Circulating micromegakaryocytes in myelodysplasia

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SUMMARY The alkaline phosphatase-antialkaline phosphatase (APAAP) immunocytochemical staining technique was used to look for circulating cells of megakaryocyte lineage in peripheral blood smears from 67 cases of myelodysplasia. Small numbers of micromegakaryocytes positive for platelet glycoprotein IIIa were found in 23 cases. These cells superficially resemble small lymphoid cells and are hence difficult or impossible to recognise in conventional Romanowsky stained smears. Circulating micromegakaryocytes were found most commonly in more aggressive types of myelodysplasia (such as refractory anaemia with excess blasts (RAEB) and refractory anaemia with excess blasts in transformation (RAEB-t)), and their presence may therefore indicate a poor prognosis. Because of the simplicity of this immunocytochemical labelling technique, it could be of wide use in the initial assessment of patients with myelodysplasia, and possibly for the early detection of acute leukaemic transformation.

Myelodysplasia usually presents in elderly patients with a combination of peripheral blood cytopenias and dysplastic changes in both blood and bone marrow (for example, Pelger-Huet anomalies, dysmegakaryopoiesis). The natural history of myelodysplasia is variable: some patients enjoy a prolonged survival; in others the disease rapidly transforms into frank leukaemia within a year of diagnosis.^{1 2}

The unpredictable clinical behaviour of myelodysplasia prompted the FAB cooperative group to define criteria for classifying it into five categories: refractory anaemia (RA), refractory anaemia with ring sideroblasts (RAS), refractory anaemia with excess of blasts (RAEB), chronic myelomonocytic leukaemia (CMML), and RAEB in transformation (RAEB-t).³ Subsequently haematological features of myelodysplastic syndrome have been analysed with the aim of providing prognostic and therapeutic guidelines.¹ Reported indicators of a poor prognosis include the presence of abnormally localised immature myeloid precursors (ALIPs) in marrow trephine biopsy specimens, circulating blast cells, and an excess of blast cells in the bone marrow smear.

This study describes a new and easily evaluated haematological feature of myelodysplasia which may be of prognostic value. It is possible to detect circulating micromegakaryocytes in a proportion of patients with myelodysplasia by staining routine

blood smears immunocytochemically with monoclonal antibodies to platelet glycoprotein IIIa. These cells are virtually undetectable in conventionally stained smears (because of their rarity and lack of distinct morphological features), but stand out clearly in immunoalkaline phosphatase labelled blood smears.⁴

Material and methods

Peripheral blood (edetic acid or heparin anticoagulated) and bone marrow samples were obtained from patients with myelodysplasia attending the following hospitals: the John Radcliffe Hospital, Oxford; the University Hospital of Wales, Cardiff; the Royal Victoria Hospital, Bournemouth; and Stoke Mandeville Hospital, Aylesbury. Peripheral blood and bone marrow smears were prepared as for routine haematological examination, and buffy coat smears were prepared following centrifugation in a Wintrobe tube. Buffy coat smears were also made from 10 haematologically normal patients with other disorders (age range 18 to 78 years, mean 58.6 years) attending the John Radcliffe Hospital. All samples were air dried at room temperature for six to 18 hours before immunocytochemical labelling or stored at -20°C, wrapped in aluminium foil.

ANTIBODIES

The monoclonal antibodies C17⁵ (antiplatelet gpIIb/IIIa) and Y-2/51 (antiplatelet gpIIIa from our

own laboratory) were used (as ascitic fluid diluted to 1/1000 and as undiluted tissue culture supernatant, respectively). Rabbit antimouse immunoglobulin was obtained from Dakopatts a/s. Alkaline phosphatase-antialkaline phosphatase (APAAP) complexes were prepared in the authors' laboratory⁶ and are available from Dakopatts a/s.

IMMUNOCYTOCHEMICAL LABELLING

Immediately prior to labelling, slides that had been stored at -20°C were brought to room temperature before unwrapping. All slides were fixed in acetone:methanol:formalin (19:19:2) for 90 seconds and immediately transferred to 0.05M, pH 7.6, "Tris" buffered saline. Immunocytochemical labelling was then performed by the APAAP labelling technique as previously described.⁷

Negative control staining was performed by omit-

ting either the primary monoclonal antibody or subsequent stages of the labelling procedure. No positive staining (of platelets or nucleated cells) was seen in the negative control preparations.

Results

The two antiplatelet glycoprotein IIIa antibodies gave strong staining of platelets in blood and bone marrow smears from both normal subjects and patients. No other cells were labelled in any of the 10 normal buffy coat smears studied.

In bone marrow smears from the patients with myelodysplasia normal megakaryocytes and those showing dysmegakaryopoietic features (including large mononuclear and multinucleated megakaryocytes) were easily visualised with both antibodies (figs 1a and b). In addition, antigen positive

Fig 1 Abnormal megakaryocytic maturation (dysmegakaryopoiesis) shown by staining bone marrow smears from cases of myelodysplasia with antiplatelet glycoprotein IIb/IIIa or IIIa. (1a) Positively labelled large mononuclear megakaryocyte and small micromegakaryocyte (arrowed) can be seen (antibody C17). (1b) Two abnormal megakaryocytic cells are shown (antibody Y-2/51). (1c) Positive staining of a small round micromegakaryocyte (superficially resembling an atypical lymphoid cell—arrowed) and many abnormal large platelets (antibody C17).

micromegakaryocytes, superficially resembling lymphocytes, were detected in 16 of 17 (94%) marrow smears. These cells had small round nuclei and very scant and often ragged cytoplasm (fig 1c).

In 23 of 67 (34%) peripheral blood samples from patients with myelodysplastic syndrome micromegakaryocytes positive for glycoprotein IIIa were detected with both monoclonal antibodies (table). These cells were seen in smears of whole blood (five of

19 cases or 26%) but could be detected more commonly (because of the higher concentration of nucleated cells) in buffy coat preparations (18 of 48 cases or 38%). The numbers of cells varied from patient to patient and even in buffy coat, smears did not exceed a few dozen per slide—that is, less than 1% of nucleated cells.

The positive cells had a variety of morphological appearances (figs 2a–e). Most commonly they

Table Correlation between the presence of circulating micromegakaryocytes and morphological subtypes in myelodysplasia

FAB subtype	No of cases studied	Prevalence of circulating micromegakaryocytes (%)
Refractory anaemia (RA)	12	1 (8)
Refractory anaemia with sideroblasts (RAS)	16	3 (18)
Chronic myelomonocytic leukaemia (CMML)	14	4 (28)
Refractory anaemia with excess blasts (RAEB)	21	11 (52)
Refractory anaemia with excess blasts in transformation (RAEB-t)	4	4 (100)
Total No of cases	67	23 (34)

Fig 2 Peripheral blood smears from cases of myelodysplasia stained for antiplatelet glycoprotein IIIb/IIIa or IIIa showing different morphological types of micromegakaryocytes. (2a) The positively labelled small round micromegakaryocyte (arrowed) is seen (C17). Note antigen negative white cells and positive platelets. (2b) Small round lymphoid-like cell with scant cytoplasm (Y-2/51). (2c) Small round cell with prominent villous projections (Y-2/51). (2d) Larger blast-like cell (Y-2/51). (2e) Large cell with folded nucleus, little cytoplasm, and adherent platelets (C17).

superficially resembled atypical lymphoid cells with round nuclei, condensed chromatin, and very scant ragged or budding cytoplasm (figs 2a and b); less commonly they were larger and had the appearance of blast cells (fig 2d), or possessed large lobulated nuclei and lacked cytoplasm (fig 2e).

When the FAB subtype of individual cases was analysed, it was found that circulating micromegakaryocytes were most commonly seen in RAEB and RAEB-t (table).

Discussion

Circulating megakaryocytes were found in about one third of patients with myelodysplasia. These cells would usually be overlooked in routinely stained blood smears because of their low numbers and non-descript morphology. It was hence only by immunocytochemical labelling, using monoclonal antibodies to platelet glycoprotein IIIa, that these cells could be recognised. The necessity to use this type of technique to detect these cells is corroborated by the contrast between our own findings and the observation (based on conventional microscopy) of the FAB group that "rarely micromegakaryocytes can be recognised in the peripheral blood."³

The circulating cells positive for platelet glycoprotein seen in myelodysplasia are similar in morphology to the micromegakaryocytes we have previously detected (also by means of the APAAP staining technique) in the circulation of some cases of acute myeloblastic leukaemia⁴ and other myeloproliferative disorders (such as chronic granulocytic leukaemia and essential thrombocythemia; unpublished observations). The present study sheds light on the origin of the circulating micromegakaryocytes seen in patients with myelodysplasia as immunocytochemical labelling of bone marrow smears revealed, in addition to the previously described dysmegakaryopoietic features (such as multinucleated and abnormally large mononuclear megakaryocytes),^{3,8} many micromegakaryocytes.

In the present study circulating micromegakaryocytes were most commonly detected in cases of the RAEB and RAEB-t FAB subtypes of myelodysplastic syndrome (table). It has previously been noted that these two diagnostic groups are most often associated with dysmegakaryopoietic features in the marrow⁹ and transformation to acute leukaemia.^{2,10} The "spill-over" of micromegakaryocytes from the marrow into the circulation in cases of RAEB and RAEB-t might possibly reflect the more aggressive nature of these forms of myelodysplasia.

In the other subgroups of myelodysplastic syndrome (RA, RAS, and CMML) circulating micromegakaryocytes were less commonly seen (table). It

will be of interest to see whether the appearance of circulating micromegakaryocytes in these myelodysplastic syndrome subtypes with better prognosis² is of clinical importance—that is, heralds transition to more aggressive forms of myelodysplasia (RAEB or RAEB-t) or to acute leukaemia. If such a correlation is found regular immunocytochemical screening of peripheral blood samples for circulating micromegakaryocytes in patients with myelodysplastic syndrome would be warranted in their routine clinical management.

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