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SHORT REPORT

Ploidy in bone marrow cells from healthy donors: a MAC (morphology antibody chromosomes) study

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Summary. The ploidy of human bone marrow cells belonging to the megakaryocytic, granulocytic-monocytic and erythrocytic lineages was studied by *in situ* hybridization using the biotin-labelled Y chromosome-specific DNA probe pY431 and the chromosome 1-specific probe pUC1.77 on cells identified morphologically and immunologically by the MAC procedure. Cells of the granulocytic-monocytic and erythrocytic lineages were seen to be 2N in ploidy, whereas the ploidy of the megakaryocytic lineage ranged from 2N to 32N, with the ploidy classes 4N and 8N being predominant. The frequency of megakaryocytes with 2N chromosomes was also high.

Keywords: ploidy, bone marrow cells, morphology antibody chromosomes technique.

Megakaryocytes account for less than 1% of all bone marrow cells. Megakaryocytes are originally diploid cells which undergo nuclear doubling without cytoplasmic partition (endomitosis), resulting in increased ploidy. Microdensitometric quantification of DNA content in Feulgen-stained megakaryocytes has yielded ploidies of 4N, 8N, 16N, 32N and 64N (Weste & Penington, 1972). However, the lower levels of polyploidy (<8N) have been difficult to evaluate previously since these cells cannot be readily distinguished from other bone marrow cell lineages by morphological criteria.

We used the MAC (Morphology Antibody Chromosomes) technique (Knuutila *et al*, 1993; Knuutila & Teerenhovi, 1989) to study ploidy level in different cell lineages from bone marrow. The cells were characterized using immunocytochemical staining and their ploidy was determined by interphase cytogenetics using *in situ* hybridization with a Y chromosome-specific and a chromosome 1-specific DNA probe.

MATERIALS AND METHODS

Human bone marrow aspirates were obtained from four karyotypically normal healthy men and one woman (aged 20–35 years) acting as bone marrow donors to leukaemic recipients (Helsinki University Central Hospital, Helsinki, Finland). Chromosome analysis was performed by standard cytogenetic methods.

Mononucleated bone marrow cells were isolated by one-step density gradient centrifugation in Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden). MAC slide preparations of mononucleated cells were made as described elsewhere (Knuutila & Teerenhovi, 1989). The phenotype of the interphase cells was determined by a modified alkaline phosphatase anti-alkaline phosphatase (APAAP) method using monoclonal antibodies and Giemsa counterstaining (Knuutila & Teerenhovi, 1989). The monoclonal antibodies Y2/51 (CD 61, provided by Dr D. Y. Mason, Oxford, U.K.), anti-glycophorin-A (GPA, provided by Dr Griffin, Boston, Mass., U.S.A.) and MY7 (CD13, Coulter Immunology, Hialeah, Fla., U.S.A.) were used to detect cells belonging to the megakaryocytic, erythrocytic and granulocytic/monocytic cell lineages, respectively.

All positive cells were located and photographed for each monoclonal antibody, except in cases 4 and 5, where the *in situ* hybridization was performed directly on the immunophenotyped cells without removal of stain (Knuutila *et al*, 1993). The APAAP stain was in patients 1–3 removed by incubating the slides in methanol : acetic acid (3 : 1, 3–5 h), and the slides

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Table I. Percentages of antibody-positive bone marrow cells with various numbers of hybridization signals for the biotin-labelled Y chromosome-specific probe pY431 and the chromosome 1-specific probe pUC1.77.*

Donor	Cell lineage†	Percentage of cells with various numbers of hybridization signals of the Y-specific probe										
		0	1	2	3	4	5	6	7	8	9	15–17
1	Erythrocytic, GPA+	4	96									
	Granulocytic/monocytic, CD13+	10	88	2								
	Megakaryocytic, CD61+	9	12	28		32		1		14		4
2	Erythrocytic, GPA+	9	91									
	Granulocytic/monocytic, CD13+	7	93									
	Megakaryocytic, CD61+	19	17	40		19		1		4		
3	Erythrocytic, GPA+	8	92									
	Granulocytic/monocytic, CD13+	7	92	1								
	Megakaryocytic, CD61+	7	15	24	2	28	1	1	2	16	1	3

Donor	Cell lineage†	Percentage of cells with various numbers of hybridization signals of the chromosome 1-specific probe																	
		0–1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18–26
4	Megakaryocytic, CD61+	36	20	5·4	16	1·5	3·7	2·5	8	0·1	0·9	0·8	1·5		0·1	0·9	1·5	0·1	0·75
	CD61−	9·5	89	1·5															
5	Megakaryocytic, CD61+	16·6	43	6·6	20	6·6	3·3	3·3	3·3			3·3	3·3	3·3				3·3	
	CD61−	8·9	91	0·1															

* For all donors but no. 5 at least 500 nucleated cells were analysed for each cell lineage. For CD61+ cells of patient 5, only 34 cells were analysed.

† The following monoclonal antibodies were used: anti-GPA for erythrocytic lineage; MY7 for CD13+ granulocytic/monocytic lineage; Y2/51 for CD61+ megakaryocytic lineage.

were air-dried at room temperature before *in situ* hybridization.

The Y-specific DNA probe pY431 recognizes a 2·1 kb Hae III repeated DNA (kindly provided by Dr K. Smith, Howard Hughes Medical Institute, Johns Hopkins University, Md., U.S.A.) of the Y chromosome. The chromosome 1-specific probe pUC1.77 (Cooke & Hindley, 1979) recognizes a tandem repeat of 1·77 kb in the pericentromeric region of chromosome 1 (1q12). The probes were labelled by nick-translation using biotin-11-dUTP (Sigma Chemical Co., St Louis, Mich., U.S.A.) and a nick-translation kit (Nick Translation Kit, Bethesda Research Laboratories, Bethesda, Md., U.S.A.) according to the supplier's instructions. Hybridization conditions were as described by Knuutila *et al* (1993). The biotinylated probes were detected indirectly with monoclonal mouse anti-biotin antibody (Dakopatts, Glostrup, Denmark), followed by peroxidase-conjugated monoclonal rabbit anti-mouse antibody and precipitation with diaminobenzidine tetrahydrochlorid acid (DAB, Sigma). The slides were counterstained with haematoxylin.

The immunologically identified interphases were localized, and the number of hybridization signals was determined using a light microscope (Leitz Laborlux D). When possible, at least 500 nucleated cells were analysed for each monoclonal antibody in each donor.

RESULTS AND DISCUSSION

The numbers of hybridization signals in cells of the different lineages are presented in Table I.

Cells of the erythrocytic and granulocytic-monocytic lineages showed a very low frequency of cells with two Y signals ($\leq 2\%$). The frequency of cells with no signal ranged from 4% to 19%, whereas the frequency of cells with one signal was 88–96% (Table I).

In megakaryocytes, the number of Y-signals ranged from 0 to 16, indicating that the highest ploidy in these cells was 32N. Megakaryocytes with two (4N) and four (8N) signals were the most frequent, although high numbers of cells with one signal (2N) were also present (Fig 1A, B). The number of chromosome 1-specific signals in the megakaryocytes ranged from 0 to 26 (Table I; Fig 1C–E). Cells with two (2N), four (4N) and eight (8N) were the most frequent.

Our study supports previous findings indicating that haemopoietic cells other than megakaryocytes are very seldom polyploid in healthy bone marrow. The fact that the ploidy of more than half the megakaryocytes in our study was 8N or less agrees with the findings of Levine (1980) and Bessman (1984), whereas de Leval (1968), Ishibashi *et al* (1986), Tomer *et al* (1988) and Rabellino & Bussel (1990) reported the majority of megakaryocytes to be 16N. Further-

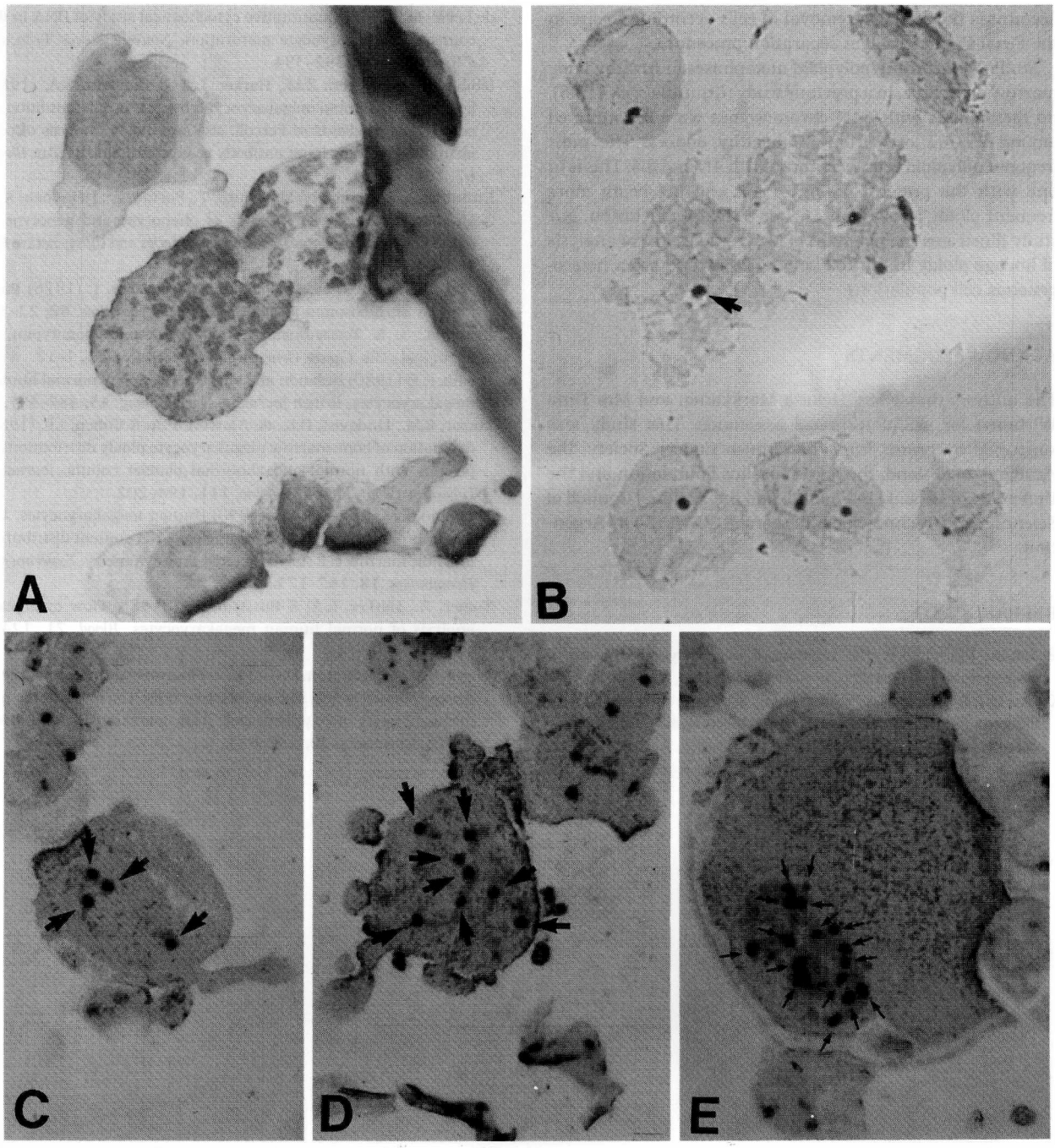

Fig 1. A, C, D, E: CD61-positive metaphase and interphase cells from healthy bone marrow donors. Cytocentrifuge preparations were stained by the alkaline phosphatase anti-alkaline phosphatase (APAAP) method. B: same view as in A after *in situ* hybridization with the Y chromosome-specific DNA probe pY431. The enzymatic detection system revealed one hybridization signal in the CD61-positive metaphase (arrow). C, D, E: *In situ* hybridization with the chromosome 1-specific probe pUC1.77 performed directly on the immunophenotyped cells. Arrows show four signals in a tetraploid (C), eight signals in an octoploid (D), and 16 signals in a 16-ploid megakaryocyte (E).

more. Mazur *et al* (1988) observed a modal megakaryocyte ploidy value of 32N. It should be noted, however, that the procedure used by us may overestimate the number of megakaryocytes of low ploidy, as the possibility of megakaryocytes of high ploidy being the most fragile in the procedure cannot be excluded. Furthermore, as large multilobular megakaryocytes were mostly those cells which did not show hybridization signals, the proportion of megakaryocytes with high ploidy may be underestimated (patients 2, 4 and 5, detailed data not shown). Low hybridization efficiency may also explain the occurrence of cells showing signals other than 2N, 4N, 8N, etc. Another possibility to be taken into

account is the selective removal of cells according to size in the Ficoll-Paque gradient separation procedure.

Studies concerning polyploid metaphases in healthy bone marrow are scarce. In a previous study (Knuutila *et al*, 1976), no metaphases with 64N chromosomes were encountered among 29 062 cells from 20 healthy donors. The most frequent polyploid cells were those with 4N and 8N. This is in line with the present finding of 4N and 8N being more frequent ploidy levels than 16N or 32N. In conclusion, our study illustrates the potential of MAC studies in the analysis of lineage ploidy in normal bone marrow and other heterogeneous cell populations.

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REFERENCES

Bessman, J.D. (1984) The relation of megakaryocyte ploidy to platelet volume. *American Journal of Hematology*, **16**, 161–170.

Cooke, H.J. & Hindley, J. (1979) Cloning of human satellite III DNA: different components are on different chromosomes. *Nucleic Acids Research*, **6**, 3177–3179.

de Leval, M. (1968) Quantitative cytochemical study of DNA in the course of megakaryocyte maturation. *Nouvelle Revue Française d'Hématologie*, **8**, 392–394.

Ishibashi, T., Ruggeri, Z.M., Harker, L.A. & Burstein, S.A. (1986) Separation of human megakaryocytes by state of differentiation on continuous gradients of Percoll: size and ploidy analysis of cells identified by monoclonal antibody to glycoprotein IIb/IIIa. *Blood*, **67**, 1286–1292.

Knuutila, S., Larramendy, M., Ruutu, T., Paetau, A., Heinonen, K. & Mahlamäki, E. (1993) Analysis of phenotype and genotype of individual cells in neoplasms. *Cancer Genetics and Cytogenetics*, **68**, 104–113.

Knuutila, S., Simell, O., Lipponen, P. & Saarinen, I. (1976) Bone marrow chromosomes in healthy subjects. *Hereditas*, **82**, 22–36.

Knuutila, S. & Teerenhovi, L. (1989) Immunophenotyping of aneuploid cells. *Cancer Genetics and Cytogenetics*, **41**, 1–17.

Levine, R.F. (1980) Isolation and characterization of normal human megakaryocytes. *British Journal of Haematology*, **45**, 487–497.

Mazur, E.M., Lindqvist, D.L., de Alarcon, P.A. & Cohen, J.L. (1988) Evaluation of bone marrow megakaryocyte ploidy distributions in persons with normal and abnormal platelet counts. *Journal of Laboratory and Clinical Medicine*, **111**, 194–202.

Rabellino, E.M. & Bussel, J.B. (1990) Human megakaryocytes. VII. Analysis of megakaryocytes for nuclear DNA content distribution in whole marrow cell suspensions by flow cytometry. *Experimental Hematology*, **18**, 167–173.

Tomer, A., Harker, L.A. & Burstein, S.A. (1988) Flow cytometric analysis of normal human megakaryocytes. *Blood*, **71**, 1244–1252.

Weste, S.M. & Penington, D.G. (1972) Fluorometric measurement of deoxyribonucleic acid in bone marrow cells. The measurement of megakaryocytic deoxyribonucleic acid. *Journal of Histochemistry and Cytochemistry*, **20**, 627–633.