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FcεR1 and CD22 mRNA are expressed in early B-lineage and myeloid leukemia cell lines.

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Abstract CD22, one of the important markers for diagnosing B-lineage acute leukemia, was expressed in mature basophil granulocytes. We then investigated the expression of CD22 and other B cell- and basophil-related molecules in 25 human acute leukemia cell lines to find the phenotype of the virtual common progenitor of B- and myeloid lineage. Surface and cytoplasmic expressions of antigens were analyzed using a flow cytometer and an essential antibody-panel used for diagnosing acute leukemia as well as cytokine receptors and basophil-related enzymes. Messenger RNA expression of FcεR1 and CD22 was also analyzed. Peroxidase-positive and -negative myeloid leukemias showed eosinophil- and basophil-type expression of enzymes, respectively. Early myeloid and B-lineage cells expressed basically similar combinations of cytokine receptors and various combinations of mRNA listed above, while T-lineage cells did not. The virtual common progenitor of B- and myeloid lineage cells may be defined as immature cells simultaneously expressing B- and basophil phenotypes.

List of abbreviations: FcεR1, high affinity IgE receptor; MPO, myeloperoxidase; EP, eosinophil peroxidase; MBP, eosinophil major basic protein; EDN, eosinophil derived neurotoxin; ECP, eosinophil cationic protein; Hist, histamine; PBGD, porphobilinogen deaminase; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; cALL, common ALL; APL, acute promyelocytic leukemia; AMgkL, acute megakaryoblastic leukemia; EL, erythro leukemia; lin$^-$ leukemia, lineage-negative leukemia; CML, chronic myelogenous leukemia; BaL, basophilic leukemia; BC, blast crisis; LBL, lymphoblastic lymphoma; pDC, plasmacytoid dendritic cells; EMeg, erythro-megakaryocytic; G- and GM-CSF, granulocyte- and granulocyte-macrophage colony-stimulating factor; CFU-GM, -GEMM, -mix and –S, colony forming units for granulocyte-macrophage, granulocyte-erythroid-megakaryocyte-macrophage, mixed lineage, and in spleen, respectively; ATRA, all-*trans* retinoic acid; MFI, relative mean fluorescence intensity; RT-PCR, reverse transcriptase-polymerase chain reaction; QRT-PCR, quantitative real time RT-PCR.

Introduction

The clinical features of bcr/abl-positive acute and chronic leukemias as well as B-lineage involvement in myelodysplastic syndrome suggest a close relationship between B- and myeloid lineage in hematopoiesis. The number of days taken to induce mature myeloid cells from progenitors in a semisolid culture system suggests the degree of immaturity of the virtual progenitors, i.e., neutrophils and monocytes, eosinophils, nucleated red cells and megakaryocytes, and basophils, in this order, from relatively mature to immature progenitors. Therefore, the study of basophil-related markers in hematopoietic progenitors may reveal the character of very immature myeloid progenitors.

Although the cytochemistry of basophils is well understood, their phenotype has not been well known, because the cells are the lowest constituent of circulating blood granulocytes in human, and the percentage is less than 1% of nucleated cells in a bone marrow aspirate. The cells are peroxidase-positive polymorphonucleated small leukocytes characterized by their basophilic-stained granules. It is known that MPO in neutrophils and EP in eosinophils are genetically different enzymes, whereas it has been unknown whether basophil peroxidase is identical to MPO, EP, or another distinct enzyme. We then established a flow cytofluorometric method

to simultaneously analyze surface phenotype and cytoplasmic enzymes related to the functions of neutrophils and eosinophils [1], and reported the expression of mRNA and antigen of EP in an immature basophil cell line, KU812F [2]. In a preliminary study using purified human basophils using anti-FcεR1 and an immunomagnetic sorting system, it was found that almost all the cells expressed MBP and Hist, and some cells expressed EP, but not MPO. However, basophils are activated by the addition of anti-FcεR1 antibody, and the phenotype will be altered. Then we established a novel technique for the direct flow cytofluorometric analysis of the cells in unseparated blood samples [3]. Cytoplasmic expressions of MBP and Hist were used as markers to define basophils, because the basophil related antigen, CD203c, recognized by a monoclonal antibody, clone 97A6 [4], was not reported at that time. The human blood intact basophils were CD11a /CD11b /CD11c /CD25 /CD38 /CD13 /CD33 /CD116 /CD123 /CD125 /CD126 /FcεR1 /MBP /Hist positive, CD71 dim positive, CD14 /CD15 /EP partially positive, and CD2 /CD3 /CD7 /CD122 /CD16 /CD56 /CD57 /CD10 /CD19 /CD20 /CD23 /CD114 /CD124 /HLA-DR /MPO negative.

CD22 has been considered to be restricted to B-lineage, however, it was expressed in human mature basophils with different conformation [5]. Antigens activate B cells and basophils through B cell receptors and IgE/FcεR1-complex, respectively. Co-activation of CD22 in B cells and basophils by CD45R of memory T cells is supposed to downmodulate the cell activation threshold through its association with PTP-1C (SHP-1). CD22 is absent in T cells, NK cells, neutrophils, eosinophils, monocytes, erythrocytes and platelets, therefore is a common antigen for B cells and basophils. FcεR1 is a functional molecule for allergic reaction expressed in basophils and mast cells, and is also expressed in eosinophils [6], monocytes [7], and Langerhans cells [8]. However, the cells purified from human peripheral blood using antibody against RcεR1 and a immunomagnetic cell sorting system are mostly basophils despite their small population [3, 5]. We hypothesized that very immature myeloid progenitors and common progenitors of myeloid and B-lineage might express basophil-related antigens, and then studied the expression of CD22 as well as basophil-related molecules in leukemia cell lines to find the phenotype of the virtual common progenitor of B- and myeloid lineage.

Materials and Methods

Cells and culture conditions

Cell lines were provided by the Japanese Cancer Research Resources Bank (JCRB: Tokyo, Japan), in addition to those originally established in our laboratory: WK93, WH94, HT93, KY821, KU812F, TK91, KAZZ, C2F8, B4D6, BPS99, TIM96 and YS-1. TOM-1 [9], ALL/MIK [10], MEG-O1 [11], UT7-GM [12], and CMK11-5 [13] were donated by Drs. Okabe M, Ogura M, Komatsu N, and Sato T, respectively. The cells were maintained in RPMI-1640 (Gibco Laboratories, Grand Island, NY) medium containing 100 U/mL penicillin G sodium (PCG; Biowhittaker, Walkersville, MD), 100 μg/mL streptomycin sulfate (SM; Biowhittaker), and 10% heat-inactivated fetal bovine serum (FBS; Dainippon Pharmaceutical, Osaka, Japan) at 37^{O}C in 5% CO_2 in humidified air. Exponentially growing cells were collected and analyzed.

Heparinized human peripheral blood was obtained from 4 healthy donors after informed consent, and utilized for the phenotype analysis of normal blood basophils after sorting procedure of the samples using a monoclonal antibody against human FcεR1 and the MACS^{TM} magnetic cell sorting system (Miltenyi Biotec, Bergisch Gladbach, Germany) as described previously [3]. The MBP-positive and Hist-positive percentages in the sorted basophil samples were 95.6 $\pm$ 3.17% and 84.0 $\pm$ 8.76% respectively. Heparinized blood was also obtained after informed consent from 4 patients with hypereosinophilia for analyzing the phenotype of blood eosinophils. Bone marrow was obtained by aspiration from a patient with chronic myelogeneous leukemia after informed consent. Appropriate informed consent according to the institution's guidelines was obtained from the donors. Bone marrow basophils and mast cells were purified from the samples using a monoclonal antibody against human FcεR1 and the MACS^{TM} magnetic cell sorting system as well, and utilized as a control for the quantitative RT-PCR of FcεR1-α. The purity of the FcεR1-positive cells in the sample was estimated as MBP-positive mononuclear cells by flow cytometry, and was 80.8%. As positive- and negative-controls for conventional RT-PCR, mature T, B cells and basophils were purified from heparinized blood obtained from a healthy donor in the magnetic field [5] using monoclonal

antibodies against CD3, CD19 and FcεR1, and the CD3, CD19 and CD203c-positive percent in the samples were 99.9, 99.9 and 83.9%, respectively.

Antibodies and controls.

Monoclonal antibodies were purchased from the providers as written in Table 1. FITC-conjugated control IgG1 and PE-conjugated control IgG1 were purchased from Becton Dickinson, San Jose, CA. MicroBeads-conjugated mouse anti-human CD3, anti-human CD19 and Streptavidine were purchased from Miltenyi Biotec. FITC-conjugated goat anti-mouse IgG1, IgG2a and PE-conjugated goat anti-mouse IgG1, IgG2a and IgG2b, and biotinated goat anti-mouse IgG2b were purchased from Southern Biotechnology, Birmingham, AL. FITC-conjugated $F(AB')_2$ fragment of goat anti-rabbit IgG was purchased from ICN Biomedicals, Aurora, OH. FITC-conjugated anti-MPO (IgG1) and control IgG1 were purchased from Caltag Laboratories, Burlingame, CA. Mouse myeloma proteins IgG1 (MOPC21), IgG2a (UPC10) and IgG2b (MOPC141) were purchased from Sigma, St. Louis, MO, and used as monoclonal antibody controls in an indirect staining method. Rabbit anti-human cMPL (IgG) was kindly provided by Kirin Brewery, Tokyo, Japan. Mouse monoclonal antibody against human basophil-related antigen, anti-CD203c (clone 97A6, IgG1) was a kind gift of Dr. Hans-Joerg Buhring [14].

Flow cytometry

The cells were stained for surface phenotype using monoclonal antibodies and standard staining methods with direct or indirect procedures after FcR blocking using human IgG. The cells were also stained for analyzing cytoplasmic antigens using monoclonal antibodies against MPO, EP, MBP or Hist, and fluorochrome-labeled second antibodies and Fix & Perm™ cell permeabilization kits (Caltag). The cells were fixed using Reagent A (fixation medium) at room temperature (RT) for 15 min, washed with phosphate-buffered saline (PBS), incubated with a monoclonal antibody and Reagent B (permeabilization medium) at RT for 15 min, washed, incubated with a second antibody and Reagent B, if necessary, at RT for 15 min, washed, and then analyzed using a flow cytometer.

Cell fluorescence was analyzed with a FACScan™ Flow Cytometer and CELLQuest™ software (Becton-Dickinson). A minimum of 10,000 events was sampled and analyzed. The following parameters were calculated for estimating the expression of basophil-related antigens. The MFI of an antigen was chosen as representative of the expression observed in most cells of a cell line, and which varied in its degree according to the type of cell line:

MFI = (mean fluorescence intensity of a monoclonal antibody) / (mean fluorescence intensity of the isotype control mouse myeloma protein at the same concentration as the monoclonal antibody).

Positive percentage of an antigen was chosen as representative of the expression observed in part of a cell line.

Quantitative RT-PCR for FcεR1α.

Total RNA was isolated from the samples with Trizol (LifeTechnologies, Tokyo, Japan). cDNA was synthesized from 5 μg of total RNA with a random primer and a Takara RNA PCR Kit (AMV) Ver. 2.1 (Takara, Tokyo, Japan) to a final volume of 20 μL. Templates of FcεR1α [15] and PBGD [16] were made as a control gene to calculate a standard concentration curve. cDNA from cell line C2F8 was amplified with each primer (FcεR1α-5'; gggaacaatttctttgaagtcagt and FcεR1α-3'; catttgtaatggagatgttgtggt for FcεR1α, PBGD-5'; agattggagagaaaagcctgttta, PBGD-3'; cgaatactcctgaactccagatg for PBGD) and directly inserted into the pGEN-T vector. Recombinant plasmid was then isolated after transforming into Escherichia coli JM109 competent cells. cDNA was diluted 100 times with DNAse free water, and 5 μL of the sample was used for FcεR1α and PBGD real-time quantitative PCR. The cDNA and the diluted recombinant plasmid were amplified with the primers and a dye; LightCycler-FastStart DNA Master SYBR Green I (Roche, Indianapolis, IN). After an initial denaturation step of 10min at 95^0C, a 3-step cycle procedure was used for PBGD (denaturation 95^0C, 0 sec, annealing 62^0C, 10 sec and extension and acquisition 72^0C, 13 sec for 40 cycles) and a 4-step cycle procedure was used for FcεR1α (denaturation 95^0C, 0 sec, annealing 62^0C, 10 sec, extension 72^0C, 13 sec and acquisition 83^0C, 1 sec for 40 cycles). A standard curve was calculated using LightCycler software and 5 concentrations of FcεR1α or

PBGD plasmid standards. A standard curve was drawn by plotting the cycle numbers at which the fluorescent signals entered the log-linear phase against the concentrations of the standards. The relative quantities of the PBGD and FcεR1α transcripts of all the samples were calculated using the LightCycler software using this standard curve. The final result was the normalized FcεR1α value, expressed as the ratio FcεR1α/PBGD.

RT-PCR analysis for FcεR1 and CD22

RT-PCR analysis was performed as described previously [17]. Total RNA was isolated from the sample with Trizol, and 5 μg was utilized for the cDNA synthesis. The cDNA was synthesized from the total RNA with random primers and murine Moloney leukemia virus reverse transcriptase in a final volume of 20 μl. The FcεR1α and CD22 cDNAs were amplified with AmpliTaq polymerase (TOYOBO, Osaka, Japan) and each primer (FcεR1α: same as the primers used for quantitative RT-PCR; CD22-5' and 3': in the exons V and VIII, respectively, as described previously [5]) from 1 μl of the cDNA according to the following amplification profile: FcεR1α: 40 cycles at 94^0C for 60 seconds, 62^0C for 90 seconds, and 72^0C for 120 seconds; CD22: 35 cycles at 94^oC for 60 seconds, 58^oC for 90 seconds, and 73^oC for 120 seconds. The amplified products were separated on 3% agarose gels and stained with ethidium bromide.

Results

Phenotype of the cell lines

The surface expression of leukemia-markers is summarized in Table 2. CD1, CD2, CD7 and CD8 were expressed in T-lymphoid cell lines, and were mostly absent in B-lymphoid and myeloid lines. CD19, CD20 and CD22 were expressed in B-lymphoid cell lines, and were absent in T-lymphoid and myeloid lines. CD13, CD15 and CD33 appeared to be expressed in MPO-positive cell lines, while CD36, CD41 and GPA were expressed by MPO-negative lines.

The expression of basophil-related bioactive molecules in the cytoplasm of human blood eosinophils and basophils is shown in Figure 1. The antigen levels of MBP and EP in mature eosinophils showed a linear correlation with each other, and also had a linear correlation with the side-scatter of the cells. Therefore, cells have a wide range in the number of eosinophilic granules in the cytoplasm, and the concentrations of MBP and EP in eosinophilic granules are approximately constant. In contrast, the expression of EP in mature basophils was observed in a subset of the cells which expressed MBP to a high level. The concentration of EP in basophilic granules was found to have a broad range, and the EP-positive percentage in human blood basophilic granulocytes varied from 16 to 57% [3]. Basophils expressed histamine, while eosinophils did not.

The representative expression pattern of basophil-related bioactive molecules is shown in Figure 2, and summarized in Table 3. No expression of MBP, Hist or EP was documented in an immature B-cell line, YS-1. A t(15;17) cell line, HT93, showed MBP and EP expression resembling to those of peripheral blood eosinophils. In contrast to HT93, a Ph1-positive immature basophil cell line, KU812F showed MBP and EP expression resembling to those of blood basophils. Histamine expression was also observed in KU812F and KAZZ. Simultaneous expressions of CD203c and FcεR1 were observed in the most MPO-negative myeloid cell lines.

The general patterns of the expression of the CSF receptors (Table 3) were as follows; coexpression of CD114, CD123 and CD126 was seen in most of the MPO-positive myeloid cells; coexpression of CD116, CD123, CD124 and CD126 was seen in the majority or many of MPO-negative myeloid and the B-lineage cells; almost no receptors were observed in the T-lineage cells. The MPO-positive AML cell lines expressed EP and MBP the most, but did not express Hist, cMPL, CD203c or FcεR1, therefore their character was hybrid progenitor of neutrophils and eosinophils, namely GFU-GM. The MPO-negative myeloid leukemia cell lines expressed CD36, CD41, GPA, MBP, CD203c and FcεR1 the most, and some of the lines also expressed EP and/or Hist, or cMPL, therefore their character was hybrid progenitor of basophils and EMeg, namely CFU-GEMM but not CFU-GM.

mRNA expression of FcεR1 and CD22

The amount of mRNA of FcεR1 α-chain measured using quantitative RT-PCR is summarized in Table 3. The conventional RT-PCR of FcεR1 and CD22 is shown in Figure 3 and summarized in Table 3 as well. Mature blood T cells did not express either of the mRNAs, B cells expressed CD22, and basophils

expressed FcεR1 and CD22. MPO-positive cell lines mostly did not express these mRNAs. All the MPO-negative myeloid cell lines expressed FcεR1, and about one half of the lines expressed CD22. All the B-lineage cell lines expressed CD22, and one half of the lines expressed FcεR1. None of the T-lineage cell lines expressed these mRNA. Therefore, MPO-negative myeloid lines and B-lineage lines showed character of hybrid progenitor of basophils and B cells to some degree.

Discussion

Human eosinophils and basophils share very similar bioactive molecules such as MBP,EP, EDN and ECP [3, and unpublished data], and some investigators have speculated that the two unique granulocytes arise from relatively mature precursors called "hybrid eosinophil/basophil granulocytes" [18]. However, eosinophils grow in CFU-GM assay conditions from human blood or bone marrow in 2 weeks [19], while basophils and mast cells grow in CFU-mix and/or CFU-S conditions after prolonged cultivation [20, 21]. Moreover, MPO-positive leukemia cell lines differentiate to eosinophils in the presence of IL-5, GM-CSF, ATRA, or sodium butyrate [1, 22], while MPO-negative CFU-GEMM-derived leukemia cell lines including an immature basophil line, KU812, differentiate to erythroid and/or megakaryocytic lineages in the presence of hemin, phorbol myristate acetate or thrombopoietin [23-26]. Therefore, basophil granulocytes are thought to arise from relatively early myeloid progenitors, but not from late progenitors of neutrophils, monocytes or eosinophils, in the serial maturation pathway of myeloid hematopoiesis. The data in this paper also support the virtual hierarchy of myelopoiesis.

All the MPO-negative myeloid lines studied in this paper expressed FcεR1 antigen and mRNA. Among these cell lines, Hist and EP were expressed on CD203c-positive cell lines. Therefore, CD203c is thought to be closely related to mature basophils as well as their progenitors. Interestingly, the expressions of cMPL (a receptor of thrombopoietin) and CD203c approximately did not overlap in this category of FcεR1-positive myeloid progenitor leukemia.

FcεR1 is a function-related receptor of basophils and mast cells. CD22 is thought to modulate cell activation of B-lymphocytes and provably basophils. All the MPO-negative myeloid lines expressed FcεR1 mRNA, and about one half of them expressed CD22, while all the B-lineage lines expressed CD22 mRNA and one half of them expressed FcεR1. Therefore, these two categories of progenitors may arise from a common B/M (B-lineage and myeloid) progenitor defined as $Fc\varepsilon R1^{+}CD22^{+}$ cells.

The most important models to explain lineage commitment of hematopoietic stem cells are as follows: 1) stochastic commitment [27], 2) a concept of common lymphoid progenitor [28], and 3) "stepwise lineage restriction" [29]. The culture conditions of classical studies by Ogawa did not support T/NK as well as pDC lineages. The culture system by Galy supported myeloid cell development in vitro, however the so-called "common lymphoid progenitor" was defined as $CD34^{hi}Lin^{-}CD10^{+}$, which might have been very heterogeneous populations including B-progenitor, T/NK-progenitor and pDC-progenitor, which might have arisen from stem cells independently. The culture system established by Katsura et al. supported the development of T/NK, B and myeloid cells from single progenitor cells in vitro. Among seven possible progenitors, i.e., multipotent p-M/T/B (capable of generating myeloid, T and B cells), bipotent p-M/T, p-M/B, p-T/B, unipotent p-M, p-T and p-B, only p-T/B type progenitor was found to be absent. This concept may require the origin of myeloid cells to p-M/T as well as p-M/B, and it is therefore somewhat strange to consider what the differences between two myeloid cells arisen from p-M/T and p-M/B are. On the other hand, this concept helps in understanding the phenotype of de novo acute leukemia and leukemia cell lines as shown in Figure 4. Some type T-I (pro-T) lines differentiate to T-lymphoid as well as monomyeloid and erythroid cells [30]. Therefore, if the very first event of lineage commitment of stem cells is prohibition against the development to one of the two (B and T/NK) lymphoid cells but not acquisition of lineage, then the concept by Katsura may support the clinical observations of biphenotypic leukemia as well as the absence of confusion and conflict in the development of T-cell receptors and B-cell receptors, despite sharing the same mechanism in part utilized to construct T- and B-restricted functions, respectively. However, because the circumstance in bone marrow does not fit to develop T-lineage, the many of transformed progenitor and precursor T cells show clinical features of extramedullary lymphoblastic lymphoma. The

$CD7^{+}CD33^{+}CD34^{+}CD56^{+}cCD3\varepsilon^{dull}$ "myeloid/natural killer cell precursor acute leukemia" [31] will be integrated into M/T biphenotypic leukemia [32, 33]. Meanwhile, little is known about the clinical feature of transformed progenitor pDC [34].

Simultaneous analysis of DNA and RNA quantity in cells revealed different profiles of cell kinetics in normal and abnormal hematopoietic components [35]. There were cells with relatively low levels of RNA in the S-phase (Type 1) and a high level in the S-phase (Type 2) [36]. Lymphoid leukemia and its normal counterparts synthesize low amounts of RNA in the G1-phase (Type 1), and myeloid leukemia and its normal counterparts synthesize high amounts of RNA in the G1 (Type 2). Normal $CD34^{+}CD38^{-/dull}$ cells as well as lineage-negative and biphenotypic leukemia of M/T and M/B show an intermediate level of RNA synthesis, namely Type 0 growth [37]. Although the biological mechanism underlying this observation is not known, lin^{-} and biphenotypic progenitors share a similar cell cycle tendency to near-stem cells.

Among primary and secondary BaL [38, 39], some cases with de novo BaL morphologically and phenotypically showed characters of B-lineage ALL [40, 41], therefore granular ALL [42] possibly includes some de novo BaL as a subset. Moreover, a detailed and sophisticated study on this category of acute leukemia may reveal the close relationship between B- and myeloid lineage through phenotypic and genotypic analyses of common B- and basophil-related molecules such as FcεR1 and CD22.

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Table 1 Monoclonal antibodies used.

cluster	antigen	antibody	provider
CD1		OKT6	Ortho
CD2		Leu-5b	B.D.
CD3		Leu-4	B.D.
CD4		Leu-3a	B.D.
CD7		Leu-9	B.D.
CD8		Leu-2a	B.D.
CD10		CALLA	B.D.
CD13		MCS-2	Nichirei
CD14		Leu-M3	B.D.
CD15		Leu-M1	B.D.
CD19		Leu-12	B.D.
CD20		Leu-16	B.D.
CD21		OKB9	Ortho
CD22		Leu-14	B.D.
CD23		Leu-20	B.D.
CD25	IL-2Ra		B.D.
CD33		Leu-M9	B.D.
CD34		HPCA-2	B.D.
CD36		IOP36	Coulter
CD38		IOB6	Coulter
CD41		P2	Coulter
CD56		Leu-19	B.D.
CD114	G-CSFR	LMM741	Pharmingen
CD116	GM-CSFRa	SCO4	Coulter
CD122	IL-2Rb	Mikb1	Nichirei
CD123	IL-3Ra	9F5, 7G3	Pharmingen
CD124	IL-4Ra	S456C9	Coulter
CD125	IL-5Ra	A14	Pharmingen
CD126	IL-6Ra	M91	Coulter
CD203c		97A6	*
	AC133	AC133/1	Miltenyi
	HLA-DR		B.D.
	GPA		Coulter
	cMPL		*
	FceR1	CRA1	Kyokuto
	MPO		Caltag
	EP	SF25.5	Nichirei
	MBP	BMK-13	Nichirei
	Histamine	D2	Biogenesis

B.D., Becton Dickinson; GPA, Glycophorin A; MPO, myeloperoxidase;
EP, eosinophil peroxidase; MBP, major basic protein.
Nichirei, Tokyo, Japan; Kyokuto Pharmaceutical, Ibaraki, Japan.
*97A6 and anti-cMPL were kind gifts as written in the text.

Table 2

Table 2 Surface phenotype analysis for leukemia diagnosis

			early markers			T-cell markers						B-cell markers						Myeloid markers							Other markers			
			CD34	CD38	AC133	CD1	CD2	CD3	CD4	CD7	CD8	CD10	CD19	CD20	CD21	CD22	CD23	CD33	CD13	CD14	CD15	CD36	CD41	GPA	CD56	CD25	CD122	HLA-DR
MPO(+)/myeloid																												
	KY821	AML(M2)	(-)	(+)	(-)	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(+)	(-)	(+)	(-)	(-)	(-)	(-)	(+)	(-)	(+)
	NB4	t(15;17)	(-)	(+)	(-)	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(+)	(-)	(-)	(-)	(+)	(-)	(-)	(-)
	HT93	t(15;17)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(+)	(-)	(-)	(-)	(+)	(-)	(-)	(-)
	HL60	APL	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(+)	(-)	(-)	(-)	(+)	(-)	(-)	(-)
MPO(-)/myeloid																												
	KAZZ	CML/BC	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(-)	(+)	(-)	(-)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)
	KU812F	CML/BC	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(-)	(+)	(+)	(-)	(-)	(+)	(-)	(-)
	C2F8	CML/BC	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(+)	(+)	(-)	(-)	(-)	(+)	(-)	(+)
	B4D6	CML/BC	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)
	UT7-GM	AMgkL	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)
	HEL	EL	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(-)
	CMK11.5	AMgkL	(+)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(-)	(+)	(+)	(-)	(-)	(-)	(-)	(-)
	Meg01	CML/BC	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(-)	(+)	(+)	(-)	(+)	(+)	(+)	(-)	(-)	(+)	(-)	(-)
	TK91	CML/BC	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)
B/lymphoid																												
	YS-1	CML/BC	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(-)	(+)	(-)	(-)	(+)	(-)	(-)	(-)	(+)
	ALL/MIK	Ph1-ALL	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
	TOM-1	Ph1-ALL	(-)	(+)	(-)	(-)	(+)	(-)	(+)	(+)	(-)	(+)	(-)	(-)	(-)	(-)	(+)	(+)	(+)	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(+)	(-)
	WK93	cALL	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(+)	(+)	(+)	(-)	(+)	(+)	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
	WH94	t(1;19)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(+)	(-)	(+)	(-)	(-)	(+)	(-)	(+)	(-)	(-)	(+)	(-)	(-)	(-)	(+)
	BPS99	t(1;19)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(+)	(-)	(+)	(-)	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
	Raji	Burkitt's	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
	Daudi	Burkitt's	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(+)
T/lymphoid																												
	Molt4	T-ALL	(+)	(+)	(-)	(+)	(+)	(-)	(-)	(+)	(+)	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	Molt3	T-ALL	(-)	(+)	(-)	(+)	(+)	(-)	(-)	(+)	(+)	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	TIM96	T-ALL	(-)	(+)	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)
	CCRF/CEM	T-ALL	(-)	(+)	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)

(-), negative; (+), positive

Table 3

Table 3 Basophil-related phenotype expression in leukemia cell lines

		CSF receptors						Cytoplasmic antigens				Other surface antigens			QRT-PCR	RT-PCR	
		CD114	CD116	CD123	CD124	CD125	CD126	MPO	MBP	EP	Hist	cMPL	CD203c	FceR1a	FceR1a	FceR1a	CD22
MPO(+)/myeloid																	
	KY821	(+)	(-)	(+)	(-)	(-)	(+)	**(+, 1.5R)**	**(+, 59.4%)**	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	NB4	(+)	(-)	(+)	(-)	(-)	(+)	**(+, 4.0R)**	**(+, 35.7%)**	**(+, 0.7%)**	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	HT93	(+)	(-)	(+)	(-)	(-)	(+)	**(+, 3.9R)**	**(+, 90.4%)**	**(+, 41.7%)**	(-)	(-)	(-)	(-)	(-)	(-)	(+)
	HL60	(-)	(-)	(-)	(-)	(+)	(+)	**(+, 4.0R)**	**(+, 90.8%)**	**(+, 6.8%)**	(-)	(-)	(-)	(-)	(-)	(-)	(-)
MPO(-)/myeloid																	
	KAZZ	(-)	(-)	(-)	(+)	(-)	(-)	(-)	**(+, 90.8%)**	(-)	**(+, 4.7%)**	(-)	**(+, 43.9R)**	**(+,1.4R)**	**(+, 0.0686C)**	(+)	(-)
	KU812F	(-)	(-)	(+)	(+)	(-)	(+)	(-)	**(+, 18.6%)**	**(+, 3.4%)**	**(+, 6.3%)**	(-)	**(+, 32.9R)**	**(+, 1.8R)**	**(+, 0.396C)**	(+)	(-)
	C2F8	(-)	(+)	(+)	(-)	(-)	(-)	(-)	**(+, 7.8%)**	**(+, 0.6%)**	(-)	(-)	**(+, 3.1R)**	**(+, 5.8R)**	**(+, 14.2C)**	(+)	(-)
	B4D6	(-)	(-)	(+)	(+)	(-)	(-)	(-)	**(+, 7.4%)**	(-)	(-)	(-)	**(+, 18.9R)**	**(+, 2.5R)**	**(+, 0.0912C)**	(+)	(+)
	UT7-GM	(-)	(+)	(+)	(-)	(-)	(-)	(-)	**(+, 18.4%)**	(-)	(-)	**(+, 1.9R)**	(-)	**(+, 12.1R)**	**(+, 2.25C)**	(+)	(+)
	HEL	(-)	(-)	(-)	(+)	(-)	(-)	(-)	**(+, 1.7%)**	(-)	(-)	**(+, 1.3R)**	(-)	**(+, 17.2R)**	**(+, 0.602C)**	(+)	(-)
	CMK11.5	(-)	(-)	(+)	(+)	(-)	(+)	(-)	**(+, 6.0%)**	(-)	(-)	**(+, 1.5R)**	**(+, 1.4R)**	**(+, 8.0R)**	**(+, 28.0C)**	(+)	(-)
	Meg01	(-)	(-)	(-)	(+)	(-)	(+)	(-)	**(+, 0.3%)**	(-)	(-)	(-)	**(+, 18.1R)**	**(+, 1.8R)**	(-)	(+)	(+)
	TK91	(-)	(+)	(+)	(+)	(-)	(-)	(-)	**(+, 1.2%)**	(-)	(-)	(-)	(-)	**(+, 1.4R)**	**(+, 7.04C)**	(+)	(+)
B/lymphoid																	
	YS-1	(-)	(+)	(+)	(+)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	**(+, 1.5R)**	(-)	(+)	(+)
	ALL/MIK	(-)	(+)	(+)	(+)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
	TOM-1	(-)	(+)	(+)	(+)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
	WK93	(-)	(-)	(+)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	**(+, 1.2R)**	(-)	(+)	(+)
	WH94	(-)	(-)	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
	BPS99	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)
	Raji	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)
	Daudi	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)
T/lymphoid																	
	Molt4	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	Molt3	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	TIM96	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	CCRF/CEM	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Mature granulocyte																	
	Neutrophil	(+)	(+)	(-)	(+)	(-)	(+)	**(+, 34.0R)**	(-)	(-)	(-)	(-)	(-)	(-)	NT	NT	NT
	Eosinophil	(-)	(+)	(+)	(-)	(+)	(+)	(-)	**(+, 98%)**	**(+, 95%)**	(-)	(-)	(-)	(-)	NT	NT	NT
	Basophil	(-)	(+)	(+)	(-)	(+)	(+)	(-)	**(+, 91%)**	**(+, 37%)**	**(+, 74%)**	(-)	**(+, 21.3R)**	**(+, 23.7R)**	**(+, *38.8C)**	(+)	(+)

(-), negative; (+), positive; (%), % of positive cells; (R), relative mean fluorescence intensity, a ratio obtained by comparison with isotype control;
(C), relative copy number of mRNA measured using quantitative real time RT-PCR (QRT-PCR), a ratio obtained by comparison with copy number of a house keeping gene, PBGD.
MPO (myeloperoxidase); MBP (eosinophil major basic protein); EP (eosinophil peroxidase); Hist (histamine); FceR1-a (FceR1 a-chain).
*, measured in purified human bone marrow basophils (purity, 80.8%)

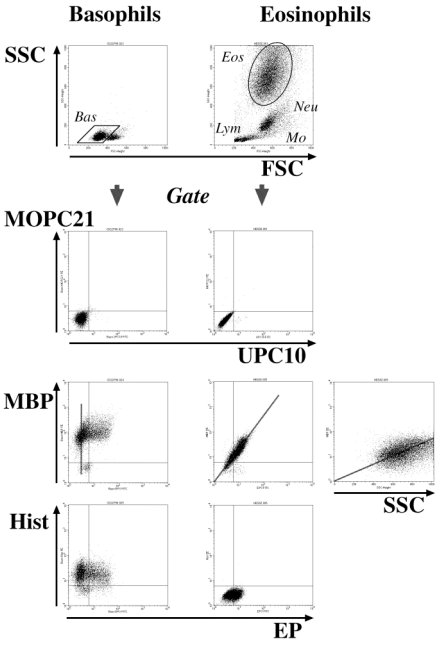

Fig. 1 Flow cytometric pattern of the eosinophil/basophil-related antigen expression in human blood eosinophils and basophils. MBP (major basic protein); Hist (histamine); EP (eosinophil peroxidase); SSC (side-scattering); FSC (forward-scattering); MOPC21 (mouse myeloma protein IgG1); UPC10 (mouse myeloma protein IgG2a); Bas (basophils); Eos (eosinophils); Neu (neutrophils); Mo (monocytes); Lym (lymphocytes).

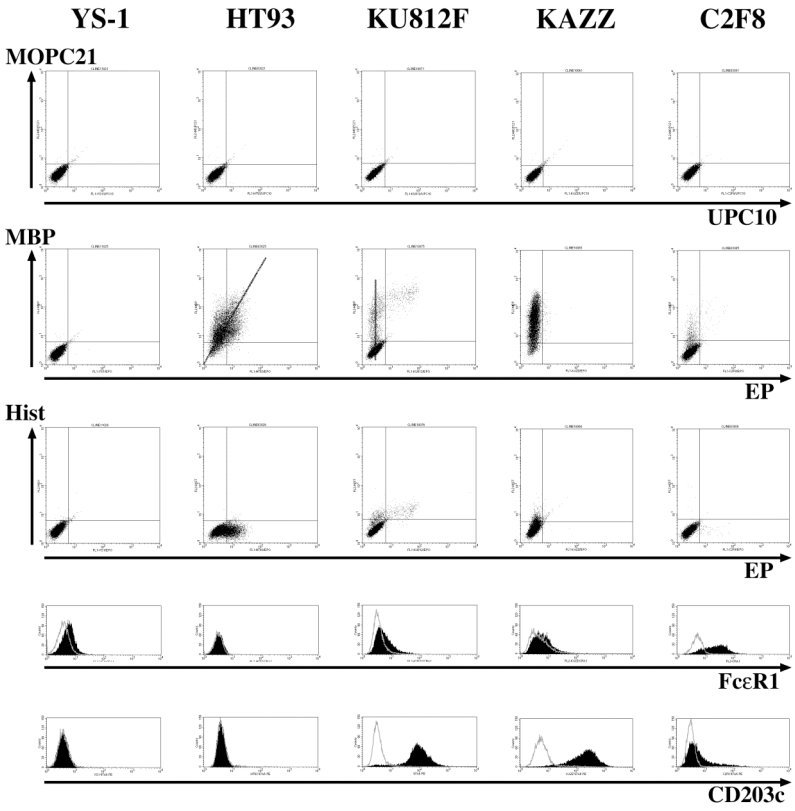

Fig. 2 Flow cytometric pattern of the eosinophil/basophil-related antigen expression in 5 leukemia cell lines, YS-1 (Ph1-positive immature B-cell), HT93 (MPO-positive t(15;17) leukemia), KU812F (Ph1-positive immature basophil), KAZZ (Ph1-positive immature erythroid), and C2F8 (Ph1-positive immature megakaryocytic). MBP (major basic protein); Hist (histamine); EP (eosinophil peroxidase); MOPC21 (mouse myeloma protein IgG1); UPC10 (mouse myeloma protein IgG2a).

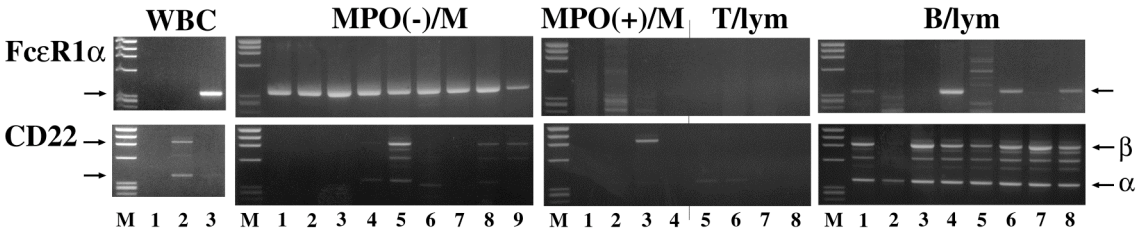

Fig. 3 RT-PCR analyses for the α-subunit of FcεR1 and CD22. Lane M, markers (1,353, 1,078, 872, 603, 310, 281/271 and 234 bp). Lanes 1, 2 and 3 of WBC, T cells, B cells and basophils purified from normal human blood, respectively. Lanes 1 to 9 of MPO(-)/M, cell lines KAZZ, KU812F, C2F8, B4D6, UT7-GM, HEL, CMK11-5, Meg-01 and TK91, respectively. Lanes 1 to 4 of MPO(+)/M and 5 to 8 of T/lym, cell lines KY821, NB4, HT93, HL60, Molt4, Molt3, TIM96 and CCRF/CEM, respectively. Lanes 1 to 8 of B/lym, cell lines YS-1, ALL/MIK, TOM-1, WK93, WH94, BPS99, Raji and Daudi, respectively.

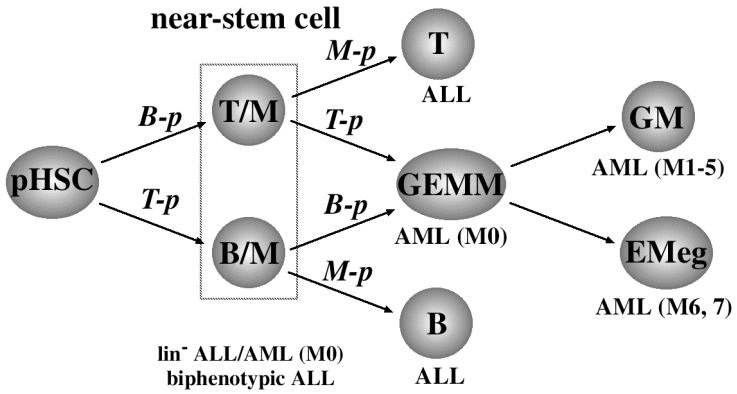

Fig. 4 A model for the virtual hematopoietic progenitors and acute leukemia. *B-p*, *T-p* and *M-p*, prohibition against B, T and myeloid-lineages, respectively. pHSC, pluripotent hematopoietic stem cells; T/M and B/M, bipotent progenitors of myeloid and T or B-lineage, respectively; GEMM and GM, CFU-GEMM and -GM, respectively; EMeg, erythromegakaryocytic progenitor. Note that this scheme is simplified by disregarding unclassified lineages as pDC and others.