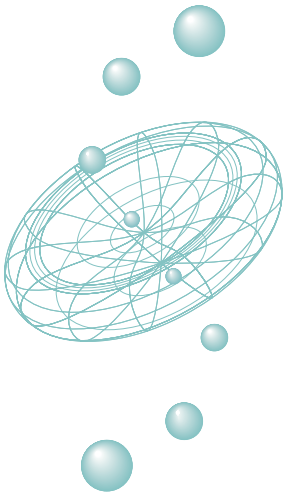
REVIEW ARTICLE



Platelet Development

Paul HARRISON

Oxford Haemophilia Centre & Thrombosis Unit,
Churchill Hospital, Oxford, OX3 7LJ, United Kingdom.
Phone: 44-(0)1865-225305 Fax : 44-(0)-1865-225299
Email: paul.harrison@ndm.ox.ac.uk

Key Words

Platelet, Megakaryocyte, Thrombopoietin, Thrombopoiesis, Immature Platelet Fraction

Received 1 June, 2007: Accepted 26 June, 2007

BACKGROUND

This review provides an overview of platelet development and will give the required background for understanding the clinical relevance of measuring the Immature Platelet Fraction (IPF) as an index of thrombopoiesis. The normal average platelet count within humans is 150-400×10^9 cells per litre and is maintained within fairly narrow limits (count and MPV) within any individual on a day to day basis. Abnormalities in platelet production results in clinically significant disorders e.g. thrombocytopenia (<150×10^9/L) or thrombocythemia (> 600×10^9/L) which can either significantly increase the risk of bleeding or thrombosis respectively. As platelets are anucleated cells with limited protein biosynthetic capability their normal lifespan is also comparatively short (~10 days) relative to other blood cells. Assuming an average adult blood volume of ~5 litres, approximately 1×10^{11} platelets are therefore produced every single day (equivalent to 1.2×10^6 platelets/second) to maintain the normal platelet count under steady state conditions. However the capacity to increase platelet production can increase to greater than ~10 fold this number if required for example in severe haemorrhagic conditions. In this review we will therefore review the current knowledge on platelet development and the regulation of platelet production or thrombopoiesis in health and disease.

ABBREVIATIONS

$\alpha_2\beta_1$ - GPIa/IIa integrin
$\alpha_{IIb}\beta_3$ - GPIIb/IIIa integrin
DMS - Demarcation membrane system
DNA - Deoxyribonucleic acid
IPF - Immature Platelet Fraction
ITP - Idiopathic Thrombocytopenic Purpura
GFP - Green Fluorescent Protein
GPIb-IX-V - Glycoprotein Ib complex
GPVI - Glycoprotein VI
MK - Megakaryocyte
MPV- Mean platelet volume
mRNA - Messenger ribonucleic acid
TPO - Thrombopoietin
VLA-4 - Very Late Antigen 4
VWF- Von Willebrand Factor

WHY DO WE NEED PLATELETS?

The normal average platelet count within humans is 150-400×10^9 cells per litre and is maintained within fairly narrow limits within any individual on a day to day basis.[1)] As platelets are anucleated cells with limited protein biosynthetic capability, their normal lifespan is comparatively short (-10 days) relative to other blood cells. This means that approximately 10% of the platelet count

(-1×10^{11}) is replenished on a daily basis to maintain the normal count in steady state conditions but this also has to be balanced by the efficient removal of an equivalent number of senescent platelets from the circulation.[1)] The system has the capacity to increase the rate of platelet production in various scenarios where the platelet count suddenly falls (e.g. in severe haemorrhage) or in conditions where there is peripheral platelet destruction (e.g. immune mediated destruction in ITP). Platelets are multifunctional and play a key role in many physiological processes (e.g. wound repair, immune responses and tumour metastasis) apart from their well known role(s) in haemostasis and thrombosis. Human platelets are small flattened discs (2-4 μm in diameter by 0.5 μm thick) that are normally marginated towards the vessel wall by the larger erythrocytes.[2)] This facilitates the localisation of the platelets to the precise vicinity where they will not only be exposed to maximal shear stresses but where they can constantly interact with the intact vascular endothelium and closely monitor vessel wall integrity. Platelets are extremely sensitive to their environment and possess many different receptors that detect the balance between many different types of molecules that can either promote platelet activation or inhibition.[3)] They are therefore primed to undergo a series of controlled activation events upon vascular wall damage resulting in the rapid formation of a stable haemostatic plug. These events therefore prevent any significant blood loss from occurring providing the level of damage is not excessive when the system may be potentially overwhelmed. Upon vessel wall damage and exposure of subendothelial matrix proteins (e.g. collagen), platelets undergo rapid adhesion mediated by VWF bridging from the exposed collagen to the platelet GPIb-IX-V complex.[3)] This facilitates the slowing down, tethering and translocation of platelets over the area of vessel well damage which allows other receptor ligand pairs (e.g. GPVI and collagen) to come into play that mediate platelet activation and firm adhesion and spreading.[3)] Platelets can therefore rapidly seal an area of damage with a monolayer of fully spread and activated cells. Platelet activation also results in the secretion of granular components (e.g. ADP) and generation of thromboxane, which results in the recruitment and activation of more platelets in the vicinity resulting in the outward growth of the platelet plug via aggregation mediated primarily via either fibrinogen or VWF bridging between activated $\alpha_{IIb}\beta_3$ on adjacent platelets.[3)] The activated platelets also provide a pro-coagulant surface upon which coagulation molecules can assemble resulting in very efficient thrombin generation and fibrin formation which serves to stabilise the haemostatic plug. Excessive growth of the haemostatic plug is regulated by negative regulators produced by normal endothelial cells adjacent to the area of vessel wall damage.[3)] Maintenance of the platelet count is therefore critical for normal haemostasis and thrombosis and there is a direct inverse relationship between the platelet count and risk of bleeding. In severe thrombocytopenia (e.g. $<10\text{-}20\times10^9$/L) the risk of spontaneous severe bleeding becomes excessive and usually requires clinical support by giving platelet transfusions. Conversely either higher or pathologically elevated platelet counts are associated with a significant increased risk of arterial thrombosis and may either require antiplatelet therapy (e.g. aspirin) to inhibit platelet function and/or long term therapy designed to reduce the platelet count to manageable levels (e.g. hydroxyurea).

WHERE DO PLATELETS COME FROM?

Platelets develop from and are released into the bloodstream by their specialised parent bone marrow precursor cells or megakaryocytes (MK) first identified by Wright in 1906.[4)] MK are very rare myeloid cells (< 1%) that are located primarily within the bone marrow but can also be found in the peripheral blood particularly within either the lung or spleen.[5)] MKs form from precursor cells originally derived from pluripotent stem cells. During MK maturation the cells can undergo dramatic enlargement to >100μm in diameter and become characteristically polyploid via multiple replication of their DNA content (up to 128N).[5)] This facilitates a dramatic increase in protein biosynthesis coupled with the maturation and expansion of the cytoplasm characterised by the presence of the demarcation membrane system (DMS) which is thought to define pre-formed "platelet territories".[6)] Each cell therefore essentially becomes a factory for the development of platelets and in terms of cell volume the largest mature MK can theoretically release >2000 platelets. Electron microscopy of mature MK indeed reveals that the cytoplasm contains all of the classical platelet organelles and structures including the alpha and dense granules, mitochondria, rough endoplasmic reticulum, surface connected canalicular system etc.[7)] A fully differentiated mature MK is therefore primed to undergo the remarkable and unique process of platelet biogenesis.

HOW ARE PLATELETS FORMED?

The transition of a mature MK into potentially thousands of anucleated platelets is a complex but poorly understood process. Several models of platelet formation have been proposed including platelet budding from the MK surface, cytoplasmic fragmentation via the DMS and proplatelet formation.[5,6)] Until the mid 1990s *in vitro* studies on cultured MK were significantly hampered by the lack of a specific maturation factor that is vital for the growth and terminal differentiation of the cell. The discovery of TPO and its receptor c-Mpl in1994 therefore led to an explosion of research into thrombopoiesis.[8)] As early as 1906, Wright suggested that platelets can bud off MK cytoplasmic extensions or pseudopods.[4)] Remarkably this model still persists today and it is now thought that platelets are assembled and released from specialised MK cytoplasmic extensions, called proplatelets. Significantly proplatelets have also been observed within the bloodstream and extending from bone marrow sinusoids *in vivo*.[6)] The availability of recombinant TPO revolutionized the ability not only to reliably culture MKs to full maturation *in vitro* but to also observe platelet formation by time lapse video microscopy.[9)] The cytoplasm of mature MKs has been shown to undergo a remarkable transformation into long proplatelets (***Fig. 1***).[9)] This

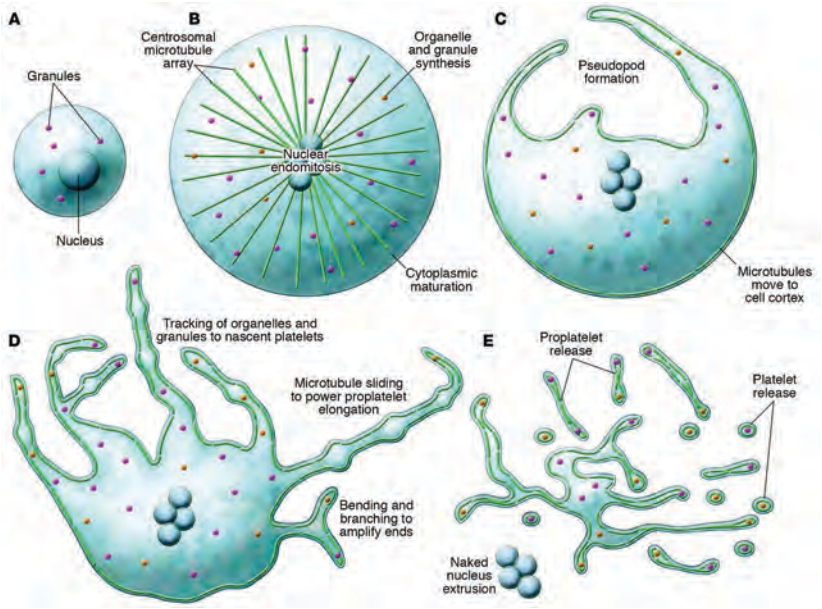


Fig. 1 *Overview of megakaryocyte production of platelets*

As megakaryocytes transition from immature cells (A) to released platelets (E), a systematic series of events occurs. (B) The cells first undergo nuclear endomitosis, organelle synthesis, and dramatic cytoplasmic maturation and expansion, while a microtubule array, emanating from centrosomes, is established. (C) Prior to the onset of proplatelet formation, centrosomes disassemble and microtubules translocate to the cell cortex. Proplatelet formation commences with the development of thick pseudopods. (D) Sliding of overlapping microtubules drives proplatelet elongation as organelles are tracked into proplatelet ends, where nascent platelets assemble. Proplatelet formation continues to expand throughout the cell while bending and branching amplify existing proplatelet ends. (E) The entire megakaryocyte cytoplasm is converted into a mass of proplatelets, which are released from the cell. The nucleus is eventually extruded from the mass of proplatelets, and individual platelets are released from proplatelet ends. Reproduced with permission from the American Society for Clinical Investigation from Fig. 4, Page 3352 in Patel SR, Hartwig JH, Italiano JE (2005) The Biogenesis of platelets from megakaryocyte precursors. Journal of Clinical Investigation, 115, 3348-3354.

process exhibits some characteristics of classical apoptosis or programmed cell death, including nuclear condensation and expression of various apoptotic markers in MK and their differential expression in platelets.[10-12] The proplatelets are now thought to serve as a transport system for the export of all the classical platelet components including granules, mitochondria, and even Golgi apparatus and rough ER from the mature MK cytoplasm along microtubules to the proplatelet ends where platelet sized swellings form and are released (***Fig. 1***).[5,9] The platelets released *in vitro* are remarkably similar in size, structure and function to their counterparts in the bloodstream suggesting that this is a valid model of platelet formation.[6,13] This model may also prove useful for generating large numbers of platelets *in vitro* for therapeutic use with the obvious advantages over blood derived concentrates.[14] Although this model of *in vitro* platelet production has provided some important novel information about the mechanism(s) of MK fragmentation there are may unresolved questions. For instance how relevant is this to the *in vivo* situation, particularly, how and where are platelets released from MKs into the circulation?

WHERE ARE PLATELETS FORMED?

Although MKs are formed and mature within the bone marrow, formation of platelets within the bone marrow environment would not facilitate their efficient delivery into the bloodstream and probably result in significant platelet activation and release of granule components into the marrow microenvironment with consequent pathology as observed in Gray Platelet Syndrome where myelofibrosis can occur due to the release of alpha granular growth factors.[15] The data from the *in vitro* culture of maturing MKs also suggests that platelet formation can occur without the confines of the special "niche" environment provided within the bone marrow. Mature MKs are highly chemotactic and electron microscopy has shown that they can extend proplatelets between the gap junctions of bone marrow sinusoidal endothelial cells. Indeed this process seems to be dependent upon a specific interaction of MK expressed VLA-4 with VCAM-1 on the endothelial cells.[6] Platelets would now be able to bud away from the extended proplatelet directly into the blood. There is also some evidence to suggest that larger MK fragments or even proplatelets themselves bud off

into the blood and then further fragment or mature into platelets within the circulation.[16,17] The extension of proplatelets into the bloodstream may also represent an intermediate step in the migration of the entire cell into the bloodstream and indeed large MKs can be identified as rare events within the peripheral blood. MKs are actually quite abundant within the pulmonary and splenic circulation. This has resulted in a theory that platelets are formed within the lung capillaries from mature circulating MKs that become lodged because of their large size. In support of this theory, platelet counts and naked MK nuclei numbers are found to be higher in the pulmonary vein than in the pulmonary artery.[6] Also the human MK count is significantly higher in the pulmonary artery than in the aorta. Patients undergoing cardiopulmonary bypass have also been shown to have increased numbers of large MKs in their peripheral blood.[18] It has been estimated that about 250,000 megakaryocytes/hour are delivered to and fragment within the pulmonary circulation and that the total contribution of this unique mechanism to the overall platelet count could be anywhere between 7-100%.[6,19] Also in pregnancy it has been hypothesized that the placenta is the site of platelet formation until the lungs become fully functional at birth.[20] It is possible that all the mechanisms described above may all contribute to the maintenance of the normal platelet count. Now that real time imaging of platelets can be performed *in vivo*, it may be possible to apply this technology to viewing real-time platelet formation from injected mature MKs cultured *in vitro* or specifically labelled (e.g. using GFP) within the bone marrow.

WHAT IS THE FATE OF THE CIRCULATING PLATELET?

As the majority of circulating platelets are not consumed for the maintenance of vascular integrity then they will continue to circulate for their normal lifespan of 10 days. During its lifespan the platelet undergoes a series of subtle changes with evidence of some loss of haemostatic function and changes in size in humans (younger platelets on average being larger) resulting in further platelet heterogeneity.[21] Therefore another important question relates to the identification and removal of senescent platelets from the circulation. This will be equally important to maintain the platelet count and remove potentially dysfunctional cells from the circulation. At the end of their life, senescent platelets are efficiently removed primarily by the macrophages in either the spleen or liver. Senescent platelets may theoretically express a range of unique marker(s) not present on younger platelets which are instantly recognised by macrophages and then removed from the circulation. This process must involve a series of closely linked mechanisms including macrophage recognition, binding, signalling and finally phagocytosis of the senescent platelet and is probably similar to mechanisms involved in recognition and removal of apoptotic cells[22] The rate of this process must also be potentially equivalent to the rate of platelet production in steady state conditions. This actual mechanism may involve recognition of clustering of the GPIb-IX-V complex (e.g. as with cold stored platelet concentrates),[23] cleavage of glycocalicin, expression of apoptotic markers (e.g. phosphatidyl serine), loss of mitochondrial membrane potential, changes in surface glycosylation, decreased levels of sialic acid, accumulation of surface bound IgG or as yet unknown marker(s).[21] However the exact mechanism(s) has yet to be fully determined. Increased understanding of this mechanism is important as it may facilitate the long term cold storage of platelet concentrates and could help in the therapeutic modulation of the lifespan of platelets in patients with thrombocytopenia.

HOW IS THE CIRCULATING PLATELET NUMBER REGULATED?

There is clearly a rapid sensory system for regulating the platelet count. Induction of thrombocytopenia results in a rapid response by bone marrow MKs with a shift in their modal ploidy distribution and the release of larger platelets.[24] The opposite occurs when the platelet count is artificially increased. Many studies have also shown a classical inverse relationship between the platelet count and the volume distribution. Many thrombocytopenic conditions are associated with larger circulating platelets and increased MPV. Conversely in patients with high platelet counts (e.g. essential thrombocythemia) there is usually a decrease in platelet size and MPV. This suggests that there is a direct feedback mechanism from the peripheral blood to the bone marrow that regulates thrombopoiesis and efficiently controls the circulating mass of platelets. In any one individual this is probably set at a particular level and may explain why the measurement of the platelet count and volume remains reproducible in the same person under normal conditions. MK maturation, platelet development and formation has been shown to be regulated by many different cytokines at multiple levels including TPO, SDF-1,GM-CSF, IL-3, IL-6, IL-11, LIF and Kit ligand.[1] However, IL-3 can drive only the early stages of development and the majority of the other cytokines tend to function in concert with TPO. TPO is therefore thought to be the principal humoral regulator of platelet development.[25] Although the name TPO was first proposed in the 1950s, due to many practical difficulties it took nearly 40 years for the protein to be eventually identified, purified and cloned by 5 separate groups.[26] This resulted in an explosion of research into MK maturation, platelet production and the potential clinical application(s) of this hormone and related molecules. TPO has been shown to regulate all stages of MK development from the stem cell through to cytoplasmic maturation. TPO is therefore now established as the predominant regulator of thrombopoiesis and is primarily synthesized within the liver, kidneys and bone marrow.[1] The rate of TPO synthesis is constitutive and remains fairly constant under steady state conditions. Blood levels of TPO have been found to be inversely related to the total platelet or MK mass and infusion of recombinant TPO massively increases platelet production.[1] TPO binds to a specific high affinity receptor (c-MPL) found within MKs and platelets. This results in signalling events, receptor mediated endocytosis and destruction of the

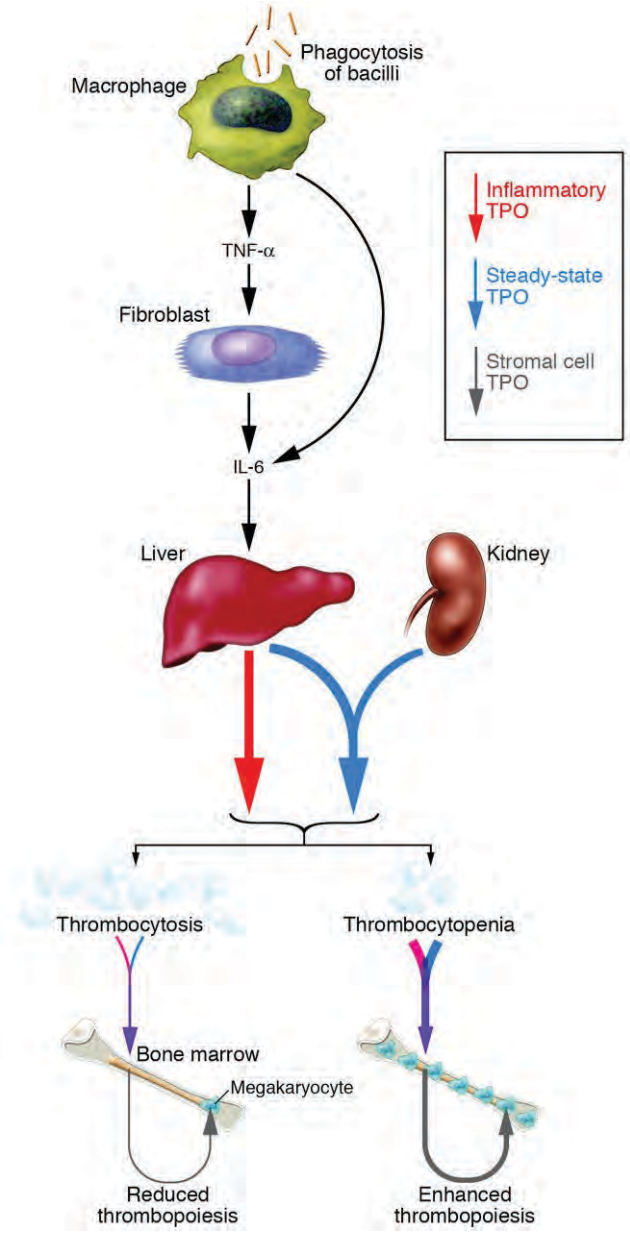


Fig. 2 *The regulation of thrombopoietin levels*
A steady-state amount of hepatic thrombopoietin (TPO) is regulated by platelet c-Mpl receptor-mediated uptake and destruction of the hormone. Hepatic production of the hormone is depicted. Upon binding to platelet c-Mpl receptors, the hormone is removed from the circulation and destroyed, which reduces blood levels. In the presence of inflammation, IL-6 is released from macrophages and, through TNF-α stimulation, from fibroblasts and circulates to the liver to enhance thrombopoietin production. Thrombocytopenia also leads to enhanced marrow stromal cell production of thrombopoietin, although the molecular mediator(s) of this effect is not yet completely understood. Reproduced with permission from the American Society Clinical Investigation from Fig. 1, page 3340 in Kaushansky K (2005) The molecular mechanisms that control thrombopoiesis. Journal of Clinical Investigation, 115, 3339-3347.

TPO and provides a key autoregulatory loop for regulating the circulating platelet number. However if the platelet count rises, more TPO is removed from the circulation resulting in decreased bone marrow MK stimulation of thrombopoiesis. Conversely, in thrombocytopenia, there are less platelets to remove TPO from the circulation so the free level increases resulting in increased stimulation of bone marrow thrombopoiesis (***Fig. 2***).[1)] Studies from either TPO or c-MPL knockout mice support the importance of this receptor-ligand pair in platelet development as these mice exhibit only 10% of the normal platelet count or MK/platelet mass.[27,28)] This suggests that there may be other additional factors that control the platelet production as the platelet counts were not reduced to zero. Double knockout mice have shown than the IL-6 family of cytokines that signal via gp130 are not critical for the controlling the production of the remaining platelets in this situation. This doesn't mean than these cytokines do not play an important role, as they can not only stimulate TPO production (***Fig. 2***) but injection of IL-6 and IL-11 can also result in the production of larger so called stressed platelets and it is possible in cardiovascular disease that the underlying inflammatory condition could dramatically alter thrombopoiesis. Indeed the MPV as been suggested to be an independent risk factor for heart disease.[29,30)] Other genetic studies have shown that the importance of a transcriptional programme that controls the differentiation of stem cells through to MK development and maturation.[5)] Important relevant transcription molecules include GATA-1 and NF-E2.[5)] However various cytoskeletal components including β1 tubulin, the GPIb-IX-V-filamin-actin linkage and non muscle myosin heavy chain all have also been shown to be important for normal platelet production.[5)]

A NEW AUTOMATED CLINICAL ASSAY OF PLATELET PRODUCTION

Newly released platelets have been shown to contain increased levels of mRNA and have been traditionally termed reticulated platelets. Measurement of reticulated platelets may therefore provide a potential measure of the rate of platelet production or thrombopoiesis and be analogous to the more established clinical measurement of the red cell reticulocyte count. They were first described in 1969 by using new methylene blue labelling of platelets on a blood film (***Fig. 3***).[31)]

They can also be detected by using nucleic acid stains (e.g. thiazole orange) in conjunction with flow cytometry.[32)] *In vivo* biotinylation experiments within animal models have provided the definitive proof that the nucleic acid positive platelets are indeed the youngest cells.[33-36)] The majority of mRNA in reticulated platelets is therefore thought to be unstable (< 24 hours), so the theoretical levels of these immature platelets in humans should be no greater than 10% of the platelet count under normal conditions of platelet production. Despite this recent evidence suggests that platelets may also contain a protected pool of specialised mRNA within the cytoskeleton which

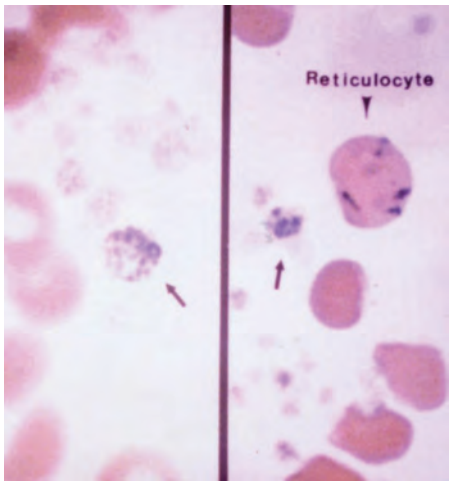


Fig. 3 *New methylene blue labelling of reticulated platelets and red cell reticulocytes on a human blood film*
Reproduced with kind permission from Dr. Kiyoaki Watanabe, International University of Health and Welfare, Japan.

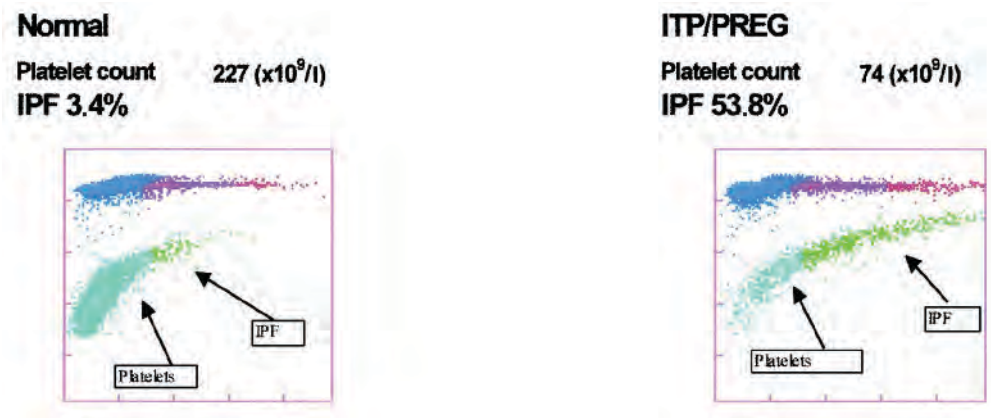


Fig. 4 *Optical platelet scattergrams from a healthy individual with a normal IPF and a patient with a high IPF*
Mature platelets appear as blue dots, green dots represent the IPF with increased cell volume and higher fluorescence intensity compared to mature platelets. Reproduced with permission from Fig. 1, page 94 in Briggs et al, (2004) Assessment of an immature platelet fraction (IPF) in peripheral thrombocytopenia. British Journal of Haematology, 126, 93-99 and published by Blackwell Publishing Ltd.

under appropriate stimulation can be translated into a set of proteins that may be important for platelet physiology.[37-39] However, it is unknown what percentage of the total mRNA this pool represents and if is stable for the entire lifespan of the platelet. Although flow cytometric measurement of reticulated platelets has been described as a measure of platelet production *in vivo* and has some clinical potential for the diagnosis of thrombocytopenia and monitoring platelet count recovery, there have been significant problems with standardisation of this methodology including widely different reported normal ranges, non-specific labelling and poor concordance between laboratories using the same method and reagents.[40-43] As the method also requires a specialised operator and flow cytometer it has therefore been largely restricted to research use. However, with the convergence of flow cytometry principles into modern haematology impedance analysers it is now possible to also use fluorescent dyes to additionally label cells in these systems. The Sysmex XE-2100 automated full blood counter when initially introduced utilised a nucleic acid dye (polymethine dye) that is excited by a red laser (at 633 nm) to not only detect immature red cell reticulocytes but to stain the entire platelet population to provide an optical fluorescent platelet count.[44] This count was shown to more accurate than the impedance count particularly in samples with macrothrombocytopenia but has also been shown to be prone to interference in certain circumstances.[44,45] It soon became apparent that in certain samples (e.g. thrombocytopenia) a proportion of the largest and most likely youngest platelets were also more highly fluorescent than the majority of platelets suggesting that the method may also detect immature platelets (***Fig. 4***). More recently in 2005, Sysmex introduced the immature platelet fraction (IPF) Master software module to allow accurate quantification of the IPF in blood samples with-

in the XE-2100 series. This software allows automated scattergram analysis (***Fig. 4***) and gating using a specific algorithm and the IPF% is expressed as a proportional value of the total platelet count and provides a rapid indication of the rate of platelet production from the bone marrow. An increased IPF% is indicative of increased thrombopoiesis and an active bone marrow. IPF Master can therefore differentiate between decreased platelet production/bone marrow failure and increased platelet consumption in the peripheral circulation as the cause of thrombocytopenia.

Data emerging from a number of different laboratories now indeed suggests that the method is precise, provides identical normal ranges and provides a potentially valuable diagnostic tool for differentiating between consumptive and aplastic causes of thrombocytopenia.[46-50] The IPF is now FDA approved and also seems more sensitive than other platelet parameters (e.g. MPV) at predicting platelet recovery and unlike such impedance parameters (which depend upon generation of a volume distribution curve) always gives a result in severe thrombocytopenic samples.[49] The IPF should not only reduce the need for invasive bone marrow examinations but provides a fully automated, inexpensive and standardised measure of the rate of platelet production that may prove to be clinically useful in discriminating between disorders of platelet production or platelet destruction and potentially reducing unnecessary platelet transfusions in severe thrombocytopenia.[46-50]

SUMMARY

Our knowledge of the regulation of the platelet production and number has dramatically increased over the last 10 years, particularly after the cloning of TPO. Video microscopy of TPO stimulated *in vitro* cultured mature MKs has provided stunning insight into the process of platelet biogenesis and it should just be a matter of time before we fully understand exactly how and where this vital process is occurring *in vivo*. These advances coupled with newly available platelet parameters such as the IPF on automated full blood counters will continue to improve the treatment and diagnosis of various thrombocytopenic and thrombocythemic disorders. Much further research into both platelet production and senescence is therefore warranted and could provide important new therapeutic options and more useful clinical measurements for monitoring and treatment of the various abnormalities of platelet number.

KEY POINTS

- Maintenance of the platelet count within normal limits ($150\text{-}400\times10^9$/L) is vital for normal haemostasis. The platelet count within a normal individual is maintained within fairly narrow limits in terms of platelet mass (count×volume) under normal conditions.
- Anucleated platelets only live for a comparatively short life span (10 days) and approximately 1×10^{11} platelets have to be produced every day to maintain the count. This has to be counter-balanced by either their consumption in haemostasis or in the case of the majority by senescence mechanisms and efficient phagocytosis by macrophages at the end of their lifespan.
- Platelets develop and are released into the bloodstream from their parent cell the bone marrow megakaryocyte (MK). Although platelet formation has been shown to occur within *in vitro* culture systems it is still unclear exactly how and where platelets are released *in vivo*.
- The platelet count is regulated predominantly by the cytokine Thrombopoietin (TPO). TPO is usually synthesized at a constant rate by the liver and can be measured within normal plasma. The free level of TPO that can stimulate thrombopoiesis is controlled by the total circulating and bone marrow platelet/MK mass. Platelets and MK express the TPO receptor or c-MPL which signals, internalises and degrades TPO on a constant basis.
- Immature or Reticulated platelets are more positive for mRNA for the first 24 hours of their life and can simply be detected using nucleic acid dyes by flow cytometry. Although their measurement provides an index of the rate of platelet production there is a significant problem with the standardization of this methodology.
- The Immature Platelet Fraction (IPF) is now available as a fully automated, precise, rapid, FDA approved and relatively cheap non invasive method of measuring the rate of platelet production. It has already been shown to be a useful diagnostic tool for differentiating between consumptive and aplastic causes of thrombocytopenia and will be potentially useful for predicting platelet count recovery and eliminating unnecessary platelet transfusions and bone marrow biopsies.

Note added during Proof Reading:
It has recently been described "that the intrinsic program for apoptosis (i.e. The antagonistic balance between Bcl-x(L) and Bak) seems to control platelet survival and dictates their life span"

- *Mason KD, Carpinelli MR, Fletcher JI et al. Programmed anuclear cell death delimits platelet life span. Cell. 2007;128(6):1173-86.*
- *Qi B, Hardwick JM. A Bcl-xL timer sets platelet life span. Cell. 2007 Mar 23;128(6):1035-6.*

References

1) *Kaushansky K. The molecular mechanisms that control thrombopoiesis. J.Clin.Invest. 2005;115:3339-3347.*
2) *George JN. Platelets. Lancet. 2000;355:1531-1539.*
3) *Watson SP, Harrison P. The Vascular Function of Platelets. In: Hoffbrand V, Tuddenham E, Catovsky D, eds. Postgraduate Haematology.: Blackwell Publishing.2005:808-824.*
4) *Wright J. The origin and nature of blood platelets. Boston Med Surg J. 1906;154:643-645.*
5) *Patel SR, Hartwig JH, Italiano JE, Jr. The biogenesis of platelets from megakaryocyte proplatelets. J.Clin.Invest. 2005;115:3348-3354.*
6) *Italiano J, Hartwig J. Megakaryocyte Development and Platelet Formation. In: Michelson AD, ed. Platelets.: Elsevier Academic Press. 2007:23-44.*
7) *Cramer EM, Norol F, Guichard J et al. Ultrastructure of platelet formation by human megakaryocytes cultured with the Mpl ligand.*

Blood.1997;89:2336-2346.

8) *Kaushansky K. Thrombopoietin: biological and preclinical properties. Leukemia. 1996;10 Suppl 1:S46-S48.*
9) *Italiano JE, Jr., Lecine P, Shivdasani RA, Hartwig JH. Blood platelets are assembled principally at the ends of proplatelet processes produced by differentiated megakaryocytes. J.Cell Biol. 1999;147:1299-1312.*
10) *Kaluzhny Y, Ravid K. Role of apoptotic processes in platelet biogenesis. Acta Haematol. 2004;111:67-77.*
11) *De BS, Sabri S, Daugas E et al. Platelet formation is the consequence of caspase activation within megakaryocytes. Blood. 2002;100:1310-1317.*
12) *Li J, Kuter DJ. The end is just the beginning: megakaryocyte apoptosis and platelet release. Int.J Hematol. 2001;74:365-374.*
13) *Choi ES, Hokom MM, Chen JL et al. The role of megakaryocyte growth and development factor in terminal stages of thrombopoiesis. Br.J.Haematol. 1996;95:227-233.*
14) *Matsunaga T, Tanaka I, Kobune M et al. Ex vivo large-scale generation of human platelets from cord blood CD34+ cells. Stem Cells. 2006;24:2877-2887.*
15) *Nurden AT, Nurden P. The gray platelet syndrome: Clinical spectrum of the disease. Blood Rev. 2007;21:21-36.*
16) *Kosaki G. In vivo platelet production from mature megakaryocytes: does platelet release occur via proplatelets? Int.J.Hematol. 2005;81:208-219.*
17) *Behnke O, Forer A. From megakaryocytes to platelets: platelet morphogenesis takes place in the bloodstream. Eur.J Haematol.Suppl.1998;61:3-23.*
18) *Woods MJ, Greaves M, Smith GH et al. The fate of circulating megakaryocytes during cardiopulmonary bypass. J Thorac.Cardiovasc.Surg. 1993;106:658-663.*
19) *Trowbridge EA, Martin JF, Slater DN. Evidence for a theory of physical fragmentation of megakaryocytes, implying that all platelets are produced in the pulmonary circulation. Thromb.Res. 1982;28:461-475.*
20) *Woods MJ, Landon CR, Wagner BE et al. Isolation of circulating megakaryocytes in man. Med Lab Sci. 1992;49:252-258.*
21) *Batar P, Dale GL. Platelet Turnover and Ageing. In: Michelson AD, ed. Platelets.: Elsevier, Academic Press. 2002:53-64.*
22) *Gregory CD, Devitt A. The macrophage and the apoptotic cell: an innate immune interaction viewed simplistically? Immunology. 2004;113:1-14.*
23) *Hoffmeister KM, Felbinger TW, Falet H et al. The clearance mechanism of chilled blood platelets. Cell. 2003;112:87-97.*
24) *Trowbridge EA, Martin JF. An analysis of the platelet and polyploid megakaryocyte response to acute thrombocytopenia and its biological implications. Clin.Phys.Physiol Meas. 1984;5:263-277.*
25) *Kaushansky K. Thrombopoietin: a tool for understanding thrombopoiesis. J.Thromb.Haemost. 2003;1:1587-1592.*
26) *Kaushansky K. Thrombopoietin: basic biology, clinical promise. Int.J.Hematol. 1995;62:7-15.*
27) *Bunting S, Widmer R, Lipari T et al. Normal platelets and megakaryocytes are produced in vivo in the absence of thrombopoietin. Blood. 1997;90:3423-3429.*
28) *Gurney AL, Carver-Moore K, de Sauvage FJ et al. Thrombocytopenia in c-mpl-deficient mice. Science. 1994;265:1445-1447.*
29) *van der LB, Martin JF. A role for changes in platelet production in the cause of acute coronary syndromes. Arterioscler. Thromb. Vasc.Biol. 1999;19:672-679.*
30) *Martin JF. Megakaryocytes and thrombopoiesis in vascular disease. Prog.Clin.Biol.Res. 1990;356:319-331.*
31) *Ingram M, Coopersmith A. Reticulated platelets following acute blood loss. Br.J.Haematol. 1969;17:225-229.*
32) *Kienast J, Schmitz G. Flow cytometric analysis of thiazole orange uptake by platelets: a diagnostic aid in the evaluation of thrombocytopenic disorders. Blood. 1990;75:116-121.*
33) *Dale GL. Platelet kinetics. Curr.Opin.Hematol. 1997;4:330-334.*
34) *Ault KA, Knowles C. In vivo biotinylation demonstrates that reticulated platelets are the youngest platelets in circulation. Exp.Hematol. 1995;23:996-1001.*
35) *Robinson M, Machin S, Mackie I et al. In vivo biotinylation studies: specificity of labelling of reticulated platelets by thiazole orange and mepacrine. Br.J.Haematol. 2000;108:859-864.*
36) *Dale GL, Friese P, Hynes LA et al. Demonstration that thiazole-orange-positive platelets in the dog are less than 24 hours old. Blood. 1995;85:1822-1825.*
37) *Weyrich AS, Lindemann S, Tolley ND et al. Change in protein phenotype without a nucleus: translational control in platelets. Semin.Thromb.Hemost. 2004;30:491-498.*
38) *Denis MM, Tolley ND, Bunting M et al. Escaping the nuclear confines: signal-dependent pre-mRNA splicing in anucleate platelets. Cell. 2005;122:379-391.*
39) *Weyrich AS, Denis MM, Schwertz H et al. mTOR-dependent synthesis of Bcl-3 controls the retraction of fibrin clots by activated human platelets. Blood. 2006*
40) *Matic GB, Chapman ES, Zaiss M et al. Whole blood analysis of reticulated platelets: improvements of detection and assay stability. Cytometry. 1998;34:229-234.*
41) *Rinder HM, Munz UJ, Ault KA et al. Reticulated platelets in the evaluation of thrombopoietic disorders. Arch.Pathol.Lab Med. 1993;117:606-610.*
42) *Robinson MS, MacKie IJ, Khair K et al. Flow cytometric analysis of reticulated platelets: evidence for a large proportion of non-specific labelling of dense granules by fluorescent dyes. Br.J.Haematol. 1998;100:351-357.*
43) *Ault KA, Rinder HM, Mitchell J et al. The significance of platelets with increased RNA content (reticulated platelets). A measure of the rate of thrombopoiesis. Am.J.Clin.Pathol. 1992;98:637-646.*
44) *Briggs C, Harrison P, Grant D et al. New quantitative parameters on a recently introduced automated blood cell counter-the XE 2100™. Clin.Lab Haematol. 2000;22:345-350.*
45) *Segal HC, Briggs C, Kunka S et al. Accuracy of platelet counting haematology analysers in severe thrombocytopenia and potential impact on platelet transfusion. Br.J.Haematol. 2005;128:520-525.*
46) *Zucker ML, Murphy CA, Rachel JM et al. Immature platelet fraction as a predictor of platelet recovery following hematopoietic progenitor cell transplantation. Lab Hematol. 2006;12:125-130.*
47) *Briggs C, Kunka S, Hart D et al. Assessment of an immature platelet fraction (IPF) in peripheral thrombocytopenia. Br.J.Haematol. 2004;126:93-99.*
48) *Briggs C, Hart D, Kunka S et al. Immature platelet fraction measurement: a future guide to platelet transfusion requirement after haematopoietic stem cell transplantation. Transfus.Med. 2006;16:101-109.*
49) *Abe Y, Wada H, Tomatsu H et al. A simple technique to determine thrombopoiesis level using immature platelet fraction (IPF). Thromb.Res. 2006;118:463-469.*
50) *Kickler TS, Oguni S, Borowitz MJ. A clinical evaluation of high fluorescent platelet fraction percentage in thrombocytopenia. Am.J Clin.Pathol. 2006;125:282-287.*