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PROTEIN SYNTHESIS IN MEGAKARYOCYTES STIMULATED BY THROMBOPOIETIN IN VITRO. K. L. Kellar, B. L. Evatt, C. R. McGrath and R. B. Ramsey. Division of Hematology, Centers for Disease Control, Atlanta, Georgia, U.S.A.

Studies in our laboratories have been concerned with the responses of megakaryocytes to thrombopoietin in vitro. We have shown that preparations of thrombopoietin stimulate DNA synthesis in guinea pig megakaryocytes. The increase in ^{3}H-thymidine incorporation correlates with an increase in the labeling index of the megakaryocytes. After 2 and 3 days of incubation an increase in the ploidy levels of the megakaryocytes has been observed in thrombopoietin-supplemented cultures compared to controls. Recent studies have examined the incorporation of ^{3}H-leucine in megakaryocyte cultures. Megakaryocytes were prepared on BSA or Percoll gradients to purities of 70-95%. ^{3}H-leucine incorporation was measured after a 15 hr incubation of the megakaryocytes in medium containing 10% thrombopoietin or control preparations of normal plasma or phosphate-buffered saline. Utilization of isotope increased over a 24 hr period and was higher in the thrombopoietin-supplemented cultures. In addition, synthesis of specific proteins was analyzed by using SDS-polyacrylamide gel electrophoresis and quantitation was achieved by employing rocket immunoelectrophoresis. The results indicate that thrombopoietin stimulates endoreduplication and protein synthesis in megakaryocytes in vitro and that this system may serve as a model for studying the mechanism of action of thrombopoietin in megakaryocytopoiesis.

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MEGAKARYOCYTIC COLONY STIMULATING ACTIVITY: A POTENTIAL REGULATOR OF HUMAN MEGAKARYOCYTOPOIESIS. Hoffman, R., Mazur, E., Bruno, E. & Floyd, V. Yale University School of Medicine, New Haven, Connecticut, USA.

The regulation of megakaryocytopoiesis is presently poorly understood. Utilizing an in vitro clonal assay system for the human megakaryocytic progenitor cell (CFU-M) we have attempted to define factors which are important in the control of megakaryocytopoiesis at the stem cell level. Sera were obtained from individuals with clinical disorders associated with quantitative platelet abnormalities and were added to plasma clot cultures of bone marrow mononuclear cells obtained from nine normal individuals. When serum samples from nine patients with aplastic anemia comprised 10% of the growth media, each significantly increased CFU-M derived colony formation (400-1250%) when compared to cultures containing normal human AB serum. Under similar conditions, neither sera from eight individuals with thrombocytosis associated with myeloproliferative disorders nor sera from eight patients with severe thrombocytopenia but with normal or increased numbers of bone marrow megakaryocytes (ITP, hypersplenism, TTP, DIC, megakaryocytic leukemia) altered colony formation. When aplastic anemia sera was added in increasing amounts (0, 5, 10, 20, 30%) to bone marrow cultures, CFU-M derived colonies per $5x10^{5}$ cells plated increased in a linear fashion (14, 42, 89, 123 and 238, respectively). Preliminary characterization of this megakaryocytic colony stimulating activity (M-CSA) reveals that it is dialyzable and its effect is not neutralized by pretreatment with rabbit anti-erythropoietin antisera. These studies have defined a newly recognized serum factor M-CSA that regulates in vitro megakaryocytic colony formation. M-CSA levels in various disease states suggest that it has physiological significance and that its production might be inversely related to bone marrow megakaryocyte numbers.

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MORPHOLOGICAL STUDY OF 5-HT ORGANELLES IN HUMAN MEGAKARYOCYTES. P. Hourdille, P. Fialon, F. Belloc, P. Bernard, M.R. Boisseau, Laboratoire d'Hémobiologie Hôpital Cardiologique, 33600 BORDEAUX-PESSAC - FRANCE -.

Human blood platelets possess dense bodies which have been clearly demonstrated to be the storage site for 5-hydroxytryptamine and the non-metabolic pool of ADP. Previous electron microscopy investigations performed on human megakaryocytes have revealed electron-opaque granules, but only in platelet-forming megakaryocytes. In this study different methods of visualizing platelets 5-HT storage organelles were applied to human megakaryocytes. The observations were facilited by the high enrichment of megakaryocytes obtained using the Percoll density centrifugation. By conventional electron microscopy we rarely observed dense bodies similar in size and opacity to those observed in platelets. Using uranaffin cytochemical method we observed typical and atypical uranaffin-positive organelles. Distribution of these organelles was heterogeneous with numerous megakaryocytes containing only few granules. Using mepacrine labelling test, human megakaryocytes could bedétected easily since they are large and have multilobulated nucleus.Their nucleus was intensely fluorescent and was surrounded by a slightly-fluorescent cytoplasm. For each sample, 50 megakaryocytes were examined 96 ± 1.4 % of the megakaryocytes exhibited greenish yellow, very fluorescent granules with a flashing phenomenon. These granules were randomly distributed in the whole cytoplasm. The other 4 ± 1.4 % detected by their morphological characteristics no granules were observed. We found no correlation between megakaryocytes size and the presence of granules.
These results indicate that mepacrine labelling test and uranaffin cytochemical electron microscopy are very useful tests for megakaryocytes 5-HT organelles study, and allow us to correlate the results obtained by these different methods.