Megakaryocytes and atherosclerosis

S. D. KRISTENSEN and J. F. MARTIN*

University Department of Cardiology, Skejby Hospital, Aarhus, Denmark, and
*Department of Medicine, King’s College School of Medicine and Dentistry, London, U.K.

INTRODUCTION

Platelets are produced from megakaryocyte cytoplasm. It was previously thought that changes in platelet density and volume occurred because of platelet ageing in the circulation [1-3], but the methodology used to reach this conclusion has been criticized [4]. When platelets are separated using continuous linear Percoll gradients [4] and radiolabelled before infusion in subhuman primates, no significant change in platelet density occurs during their lifespan in the circulation [5, 6]. Thompson et al. [7] separated platelets into volume fractions by centrifugal elutriation. After radiolabelling, the platelet fractions were infused into subhuman primates and the lifespans of all volume-determined fractions were found to be of approximately the same duration [7]. Thus, the weight of evidence now suggests that the density and volume of the circulating platelets are determined at the time of thrombopoiesis [4-7].

The megakaryocyte is unique among mammalian cells in that it can undergo endomitosis. By this process the megakaryocyte can double its DNA content without dividing. Therefore, megakaryocytes are polyploid, having either 4N, 8N, 16N, 32N, 64N or even 128N content of DNA, whereas normal cells have 2N. Endomitosis requires less energy than mitosis to increase protein production and occurs in insects when more secretory products are required rapidly [8]. Platelets, which have no nucleus, contain no DNA and only scarce amounts of RNA, and have a negligible capacity for synthesis of proteins [9]. The large polyploid megakaryocytes have a high capacity for protein synthesis and have been shown to contain growth factor(s) for fibroblasts [10] and smooth muscle cells [11].

Experimental studies indicate that platelets are involved in atherogenesis [12-14]. Ross & Glomset [15] proposed that platelets adhere to the arterial subintima where the endothelium is damaged and secrete platelet-derived growth factor (PDGF) [16], which causes proliferation and migration of smooth muscle cells from the media into the intima [15, 16]. Messenger RNA for PDGF β-chain has now been demonstrated to be present in human megakaryocytes [17]. We postulate that changes in the synthesis of PDGF within megakaryocytes may be causally important in atherogenesis.

PHYSIOLOGY OF THE PLATELET-MEGAKARYOCYTE RELATIONSHIP

Thrombopoiesis in conditions of altered platelet demand

Changes in the number of circulating platelets or the platelet biomass can cause changes in the DNA content and size of the megakaryocyte. This is probably controlled by one or more circulating hormones [called by some thrombopoietin(s)] [18]. Transfusion of platelets into animals is followed by a decrease in megakaryocyte DNA content and size [19, 20], whereas destruction of the circulating platelets by injection of anti-platelet antibodies causes an increase in the DNA content and the size of the megakaryocytes in the bone marrow 3-4 days later [20-22]. The platelets produced by these big megakaryocytes with an increased DNA content are more active haemostatically and have a higher capacity for thromboxane A2 production than normal platelets [21]. Furthermore, the platelet half-life is decreased in idiopathic thrombocytopenic purpura and in these patients the megakaryocyte size [23] and DNA content [24] are increased. Thus, changes in megakaryocytes occur in response to both acute and chronic consumption of platelets.

MEGAKARYOCYTES IN VASCULAR DISEASE

Thrombopoiesis in experimental atherosclerosis

The platelet half-life is shortened in rabbits fed a high-cholesterol diet [25]. After 12 weeks on the diet, the lipid accumulation occurring in the arteries is associated with...
an increase in bone marrow megakaryocyte DNA content and in megakaryocyte size [26]. Guinea-pigs fed a high-cholesterol diet for 5 days have been shown to have larger megakaryocytes than control animals [27]. These changes in the megakaryocytes could be due either to a response from the bone marrow to an increased platelet demand or to a direct effect of cholesterol on the megakaryocytes.

The control mechanisms regulating thrombopoiesis are complex. Dupont et al. [28] studied megakaryocyte DNA content and maturation in rabbits fed a hypercholesterolaemic diet for 6 months and then maintained on a normal diet for another 6 months. At this time these animals had normal blood cholesterol levels and had developed fibrous lipidic lesions in the aorta. The megakaryocyte DNA content was decreased compared with that of control animals, and histological examination suggested that this was due to an increase in megakaryocyte turnover with activation of committed stem cells. In another study, designed to investigate the initiation of atherosclerosis, rabbits were fed a high-cholesterol diet for 7 days only [29]. At that time, cells resembling smooth muscle cells were found in the subintima. The megakaryocyte size was decreased in the cholesterol-fed animals compared with control animals, probably reflecting an increased influx of megakaryocytes from the committed stem cells or an increased efflux of large megakaryocytes in response to increased platelet demand. These studies illustrate that the DNA content and the size of the bone marrow megakaryocytes at a given time reflect a snapshot of a dynamic situation. However, complex dynamic megakaryocyte changes are associated with the occurrence of atherosclerosis in animal models.

There is also evidence that activation of the platelet-megakaryocyte axis is associated with accelerated atherogenesis. When rabbits were injected with anti-platelet antibody, 95% of the circulating platelets were destroyed [21]. This was followed by an increase in megakaryocyte DNA content and size, which gave rise to a rebound thrombocytosis apparent 4–7 days after the injection. In a recent study [22], we injected rabbits fed a high-cholesterol diet for 12 weeks with goat serum containing anti-platelet antibody or goat serum alone (control group). The animals were killed 7 days after the injection and their aortas were examined. The animals that had received anti-platelet antibody had a 2–3-fold increase in the percentage of atheroma in the aorta compared with the control group. Histological examination revealed that more extracellular fat and subintimal connective tissue and a higher number of myointimal cells were present in the animals receiving anti-platelet antibodies than in control animals. The lesion in the cholesterol-fed animals therefore appeared more like developed human atherosclerosis than that found in the control animals.

In a second experiment in cholesterol-fed rabbits in which platelet destruction was caused, the accelerated atherogenesis was preceded by an increase in megakaryocyte DNA content and size [22]. A likely explanation for these findings is that an increase in megakaryocyte-derived PDGF caused the accelerated atherogenesis.

**Megakaryocytes in myocardial infarction in man**

Patients with ongoing acute myocardial infarction have a shortened bleeding time [30, 31] and an increased platelet volume [32–35] and density [32]. The bleeding time, a measurement of platelet behaviour *in vivo*, has been shown to be inversely correlated with megakaryocyte DNA content and size in man [36]. In a study where bone marrow biopsies were taken from patients approximately 18 days after myocardial infarction, it was found that megakaryocyte DNA content and size were increased in these patients when compared with patients admitted with chest pain and no myocardial infarction [37]. In a second study, megakaryocyte size was measured in men that had suffered sudden unexpected cardiac death and compared with that in age-matched men that had suffered sudden traumatic death with no evidence of vascular disease at autopsy [37]. The size of the megakaryocytes was significantly larger in the sudden cardiac death group and was not significantly different from that in the myocardial infarction group described above [37]. Since in myocardial infarction the major association of coronary artery thrombosis is atherosclerosis, there may be a causal association in man between atherosclerosis and increased bone marrow megakaryocyte size and DNA content.

**CONCLUSION**

There is therefore evidence from animal models and man that atheromatous arteries are associated with large, high ploidy megakaryocytes in the bone marrow. We propose that the megakaryocyte changes may precede the vascular changes and be casually related by increased production of PDGF in megakaryocytes giving rise to more proatherogenic platelets. The control of megakaryocyte biology may have future therapeutic application.

**ACKNOWLEDGMENTS**

S.D.K. was sponsored by a Wellcome-Carlsberg Travel Fellowship. J.F.M. is the British Heart Foundation Professor of Cardiovascular Science, King’s College School of Medicine and Dentistry, London.

**REFERENCES**


