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References

1. Bilgrami S, Feingold JM, Dorsky D, et al. Incidence and outcome of Clostridium difficile infection following autologous peripheral blood stem cell transplantation. Bone Marrow Transplant 1999; 23:1039-42.
2. Wingard JR, Yen-Hung Chen D, Burns WH, et al. Cytomegalovirus infection after autologous bone marrow transplantation with comparison to infection after allogeneic bone marrow transplantation. Blood 1988; 71:1432-7.
3. Sakr M, Hassanein T, Gavaler J, et al. Cytomegalovirus infection of the upper gastrointestinal tract following liver transplantation-incidence, location and severity in cyclosporine and FK506- treated patients. Transplantation 1992; 53:786-91.
4. Lund MB, Simonsen S, Foerster A, Rollag H, Geiran O. Gastrointestinal cytomegalovirus infection in patients with heart transplants. Tidsskr Nor Laegeforen 1991; 111:3385-7.
5. Michiels JF, Saint-Paul MC, Hofman P, et al. Cytological study of the duodenal mucosa during chronic diarrhea in HIV-positive patients. Gastroenterol Clin Biol 1993; 17:277-82.
6. Oshima Y, Nishida K, Kawazoye S, et al. Successful treatment of cytomegalovirus colitis with ganciclovir in a patient with adult T-cell leukaemia lymphoma:case report. J Chemother 1999; 11:215-9.
7. Bode U,Pizzo PA. Association of cytomegalovirus with acute colitis in a patient with lymphoma. Am J Pediatr Hematol Oncol 1979; 1:185-7.

Expression of CD34 by megakaryocytes in myelodysplastic syndromes

We report that increased numbers of megakaryocytes in bone marrow biopsies from patients with myelodysplastic syndromes express the CD34 antigen. A significant correlation was observed between CD34 positivity on megakaryocytes and a low platelet count, as well as the occurrence of cytogenetic abnormalities.

Sir,

The CD34 antigen is expressed by hematopoietic progenitors, endothelial cells and bone marrow (BM) stromal precursors.[1] In myelodysplastic syndromes (MDS) the number of CD34$^+$ blasts varies widely, exceeding in most cases the normal threshold of 1%,[2] and their number has been associated with an unfavorable clinical outcome.[2] Studying BM biopsies we noticed that in MDS, CD34 positive cells included megakaryocytes (Mks); this phenomenon was

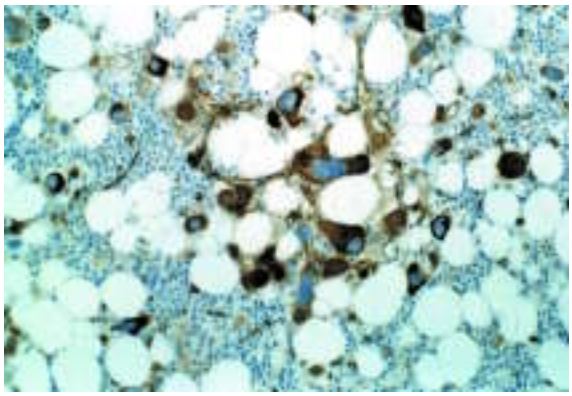

Figure 1. Bone marrow biopsy in a case of MDS, stained for CD34: the numerous dysplastic megakaryocytes, including several micro-megakaryocytes, are strongly CD34 positive. Note that only a few scattered CD34$^+$ small blasts are present in the biopsy.

analyzed in detail in 57 BM biopsies obtained from 22 patients with MDS (diagnosed and classified according to FAB criteria)[3] (Table 1) and compared with biopsies from age-matched patients without hematologic disorders (19 cases) and from patients with idiopathic thrombocytopenic purpura (ITP)(9 cases). In normal marrows and in the ITP patients rare mature Mks were found to express CD34, but only in 1

Table 1. Clinical and cytogenetic features, and bone marrow findings in patients with myelodysplastic syndrome.

Pts	Gender/ age (yrs)	Diagnosis at onset (FAB)*	Plts (10^9/L)°	Cytogenetic characteristics	BM biopsy: CD34$^+$ small immature cells (%)	BM biopsy: CD34$^+$ Mks on the total no. of Mks (%)
1#	F/65	RA	56	+8	15	10
2#	M/62	RA	285	Normal	70	3
3#	M/60	RAEB-T	91	Normal	4	60
4#	F/55	RAEB-T	55	t(14;14)	30	5
5#	M/65	RAEB	88	nd	1	0
6#	F/49	RAEB-T	24	-5	0	0
7	M/48	RAEB-T	38	Normal	15	95
8#	F/71	RA	96	nd	1	0
9	M/72	RAEB-T	215	nd	15	8
10#	M/53	RA	179	nd	0	0
11	M/76	RAEB-T	28	4p+/der(12)/mar	10	25
12	F/89	RAEB-T	23	Complex rearrangements	4	30
13	F/73	RA	120	+8	25	60
14	F/70	RA	76	Normal	0	0
15	F/80	RA	140	5q-	25	15
16	F/85	RARS	242	Normal	0	2
17#	M/31	RAEB-T	13	t(5;9)	10	10
18	M/76	RAEB-T	71	nd	15	10
19	M/75	RARS	6	der(5)/-7/12p-/ -19/miscellaneous defects	1	10
20	F/75	RA	301	Normal	0	0
21	F/69	RA	na	nd	0	2
22#	M/42	RAEB	16	Normal	0	0

*#patients with multiple biopsies; *RA: refractory anemia, RARS: refractory anemia with ringed sideroblasts, RAEB: refractory anemia with excess of blasts, RAEB-T: RAEB in trasformation; °values at the time of first bone marrow biopsy; Pts: patients; Plts: platelets; nd: not determined.*

out of 19 normal cases and 0/9 ITP cases did more than 10% of Mks express CD34; in contrast, 10 of 22 patients with MDS had 10% or more CD34+ Mks in their BM (Fisher's exact test: p=0.004 and 0.03, MDS versus normal controls and ITP, respectively); in three MDS cases more than 50% of Mks expressed CD34 and in a single case the vast majority of them were CD34 positive (Figure 1). The CD34+ Mks in MDS included mature forms as well as dysplastic and small Mks. Using immunostains on serial sections, we found that the CD34+ Mks also expressed FVIII-RA and CD61 (data not shown). *In vitro* studies have demonstrated that CD34 expression on Mks is limited to earlier progenitor cells and declines during maturation, while CD61 expression progressively increases;[4,5] in normal conditions, the CD34+CD61+ phenotype is found on a small subset of Mks precursors, morphologically identifiable as immature blasts.[4,6] Late Mks precursors still expressing CD34 have been recovered from peripheral blood of patients with acute myeloid leukemia after intensive chemotherapy,[7] and peripheral blood CD34+ stem cells from normal individuals contain a minor subset that expresses CD61.[8] The rare mature CD34+ Mks we found in a minority of biopsies from normal individuals might correspond to these circulating Mks precursors. In MDS the CD34 positivity on Mks correlated with the platelet count (mean platelet count of patients with <10% and ≥ 10% CD34+ Mks: 143.4±31.5 and 58.6±14.6; unpaired t test: p=0.03). This observation suggests that the CD34+ CD61+ FVIII-RA+ Mks in MDS represent neoplastic Mks showing asynchronous phenotypic differentiation and poor platelet production; the very low numbers of CD34+ Mks occurring in ITP, a condition characterized by effective thrombocytopoiesis and increased number of marrow Mks, further supports this hypothesis. The clinical significance of CD34+ Mks in MDS is unclear. Patients with a high percentage of CD34+ Mks showed a higher frequency of marrow karyotype abnormalities, which are usually associated with a more aggressive course of disease.[9] However, the number of CD34+ Mks appeared to be unrelated to the number of CD34+ small immature cells, with the different FAB subclasses and with the clinical evolution of the disease.

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Key words

CD34, megakaryocytes, myelodysplasia, bone marrow, immunohistochemistry.

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References

1. Civin C, Gore S. Antigenic analysis of hematopoiesis: a review. J Hematother 1993; 2:137-44.
2. Oriani A, Annaloro C, Soligo D, Pozzoli E, Cortelezzi A, Lambertenghi D. Bone marrow histology and CD34 immunostaining in the prognostic evaluation of primary myelodysplastic syndromes. Br J Haematol 1996; 92:360-4.
3. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 1982; 51:189-99.
4. Debili N, Issaad C, Massè JM, et al. Expression of CD34 and platelet glycoproteins during human megakaryocytic differentiation. Blood 1992; 80:3022-35.
5. Ayala I, Tomer A, Kellar K. Flow cytometric analysis of megakaryocyte-associated antigens on CD34 cells and their progeny in liquid culture. Stem Cells 1996; 14: 320-9.
6. Gehling UM, Ryder JW, Hogan CJ, et al. Ex-vivo expansion of megakaryocyte progenitors: effect of various growth factor combinations on CD34+ progenitor cells from bone marrow and G-CSF-mobilized peripheral blood. Exp Hematol 1997; 25:1125-39.
7. Delfini C, Centis F, Annibali M. Whole megakaryocytes are present among CD34+ cells in the peripheral blood of patients with acute myeloid leukemia after intensive chemotherapy. Haematologica 1996; 81: 284-5.
8. Bojko P, Hester JP, Durett AG, Maadani F, Körbling M, Champlin RE. Identification of megakaryocyte precursors in peripheral blood stem cell collections from normal donors. J Clin Apheresis 1998; 13:7-15.
9. Sanz GF, Sanz MA, Greenberg PL. Prognostic factors and scoring systems in myelodysplastic syndromes. Haematologica 1998; 83:358-68.

Kinetics variation of CD34+ and CD34+CD90+ in subjects following different mobilizing protocols

Since CD34+/CD90+ cells could represent an index of primitive hematopoietic progenitors, we designed a study to analyze the yield of CD34+/CD90+ cells during leukapheresis courses, in response to different mobilization regimens employed in healthy donors and in patients with hematologic neoplasias for allogeneic and autologous hematopoietic stem cell transplantation, respectively.