Blood platelets and Charles Darwin's natural selection

Stefania Momi, Paolo Gresele

Section of Internal and Cardiovascular Medicine, Department of Medicine and Surgery, University of Perugia, Italy

INTRODUCTION

Blood platelets are indubitably one of the wonders of the world, whether the first of the eight may be a matter of debate, but they certainly are. Compared with all other cells they are much smaller and, like few others, devoid of a nucleus but despite this, they are extremely complex and provided with multiple functions.[1,2]

Their peculiar shape and dimensions lend them special skills. The discoid shape allows platelets to have a much larger surface area compared with a normal globular cell of the same size and their small dimensions, by reducing the distance their granules must travel in the cytoplasm before reaching the plasma membrane, favor the rapid secretion of several molecules crucial for primary hemostasis.[3-5]

In the animal kingdom, platelets are unique to mammals, which include around 6,400 extant species out of 1.5-1.8 million living species on earth, *i.e.* only 0.35-0.42% of all living organisms.

WHAT IS KNOWN ABOUT THE PHYLOGENESIS OF MAMMALIAN PLATELETS

Several aspects of the phylogenesis of mammalian platelets have been clarified in the last decades.[6] In all *mammalia*, platelets have similar functions and structure, as small anucleate secretory cells derived from precursor polyploid megakaryocytes. Their ability to exert a wide repertoire of complex functions, which span from adhesion and aggregation at a site of vessel wall damage, to chemotaxis, phagocytosis, antigen presentation, regulation of cell growth and differentiation, participation in atherosclerosis, *etc.*,[7-9] likely dates back to an era in which all innate host defenses were accomplished by one single cell type, or at most by a small number of cells, instead than by the extensive and specialized cellular repertoire which has then developed in mammalians' blood (Figure 1).

Invertebrates possess an open circulatory system (the hemolymph) with only one type of circulating cell, the hemocyte, that among others also plays the hemostatic role being able to aggregate and seal wounds. Coelomocytes, the primary immune cells of earthworms, have many functions resembling mammalian platelets, including chemotaxis, and phagocytosis,[10] and express Toll-like receptors and favor the formation of extracellular traps.[11] The hemolymph of many insects contains coagulocytes which, upon contact with a foreign surface, extrude long processes containing cytoplasmic granules able to create a hemostatic plug.[12] In *Drosophila melanogaster* more than 90% of all hemocytes are plasmatocytes,[13] which perform important functions during animal development and, in the response to infection and tissue damage. Sea urchin coelomocytes accumulate at sites of injury in response to chemotactic stimuli, generate sealing and encapsulating clots that are dependent on cell-cell adhesion, and have secretory and phagocytic functions.[14] In *Arthropoda*, amoebocytes are responsible for hemostasis. In the *Limulus polyphemus*, the American horseshoe crab, the major

Correspondence: Stefania Momi, Department of Medicine and Surgery, Section of Internal and Cardiovascular Medicine, University of Perugia, Centro didattico - Edificio B piano 1, Strada vicinale Via delle Corse, 06132, Perugia.
Tel.: +39.0755783989.
E-mail: stefaniamomi@gmail.com

Citation: Momi S, Gresele P. Blood platelets and Charles Darwin's natural selection. Bleeding, Thrombosis, and Vascular Biology 2023;2:63.

Key words: platelets; phylogeny.

Conflicts of interest: the authors declare no potential conflict of interest.

Funding: none.

Received: 7 February 2023.
Accepted: 1 March 2023.

Bleeding, Thrombosis and Vascular Biology 2023; 2:63
doi:10.4081/btvb.2023.63

defense systems in hemolymph are carried out by the so-called amoebocyte/granulocyte,[15] which has primitive wound sealing functions and is able to aggregate in response to lipopolysaccharide (LPS) and to release a cascade of antimicrobial substances, such as the anti-LPS factor, lectins, protease inhibitors (including trypsin inhibitor and α2-macroglobulin), and also coagulation factors (Factor C, Factor B, Factor G, proclotting enzymes, coagulogen) involved in the engulfing and killing of invading microbes and in preventing hemolymph leakage.[16]

Except for *mammalia*, in all other classes of the animal kingdom cells involved in hemostasis are nucleated. Non-mammalian vertebrates have nucleated, often spindle-shaped, thrombocytes, that represent the first cells that specialized in haemostasis during evolution.[17] Zebrafish (*Danio rerio*) and other fish species have nucleated thrombocytes that, although broadly similar to platelets in terms of function,[18] do not aggregate in response to adenosine diphosphate and epinephrine,[19] but still play a role in the development of arterial thrombi.[20] Reptiles, birds, and amphibians also have nucleated thrombocytes that differ from one another for cytoplasmic granules. The cytoplasm in reptiles contains actin-like filaments in the perinuclear area and a smooth endoplasmic reticulum throughout the peripheral cytoplasm.[21] An extensive ultrastructural study of six domestic species of birds showed that their thrombocytes are similar in size to lymphocytes but have a denser nucleus, a very highly vacuolated cytoplasm and a membrane surface-connected canalicular system similar to mammalian platelets. Thrombocytes are the most common blood cell in the chicken after erythrocytes, contain 5-hydroxytryptamine,[22] and release proteins including a fraction that seems to correspond to β-thromboglobulin.[23] Bird thrombocytes do not form vaso-occlusive thrombi after arterial vessel wall injury *in vivo* and indeed birds have more prolonged bleeding.[24] A recent study comparing the hemostatic cells of five mammals with 48 bird species across the avian phylogeny, 12 reptiles, three amphibian and three fish species, showed that the cytoplasmic domain of GPIbα is lacking in birds and this might help to explain why, unlike mammalian platelets,

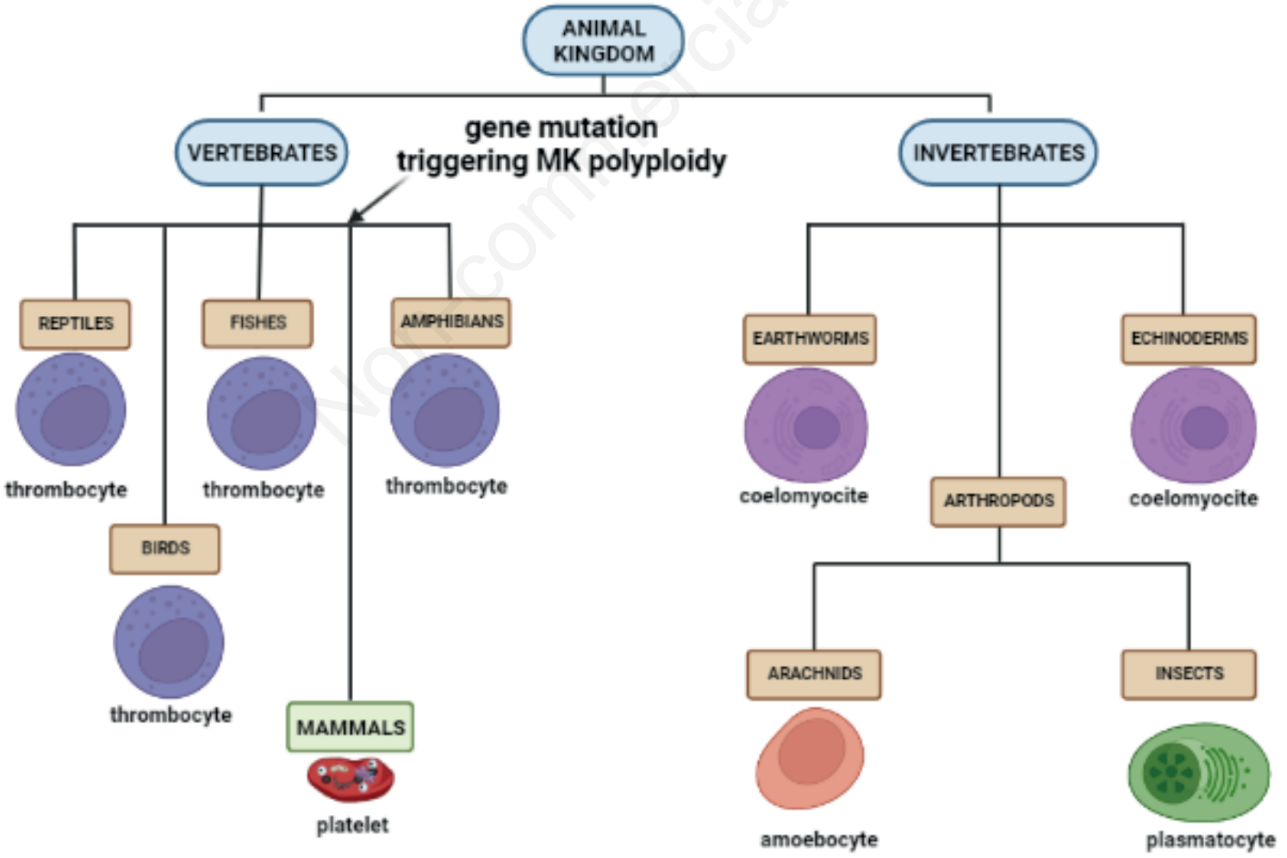

Figure 1. Simplified phylogenetic tree showing the evolutionary relationship between animal species and the relative evolution of multifunctional innate defensive and hemostatic cells to mammalian blood platelets. The hypothesized gene mutation event at the origin of mammalian platelets is shown. Created with BioRender.com.

avian thrombocytes take longer to produce a clot able to control bleeding.[25]

Non-nucleated platelets, and presumably their polyploid precursor bone marrow megakaryocytes, are present only in mammals, suggesting that this unique mechanism producing an unprecedented, highly specialized anucleated cell from the cytoplasm of a larger cell, allows to provide them with a highly efficient hemostatic function. Although platelets from different mammalian species show some dissimilarities in their biochemistry, physiology and morphology, their main functions are conserved.[26]

The evolutionary events that resulted in the appearance of mammalian megakaryocytes and platelets, as well as the potential biological advantage of this system, remain to be further clarified.

WHAT A NEW INTRIGUING HYPOTHESIS SUGGESTS

A recent fascinating new hypothesis, based on a series of new but scattered information on the structure and generation of platelets acquired over the last decade, places a gene mutation occurred around 220 million years ago at the origin of current mammalian platelets.

This hypothesis puts emphasis on two distinctive characteristics of blood platelets: the polyploidy of their bone marrow precursor cells, megakaryocytes, and the log Gaussian cell volume distribution of platelets. Indeed, megakaryocytes are one among the very few non-pathologic polyploid mammalian cells and all mammalian cells generated by a mitotic event have a Gaussian volume distribution.

An increase in ploidy is always associated with an increase in cell size, and in fact megakaryocytes are gigantic cells. An old theory of thrombopoiesis, recently revitalized by astounding *in vivo* imaging studies in mice, maintains that blood platelets are generated in lungs by megakaryocytes penetrated in the circulation through bone marrow sinusoids and fragmentated by the impact with the lung microcirculatory bed.[27,28]

The new hypothesis suggests that a random inheritable variation in a gene controlling cell division occurred in a nucleated cell precursor of current megakaryocytes in a single animal, most likely a predecessor of the modern monotremes, around 220 million years ago and altered the control of cell division causing polyploidization. Consequently, megakaryocytes became giant cells which, when passing in the bloodstream, would be fragmented in myriads of cytoplasmatic pieces generating platelets with much more efficient hemostatic properties than the previous, larger nucleated thrombocytes, thus conferring to the animal carrying this mutation an evolutionary advantage.[29] The observations that the nucleated chicken thrombocytes indeed form aggregates less resistant to high fluid shear forces than mammalian platelets,[24] and that experimental cell fragmentation generated by the application of physical forces gives rise to a collection of particles which follow a log Gaussian volume distribution[30] are in agreement with this hypothesis. Given that the new characteristics of platelets favoured a more efficient arrest of bleeding, the progeny of the animal in which the gene mutation initially occurred would have had an evolutionary advantage through natural selection, representing a key step in the evolution of mammals.[29] Subsequent further adaptive evolutionary events would have then brought to nowadays mammalian megakaryocytes and platelets.

CONCLUSIONS

Insight into the structure, function and role of platelets in health and disease continues to expand and from the initial belief that they were rudimentary cytoplasmic fragments we have now arrived to see that platelets are highly complex, multitasking cells provided with fundamental roles in hemostasis, immunity, inflammation, angiogenesis, cancer, *etc.*[31] This complexity is probably the consequence of the increased number of genes (over 5000) present in megakaryocytes[32] thanks to polyploidy[2,33] with the related increased protein production (over 3000 functional proteins). Blood platelets, thus, might represent a paradigmatic example of the way species adapt to the environment and change thanks to advantageous random genetic mutations, providing one further proof of Charles Darwin's theory on the origin of species and of the events that have led to the evolution of mammalians, a tiny group of animals within the animal kingdom, and ultimately to the appearance of man.

REFERENCES

1. van der Meijden PEJ, Heemskerk JWM. Platelet biology and functions: new concepts and clinical perspectives. Nat Rev Cardiol 2019;16:166-79.
2. Bury L, Gresele P. The amazing genetic complexity of anucleated platelets. Bleed Thromb Vasc Biol 2022;1. doi.org/10.4081/ btvb.2022.33
3. Mumford, Frelinger AL 3rd, Gachet C, et al. A review of platelet secretion assays for the diagnosis of inherited platelet secretion disorders. Thromb Hemost 2015;114: 14-25.
4. Rendu F, Brohard-Bohn B. The platelet release reaction: granules' constituents, secretion and functions. Platelets 2001;12:261-73.
5. Holmsen H, Day HJ. Thrombin-induced platelet release reaction and platelet lysosomes. Nat 1968;219:760-1.
6. Momi S, Wiwanitkit V. Phylogeny of blood platelets. In: Platelets in Thrombotic and nonthrombotic disorders: an update. Gresele P, Kleiman NS, Lopez JA, Page CP Eds. Springer 2017:11-9.

7. Gaertner F, Ahmad Z, Rosenberger G, et al. Migrating Platelets Are Mechano-scavengers that Collect and Bundle Bacteria. Cell 2017;171:1368-82e23.
8. Petito E, Momi S, Gresele P. Platelet chemotaxis and the interaction with other migrating cells. In: Platelets in Thrombotic and nonthrombotic disorders: an update. Gresele P, Kleiman NS, Lopez JA, Page CP, Eds. Springer 2017: 337-51.
9. Pitchford SC, Momi S, Baglioni S, et al. Allergen induces the migration of platelets to lung tissue in allergic asthma. Am J Respir Crit Care Med 2008;177:604-12.
10. Cooper EL, Kauschke E, Cossarizza A. Digging for innate immunity since Darwin and Metchnikoff. Bioessays 2002;24:319-33.
11. Joanna Homa J. Earthworm coelomocyte extracellular traps: structural and functional similarities with neutrophil NETs. Cell Tissue Res 2018;371:407-14.
12. Theopold U, Schmidt O, Soderhall K, Dushay MS. Coagulation in arthropods: defence, wound closure and healing. Trends Immunol 2004;25:289-94.
13. Rizki T, Rizki R. The cellular defense system of Drosophila melanogaster. Insect Ultrastruct 1984;2:579-604.
14. Rothenberg E, Davidson EH. Regulatory co-options in the evolution of deuterostome immune systems. In: Ezekowitz RAB, Hoffmann JA (eds). Innate Immunity. Totowa: The Humana Press 2003;61-87.
15. Iwanaga S, Kawabata. Evolution and phylogeny of defense molecules associated with innate immunity in horseshoe-crab. Front Biosci 1998;3:D973-84.
16. Levin J, Bang FB. A description of cellular coagulation in the Limulus. Bull Johns Hopkins Hosp 1964;115:337-45.
17. Ratnoff OD. The evolution of hemostatic mechanisms. Perspect Biol Med 1987;31:4-33.
18. Jagadeeswaran P, Sheehan JP, Craig FE, Troyer D. Identification and characterization of zebrafish thrombocytes. Br J Haematol 1999;107:731-8.
19. Belamarich FA, Fusari MH, Shepro D, Kien M. In vitro studies of aggregation of non-mammalian thrombocytes. Nature 1996;212:1579-80.
20. Thattaliyath B, Cykowski M, Jagadeeswaran P. Young thrombocytes initiate the formation of arterial thrombi in zebrafish. Blood 2005;106:118-24.
21. Wood FE, Ebanks GK. Blood cytology and hematology of the green sea turtle, Chelonia mydas. Herpetologica 1984; 40:331-36.
22. Kuruma I, Okada T, Kataoka K, Sorimachi M. Ultrastructural observation of 5-hydrozytryptamine-storing granules in the domestic fowl thrombocytes. Z Zellforsch Mikrosk Anat 1970;108:268-81.
23. Wachowicz B, Krajewski T. The released proteins from avian thrombocytes. Thromb Haemost 1979;42:1289-95.
24. Schmaier AA, Stalker TJ, Runge JJ, et al. Occlusive thrombi arise in mammals but not birds in response to arterial injury: evolutionary insight into human cardiovascular disease. Blood 2011;118:3661-9.
25. Ribeiro ÂM, Zepeda-Mendoza ML, Bertelsen MF, et al. A refined model of the genomic basis for phenotypic variation in vertebrate hemostasis. BMC Evol Biol 2015;30:124.
26. Dodds JW. Platelet function in animals: species specificities. In: De Gaetano G, Garattini S. Eds. Platelets: a multidisciplinary approach. Raven Press, New York 1978:45-59.
27. 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;28:461-75.
28. Lefrancais E, Ortiz-Muñoz G, Caudrillier A, et al. The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors. Nature 2017;544:105-9.
29. Martin JF, D'Avino PP. A theory of rapid evolutionary change explaining the de novo appearance of megakaryocytes and platelets in mammalians. J Cell Sci 2022;135: jcs260286.
30. Sommer M, Stengerb F, Peukerta W, Wagner NJ. Agglomeration and breakage of nanoparticels in stired media mils – a comparison of different methods and models. Chem Eng Sci 2006;61:135-48.
31. Gresele P, Kleiman NS, Lopez JA, Page CP, Eds.Platelets in Thrombotic and nonthrombotic disorders: an update. Springer, 2017.
32. Fisher MH, Di Paola J. Genomics and transcriptomics of megakaryocytes and platelets: Implications for health and disease. Res Pract Thromb Haemost 2018;2:630-9.
33. Chouldry FA, Bagger FO, Macaulay IC, et al. Transcriptional characterization of human megakaryocyte polyploidization and lineage commitment. J Thromb Haemost 2021;19:1236-49.