740

REORGANIZATION OF ACTIN AND MYOSIN IN THE ACTIVATED PLATELETS. N.Shibata, K.Tanaka, K.Okamoto and T.Onji. The Center for Adult Diseases, Osaka, Japan.

This study was done to clarify the intracellular dynamic arrangements of myosin(My) and actin(Ac) in activation process of human platelets(PLs) from unactivated to activated stage (clot retraction) in electron microscopy. The observation of unactivated PLs was done either in the fresh whole blood fixed directly with 0.1 % glutaraldehyde or in PLs isolated by gel filtration of platelet rich plasma(PRP) containing prostaglandin I, (10 ng/ml). The isolated PLs mounted on a glass cover slip were used as activated PLs (adrerent ones). The contracted PLs were prepared in PRP incubated with thrombin (0.5 u/ml) and 20 $\,$ mM CaCl, for 10-60 min. Treating PLs with 0.15 % Triton X-100 containing 0.05 % glutaraldehyde produced cytoskeleton. My and P-Ac were identified by an indirect immuno-cytochemical method using the specific antibody (rabbit IgG) against PL-My and protein A-gold and by demonstration of in "arrow-head" decoration by Ishikawa's method using skeletal meromyosin (HMM), respectively. [Results] (1) Unactivated PLs. Mys in monomer or oligomer distributed homogenously in scare association with cytoskeleton. Cytoskeletons were exclusively composed of F-Ac networks of crossolinked short filaments which were thinly distributed in the cytoplasm with partial connection to the cell membrance. (2) Surface activated spreading PLs. PLs adhered to the glass cover slip in dendritic forms. Mys were densely located around granulomere and formed linear arrays associated with F-Ac filaments of the cytoskeleton surrounding the granulomere and running straightly in cytoplasm. (3) Contracted PLs. Activated PLs protruded several filopodia in which networks or bundles of F-Ac filaments were found connecting to extracellular fibrin strand through cell membrane. Microfilaments formed arrow-head decoration with HMM pointing toward the cell body. The cytoskeleton in contracted PLs contained thick filaments of My-polymers attaching to F-Ac filaments end by end. It is concluded that the reorganization of Ac-My is the basis for the shape change, secretion and clot retraction of activated PLs.

MEGAKARYOCYTES

741

ON THE DOUBLING DISTRIBUTIONS OF DAY 7-MEGAKARYOCYTE (MKC) PROGE-NITORS. J.M. Paulus, R. Fernandez-Delgado, J.C. Grosdent, D. Lecocq and M. Prenant. Inst. Pathologie Cellulaire, INSERM U.48 Hôpital de Bicêtre, Paris, France; Laboratoire d'Hématologie, CHU du Sart Tilman, Univ. of Liège, Belgium; and Dept. of Pediatrics, Univ. of Valence, Valence, Spain.

It has often been assumed that hemopoietic progenitors under-go a rather uniform, deterministic number of proliferative cycles before they generate the elements which are recognizable by panoptic and cytochemical methods. We now confirm and extend previous data on the distribution of the number of doublings undergone before polyploïdization by day 7-MKC progenitors. Cultures of mouse bone marrow were stimulated by erythropoietin, WEHI conditioned medium (CM) and/or pokeweed CM. In all cases, cumulative doubling distributions of pure MKC colonies could be precisely fitted by exponential lines, whose slope and length depended on the stimulus or combination of stimuli used. The frequency of single MKC could be fitted by the same line as that plotting the number of doublings in MKC colonies, suggesting that single MKC and MKC colonies can be generated by the same class of progenitors. Expo nential shape implies that the coefficient of variation (SD/mean) of doubling numbers is one, indicating significant variability in proliferative behavior among day 7-MKC progenitors. Such exponen-tial distributions are best explained in the framework of Renewal Theory (Cox, Methuen, 1962). Whatever the distribution of the number of progenitor renewals in fixed time, the distribution of renewals will be geometric (i.e discontinuous exponential) if the total time spent by individual colonies in the proliferation phase (1) is distributed exponentially, and (2) is independent from the renewal process itself. These results suggest that the wave of MKC progenitor mitoses is randomly arrested by a differentiative event which strikes progenitor clones independently from their proliferative past.

ABNORMALITIES OF MEGAKARYOCYTE (MK) SIZE AND MOR-PHOLOGY IN PRIMARY AND SECONDARY THROMBOCYTOSIS A.M.Gladwin, E.A.Trowbridge, D.N.Slater, J.M.Adams, J.Martin, The University of Sheffield, Medical School, Glossop Road, Sheffield, S10 2JF, UK.

Platelet count elevations are observed in various clinical situa-tions. In essential thrombocythaemia (ET), this is believed to reflect a primary disorder of megakaryocytes (MK). In contrast, thrombocytosis in malignant lymphoma (ML) is thought to arise via a secondary effect on these cells. In this study, MK parameters of whole cell, cytoplasmic and nuclear volumes were evaluated in these disorders. A trephine biopsy was obtained from the iliac crest of 10 subjects with ET, and from 10 subjects with ML. All exhibited a thrombocytosis (considered to be a platelet count in excess of 400x10^[L]). In addition, a biopsy was obtained from 5 volunteer, haematologically normal subjects. Platelet counts in all subjects were measured using Coulter S+. Biopsies were processed as described elsewhere(1). MK and MK nuclear planimetric areas were measured and converted to associated volumes(1). Mean MK, MK cytoplasmic and nuclear volumes were significantly larger in ET(p<0.05) and ML(p<0.05) when compared to those in

	Normals (n=5)	ML (n=10)	ET (n=10)
Platelet_Count (x10 [°] L ⁻¹)(x + SE)	269 <u>+</u> 71	587 <u>+</u> 95	972 + 156
MK volume (fl) Cytoplasmic volume (fl) Nuclear volume (fl)	$\begin{array}{r} 9803 + 432 \\ 6996 + 220 \\ 2807 + 125 \end{array}$	17432 <u>+</u> 2149 13884 <u>+</u> 1578 3584 <u>+</u> 367	$\begin{array}{r} 14675 + 1975 \\ 11231 + 1364 \\ 3444 + 383 \end{array}$

In addition, MKs in ET showed morphological controls. abnormalities which were not exhibited in ML. These results suggest that platelet count elevations in ML and ET arise from large MKs with increased amounts of cytoplasm. The increased nuclear size observed further suggests that this may be mediated by an increased DNA content. (1) E. A. Trowbridge et al. Clin.Phys.Physiol.Meas. 5(3):

(1) E. A. Tr 145-170 (1984).

742