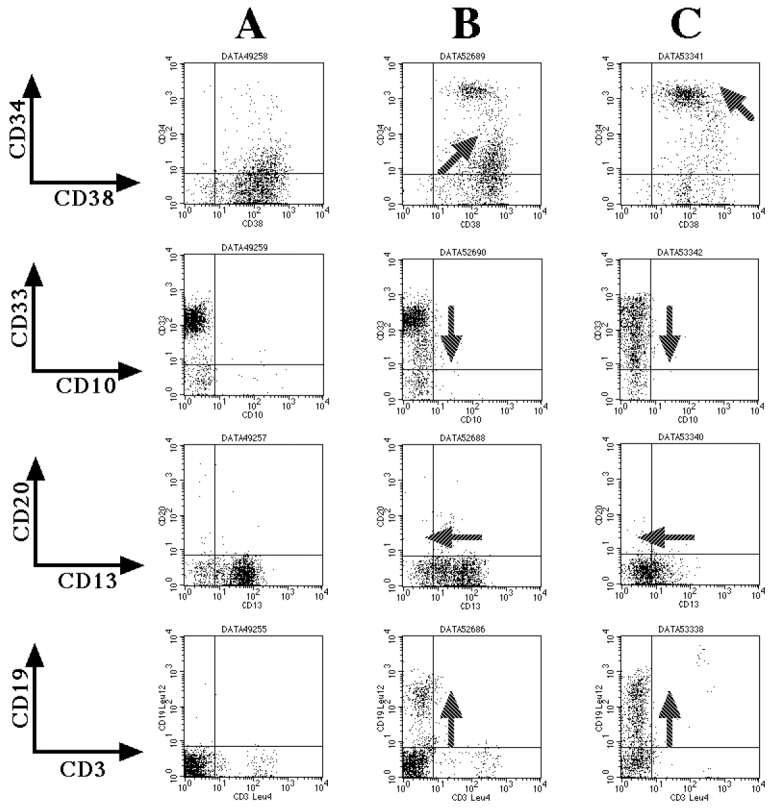

Figure 4 The alternating phenotypes of the blasts in case #03. The horizontal and vertical axes show FITC- and PE-fluorescence, respectively. A, May 21; B, September 9; C, September 30, 1997. A blast-gate was set on a FSC/SSC dot plotgram. The percentages of the CD45-dim blasts in the gate were 85.5%, 88.1% and 88.2% in panels A, B and C, respectively.