

Thursday, July 16, 1981

Poster Presentations

Megakaryocytes - I

11:00-12:30 h

Grand Ballroom Lobby Boards 211-216

0716

MEGAKARYOCYTE SIZES R.F. Levine, V.A. Medical Center, Washington, D.C. 20422

The size range of megakaryocytes (megas) is widely given as 30-100 μm ; however, extensive direct data are lacking. Large numbers of guinea pig (gp) and human megas have been examined at 1000X by phase contrast microscopy with an eyepiece micrometer. In a suspension of gp bone marrow cells megas had an average diameter of 26.0 ± 10.2 (SD) μm with a range of 10-51 (N=1037). Nonmegas had a mean diameter of 8.5 ± 1.9 with a range of 3-17 μm (N=1120). A threshold value of 13 μm was found to separate these two populations with no more than 2% overlap. Electronic size analysis confirmed the above range and distribution of gp mega sizes and the existence of a threshold at 13 μm . To evaluate the sizes of human megas in three marrow aspirates megas were measured on clinical smears and in cell suspensions from the same specimens. The megas in suspension (N=247-289) were 24.0 ± 8.9 μm (range 10-58) and the nonmegas were 9.3 ± 2.0 μm . The smeared cells had two-dimensional diameters of 31.7 ± 10.1 (range 8-82) and 10.8 ± 3.2 , respectively. On the clinical smears the threshold shifted from 13.0 to 18.4 μm (approx. two neutrophil diameters) and separated 93% of the megas from 98% of the nonmegas. Relative DNA content was determined by microdensitometry on Feulgen-stained cells. Megas of different ploidy levels had different size distributions (32N>16N>8N>4N) but 4N megas had the same size range as 4N nonmegas. Within each ploidy class size was related to maturation stage, judged by nuclear configuration. The data indicated that maturation and ploidy state are not completely independent variables. In summary, mega sizes in suspension were mostly 10-50 μm for gp and human samples. On clinical smears some flattening had apparently occurred, but most megas were 10-65 μm . The smaller megas, often missed in past studies, were still larger than most of the nonmegas; the size differences between megas and the other cells are probably related to the higher ploidy levels of the megas. The size variation among the megas is related to both cell maturation and ploidy state.

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PURINE METABOLISM IN MEGAKARYOCYTES AND PLATELETS
R.F. Levine and H.K. Webster, Hematology Section, VA Medical Center, and Dept. of Hematology, Walter Reed Army Institute of Research, Washington, D.C.

Levels of purine nucleotides (PU NTD), incorporation of labelled adenosine (Ar) and hypoxanthine (Hyp), and the distribution of these labels in the purine pathways were studied in intact human, monkey, and guinea pig (gp) platelets (plts) and in megakaryocytes (megas) from monkeys and gp. Following incubations of 5-180 min., perchloric acid extracts were made of 10^9 plts or of $0.6-1.0 \times 10^6$ megas per experiment. PU NTD in plts were separated by gradient anionic exchange HPLC; PU nucleosides and bases were separated by reversed-phase HPLC. Simultaneous quantitation was accomplished by on-line UV and radioactivity detectors. Concentrations of PU NTD in plts were comparable in all 3 species and to those in the literature for human plts. No major differences were found in the profiles of PU NTD of megas from monkeys and gp. The concentrations of adenylates in 10^6 megas were the same as in 10^9 plts. Differences were observed in purine interconversion pathways between plts and megas for Hyp, the only purine base available in a measurable quantity for salvage. Megas synthesized both adenylates and guanylates via IMP from HYP.

Incorporation of ^{14}C Hyp into PU NTD of gp megas

	AMP	ADP	ATP	GDP	GTP
nmoles/ 10^6	5	33	47	6	8
radioactivity	1494	1203	1065	69	53
specific act.	299	37	23	12	7

Pathway: Hyp \rightarrow \rightarrow IMP \rightarrow AMP \rightarrow ADP \rightarrow ATP
XMP \rightarrow GMP \rightarrow GDP \rightarrow GTP

No significant incorporation of Hyp was found in plts studied under the same conditions. In contrast, Ar was incorporated in both plts and megas, and then deaminated or directly converted to AMP. These studies found that megas have retained the capacity to synthesize PU NTD by salvage of Hyp. They are thus closer to other kinds of rapidly proliferating cells than to end stage cells such as plts, in which this pathway did not appear to be significant.

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IDENTIFICATION OF HUMAN MEGAKARYOCYTES GROWN IN VITRO BY TWO MONOCLONAL PLATELET ANTIBODIES. W. Vainchenker^o, J. Bouquet^o, J. Guichard^o, A. Mc Michael⁺, and J. Breton-Gorius^o ^oUnité INSERM U.91. Hôpital Henri Mondor, 94010 Créteil, France ; ⁺John Radcliffe Hospital, Headington, Oxford OX3 9DU, England.

The absence of specific cytochemical markers of the megakaryocyte (MK) lineage at light microscopy level has rendered difficult studies of human megakaryocyte colonies, particularly in pathology. In order to solve this problem, we have used two mouse hybridoma platelet antibodies on culture of human CFU-M. One of them, AN51 recognizes platelet glycoprotein I. CFU-M from human adults, newborns and fetuses have been grown in plasma clot in the presence of a stimulating factor (PHA-LCM). Indirect immunofluorescence was performed from day 10 to day 14 of culture on unfixed preparations. The results have shown that AN51 stains cultured MK. However, this staining was heterogeneous among the megakaryocytes. By comparison with a simultaneous study by electron microscopy (EM), it could be demonstrated that this heterogeneity was mainly related to the degree of maturity of the MK. This result suggests that synthesis of glycoprotein I occurs during MK maturation. In contrast, the second antibody stains more immature MK. Study of fetal and neonatal cultures has confirmed our previous results that a large number of these MK are small mature MK (micromegakaryocytes). In myeloproliferative disease, particularly in chronic myeloid leukemic, the number of MK colonies was extremely high with the presence of a large proportion of micromegakaryocytes which otherwise could only be identified by electron microscopy. Studies of human MK culture with monoclonal antibodies provide a new powerful tool to understand normal and pathological megakaryopoiesis.