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COMMENTARY

Megakaryocyte apoptosis: sorting out the signals

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Of all the haemopoietic processes occurring in the bone marrow, the production of megakaryocytes (MKs) and, subsequently, platelets is perhaps the most complex and unusual. Beginning with the haemopoietic stem cell, a sequence of proliferation and differentiation steps produces MK progenitors, megakaryoblasts and eventually MKs. Unique among blood precursors, the MK undergoes a process of endomitosis, producing a polyploid cell with a multiple of the normal chromosome complement (up to 64*N*). Following this, the focus of the maturation process moves to the cytoplasm. Complex invagination of the plasma membrane causes the cytoplasm to become intricately subdivided by a system of demarcation membranes. These provide a source of membrane material which, together with granules and organelles, is transported into 'proplatelets', pseudopodia elongating from the MK and producing numerous platelet-sized swellings, which bud off to be released as functional platelets. A complex reorganisation of the cytoskeleton allows the sequence of proplatelet formation, platelet budding and detachment to be completed successfully.

Predictably, the senescent MK nucleus left after platelet release is disposed of by apoptosis and phagocytosis. It has become evident, however, that a specialised form of apoptosis is engaged during the process of proplatelet formation and the release of mature platelets. In cultured MKs derived from $CD34^+$ bone marrow cells, activation of caspase-3 and -9, mitochondrial membrane permeabilisation and cytochrome *c* release were all evident in maturing MKs, suggesting a process of apoptosis. Proplatelet formation could be diminished either by exposure of cells to caspase inhibitors or by overexpression of Bcl-2 (De Botton *et al*., 2002). Intriguingly, caspase activation prior to proplatelet maturation showed a *localised* distribution, rather than the diffuse pattern encountered later in the senescent MK. Further evidence of the highly compartmentalised nature of these apoptotic events comes from observations of an exclusion from the proplatelet pseudopodia and budding platelets of both mitochondrial permeability transition and caspase-9 (Clarke *et al*., 2003), allowing released platelets to remain viable despite emerging from a dying MK. Thus, platelets differ from the nonfunctional, thrombogenic and short-lived apoptotic bodies produced during a conventional apoptosis.

What are the signals controlling these events? MK survival, proliferation and differentiation are coordinated and controlled by combinations of cytokines and mediators presented within specialised bone marrow 'niches'. Interaction with a vascular niche appears to be required for the final stages of MK maturation and platelet release (Avecilla *et al*., 2004). Thrombopoietin (TPO) is the essential growth factor for adequate platelet production, but stem cell factor, IL-3, IL-6 and IL-11 all play important roles at different developmental stages. Mouse knockouts for either TPO or its ligand c-mpl show a profound thrombocytopenia; nevertheless, there remains a residual thrombopoiesis that produces MKs and platelets which are morphologically and functionally normal (Bunting *et al*., 1997). The principal role of TPO therefore appears to be the maintenance of MK numbers, but the final differentiation of MKs to proplatelets and mature platelets depends upon other signalling systems. Chemokines such as stromal-derived factor 1 and fibroblast growth factor 4 help localise MKs to the vascular niche within the bone marrow (Avecilla *et al*., 2004) and glutamate signalling *via* the *N*-methyl-D-aspartate (NMDA) receptor is implicated in the terminal differentiation of MKs (Hitchcock *et al*., 2003). In addition to these signal systems, several papers have indicated a role for nitric oxide (NO). MK apoptosis is promoted by NO, whether exogenously supplied by donor compounds or endogenously produced by MKs themselves following up-regulation of inducible NO synthase by inflammatory cytokines (Battinelli & Loscalzo, 2000; Schattner *et al*., 2001). The picture emerging suggests that NO performs a dual function, promoting apoptosis *via* a cyclic GMP-dependent mechanism (an activity opposed by TPO) and enhancing terminal differentiation and platelet release *via* a mechanism independent of cyclic GMP (Battinelli *et al*., 2001).

In this issue of the *British Journal of Pharmacology*, Pozner and co-workers have further explored this web of signalling by documenting the interplay between cyclic nucleotides in MK apoptosis. They showed that, in contrast to the well-documented synergistic inhibition of platelet aggregation by NO and prostacylin (PGI_2), these two agents exert opposing influences on MK apoptosis. This opposition mirrored the intracellular balance between cyclic nucleotides. PGI_2 raised intracellular cyclic AMP but suppressed accumulation of cyclic GMP stimulated by the NO donor drug PAPA/NO. Elevation of cyclic AMP with either permeable analogues or selective phosphodiesterase inhibitors protected MKs from PAPA-/NO-mediated apoptosis, whereas pretreatment of MKs with inhibitors of either adenyl cyclase or of protein kinase A diminished the protective action of PGI_2. Cyclic AMP thus inhibited apoptosis in this experimental model. In contrast, intracellular cyclic GMP accumulation *via* permeable analogues, activators of guanylate cyclase or inhibitors of cyclic GMP-specific phosphodiesterase, served to enhance apoptosis.

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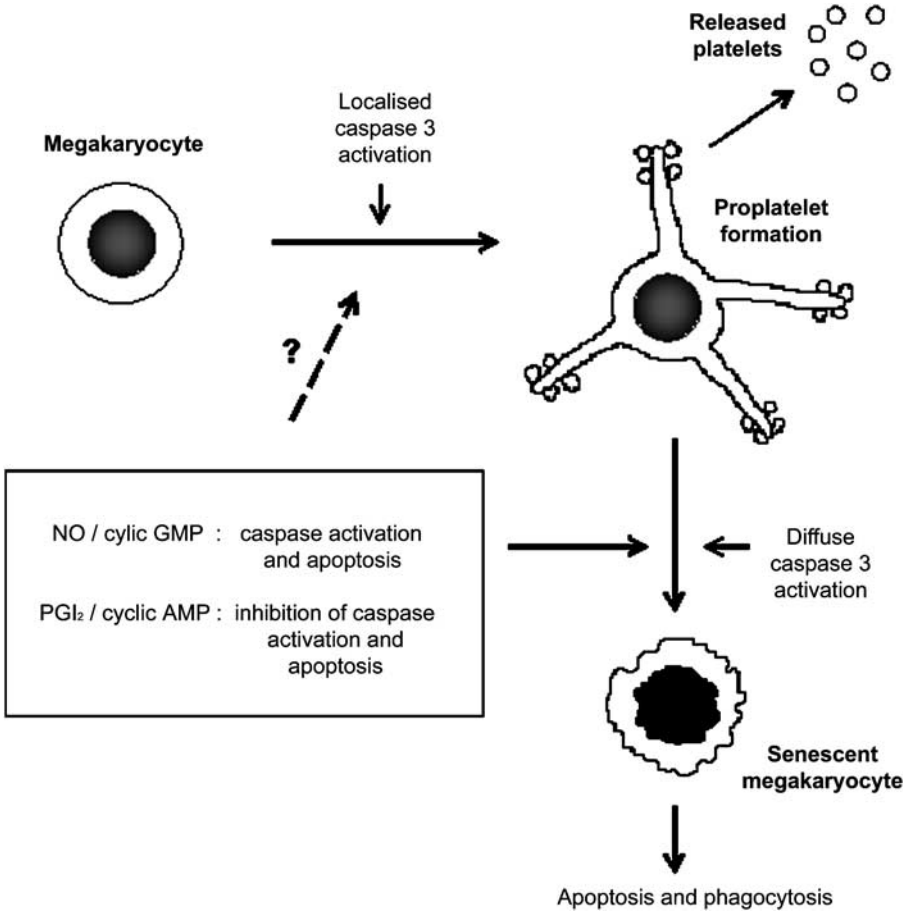

Figure 1 PGI_2 opposes the caspase activation and nuclear changes evident during NO-mediated apoptosis of MKs. It is not yet clear whether prostacylin exerts a similar influence on proplatelet formation and platelet release.

Significantly, the antiapoptotic action of PGI_2 was accompanied by a loss of caspase-3 activation.

Of course, the significance of caspase activation and apoptotic changes in MKs will depend upon the stage of MK differentiation and the intracellular location. Does PGI_2-mediated cyclic AMP accumulation modulate the compartmentalised apoptosis characterising the terminal MK differentiation stages, or the more conventional machinery of senescent MK disposal? (Figure 1). It is of interest that both PGI_2 receptor expression (Sasaki *et al*., 1997) and protein kinase A-mediated Ca^{2+} sequestration (den Dekker *et al*., 2002) are late events in MK maturation. This temporal distribution may indicate that cyclic AMP signalling is recruited to modulate the later stages of thrombopoiesis, once MKs have localised to the vascular niche. It will be important that the observations of Pozner *et al*. are followed up to see how the suppression of MK apoptosis by PGI_2 and cyclic AMP relates to proplatelet development and platelet release.

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