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ARACHIDONIC ACID (AA) INDUCED AGGREGATION OF RAT PRO- MEGAKARYOBLASTS (RPM) A.M.Gladwin, and J.Martin, The University of Sheffield, Medical School, Glossop Road, Sheffield, S10 2JF, UK.

Platelet aggregation can be induced in vitro by a variety of platelet agonists acting upon membrane receptors. Since platelets have only a limited ability to synthesise proteins, these receptors must be present in megakaryocytes. This was investigated using an eternal line line of RPMs. Cells were suspended in rat platelet free plasma (PFP) at a concentration of 10^5 cells ml^{-1}. 200µl aliquots of this were placed in a light aggregometer. For this suspension, and for an aliquot of PFP, light transmission was adjusted to zero and 100% respectively. Addition of ADP (plasma concentrations 1-100µM), thrombin (0.5-5 I.U. ml^{-1}), and adrenaline (0.1-1 I.U. ml^{-1}) to the suspension caused no change in transmission. However, addition of AA (1.5-6mM) increased light transmission, indicating RPM aggregation. Radioimmunoassay (RIA) on the resultant supernatant showed no thromboxane B2 was produced. Scanning and transmission electron microscopy showed the aggregate to be composed of non-lysed cells. Aggregation of RPMs was not inhibited by preincubation with PGI_2 (1500ng ml^{-1}) indomethacin (100µg ml^{-1}) or fenoprofen (100µg ml^{-1}). However, preincubation with aspirin (30µM) blocked aggregation.

Final Plasma Concentration of AA	1.5mM	3mM	4.5mM	6mM
%age aggregation of RPMs (n=5) (x̄±S.E.)	31.8±5.6	43.8±5.1	66.6±4.4	68.3±1.8
%age aggregation after preincubation with aspirin (n=5) (x̄±S.E.)	3.0±0.9	2.6±1.8	3.4±2.0	2.3±1.1

These results indicate that RPMs can aggregate in response to AA. Mechanism of this is unlike that observed for platelets, since PGI_2, indomethacin and fenoprofen did not block aggregation. The response was only inhibited by aspirin. Indomethacin, fenoprofen and aspirin are all known inhibitors of cyclo-oxygenase. In addition, aspirin also blocks 12-lipoxygenase. Therefore, this may suggest that the effect of AA on RPMs is mediated via 12-lipoxygenase pathway. Further investigations are in progress.

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INCREASED SPONTANEOUS NUMBER OF MEGAKARYOCYTE COLONIES IN ESSENTIAL THROMBOCYTHEMIA (ET). J.F. Deschamps, E. Bodevin and J.P. Caen U. 150 INSERM, UA 334 CNRS, Hôpital Lariboisière 75475 Paris Cedex 10, France

Megakaryocytes were grown from medullary progenitor cells using the plasma clot technique, with 5 % human serum and with or without 2,5 % PHA-LCM. Using this technique megakaryocyte cultures were done in 5 patients with essential thrombocythemia (ET) (platelet count :0,6 x 10^6 to 1,4 x 10^6 /µl) before chemotherapy and antiplatelet agents and in 2 patients with secondary thrombocytosis (ST) (platelet count : 0,65 x 10^6 and 0,8 x 10^6 /µl) corrected after effective anti-bacterial or iron therapy. The results were as follows :

	PHA-LCM 2.5 %	
	-	+
ET n = 5	27.6 ± 12.9 x	36 (17 ± 10)*
ST n = 2	5.5 ± 3.5	34 (9 ± 3)
	p < 0.01	

x number of colonies
* mean number (in brackets) of cells per colony

It appears therefore that in ET, megakaryocyte progenitors grow without PHA-LCM and show however a better proliferation in its presence. On the contrary in ST, PHA-LCM is required for obtaining a 6 times increase of MK colonies. The effect of MK growth under chemotherapy is reported in some of the patients studied before treatment and results analyzed and compared to platelet function involvement.

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DOES THE MEGAKARYOCYTE CYTOSKELETON REGULATE THROMBOPOIESIS? M.J. Heynen (1), R.L. Verwilghen (1) and J. Vermylen (2). Dept. Hematology (1) and Centre for Thrombosis and Vascular Research (2), University of Leuven, Belgium.

A widely held view of thrombopoiesis is that platelets arise from fragmentation of the periphery of mature megakaryocytes (MK's). Evidence against this concept was provided by Zucker-Franklin, who showed that platelet plasma membrane is different from that of the MK (freeze-fracture, membrane antigens). Several authors have described contractile processes in MK's.

We have performed detailed electronmicroscopic studies of the numerous small MK's of a subject with congenital macrothrombocytopenia. In the young granular MK's a central zone with organelles and a thick peripheral zone without organelles can be observed. The absence of elements of the demarcation system in the peripheral zone argues against derivation of the demarcation system from the megakaryocyte plasma membrane. In the mature granular MK's the heart of the central zone is not occupied by the nucleus, but by an area, free of organelles and membranes, containing fibrillar structures and the centrioles. Elements of the demarcation system, delineating platelet territories, radiate from this fibril-rich area, but do not extend into the very thin peripheral zone. In the platelet producing MK's the peripheral zone is thicker and the central fibril-rich area is surrounded by separated platelet territories. The peripheral zone shows several openings. In the old MK's the nucleus is only surrounded by a markedly thickened peripheral zone, which seems to result from contraction of the more extended peripheral zone of the platelet producing MK's.

From these observations we conclude that, at least in this patient, platelets are formed inside the MK and are extruded through openings of the peripheral zone. Further studies with cytoskeleton markers are required to confirm that (1) the fibril-rich heart governs the organisation of platelet territories and (2) that platelet extrusion results from contraction of the peripheral zone, the latter however not giving rise to platelets.

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CHANGES IN PROTEIN SYNTHESIS PROFILES OF MEGAKARYOCYTES (MK) DURING MATURATION. C.W. Jackson, N.K. Hutson and S.A. Steward. St. Jude Children's Research Hospital, Memphis, TN, USA.

Several key differentiation events occur within the recognizable MK compartment; however, little is known about the macromolecular changes responsible for these events. In this study, protein synthesis profiles of morphologically immature and mature guinea pig MK populations have been analyzed by two-dimensional gel electrophoresis after in vivo labeling with ^{35}S-methionine. MK were enriched by a bovine plasma aggregation enrichment procedure (Blood 69:173, 1987) and then fractionated into immature and mature populations based on differences in their respective buoyant densities (Brit. J. Haematol. 64:33, 1986). With this protocol, immature and mature MK populations were obtained in which MK constituted 95% of the cell mass. Ninety percent of the MK in the immature population had basophilic, immature morphology while ≥90% of those in the mature population had acidophilic, mature staining characteristics after Wright's staining. Protease inhibitors were used throughout the isolation procedure. The cells were solubilized and proteins subjected to two-dimensional electrophoresis according to O'Farrell (J. Biol. Chem. 250:4007, 1975). To examine basic proteins, proteins were electrophoresed in the first dimension under nonequilibrium conditions in a pH gradient as described by O'Farrell et al. (Cell 12:1133, 1977). Analyses of fluorograms revealed both qualitative and quantitative differences in synthesis profiles between these two MK populations. Among acidic proteins whose synthesis was readily detected in immature but not mature MK were ones whose MW and pI were respectively: 120K, 6.4; 70K, 5.9; 70K, 6.9; 65K, 6.8; 55K, 6.2; 55K, 6.0; 53K, 5.8; 53K, 6.5; 52K, 6.7; 50K, 6.8; 41K, 5.5 and 33K, 6.7. Acidic and neutral proteins prominently synthesized in mature but not immature MK were found at MW and PI of: 110K, 5.7; 110K, 5.8 and 80K, 7.2. Basic proteins prominently synthesized in immature but not mature MK were found at MWs of: 110K; 70K; 52K; 48K; 39K and 18K. Basic proteins actively synthesized by mature but not immature MK had MWs of: 83K; 43K and 17K. These findings demonstrate that differences in protein synthesis patterns can be readily detected between immature and mature MK and provide baseline data with which to explore the role of these proteins in MK differentiation.