Platelet Storage Lesion: A New Understanding From a Proteomic Perspective

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Platelet storage and availability for the purposes of transfusion are currently restricted by a markedly short shelf life of 5 to 7 days owing to an increased risk of bacterial growth and storage-related deterioration called the *platelet storage lesion*. Because most bacteria grow to confluence within 5 days during storage at room temperature, there is little increased risk of bacterial overgrowth with testing in place, and the only remaining issue is the quality of platelets during the extended storage. Although the manifestations of the storage lesion have been well studied using a variety of in vitro measures, the precise biochemical pathways involved in the initiation and progression of this process have yet to be identified. Proteomics has emerged as a powerful tool to identify and monitor changes during platelet storage and, in combination with biochemical and physiologic studies, facilitates the development of a sophisticated mechanistic view. In this review, we summarize recent experimental work that has led to a detailed overview of protein changes linked to platelet functions and signaling pathways, providing potential targets for inhibitors to ameliorate the storage lesion.
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PROTEOMICS, THE ANALYSIS of all proteins of a system at a defined state, has gained increasing interest in hematology as a diagnostic tool. The application of proteomics in transfusion medicine[1,2] holds promise to revolutionize quality assessment and therapeutic monitoring.[3] Several studies have been published on systematic in-depth analysis of the protein content of various blood products,[4] such as plasma,[5] red blood cells,[6] as well as platelets under resting conditions[7-12] or activated by thrombin receptor activation peptide (TRAP) or collagen.[13,14] To reduce the complexity of the proteomic sample, as well as improve assessment of low-abundance proteins, studies on platelet subproteomes—specifically the membrane,[15] microparticles,[16] α-granules,[17] and dense granules[18]—have been undertaken. Observations of changes in signaling proteins have since triggered the analyses of the phosphoproteome under resting[14] and activated conditions,[19] as well as the determination of *N*-glycosylation sites on platelets.[20]

Because the proteomics approach yields information that must be placed in a biochemical and physiologic context, it is worth reminding ourselves of some basic facts about platelets. Human platelets are the smallest formed elements of the blood; they are anucleate, disc-shaped, membrane-encapsulated cell fragments that are formed and released into the bloodstream primarily by bone marrow megakaryocytes.[21] In whole blood, platelet concentrations range from 150 to 400 × 10^9 cells per liter, with approximately one third of the total platelet pool sequestered in the spleen and two thirds present in circulation. The life span of a human platelet is roughly 7 to 10 days,[22] and its death is assumed to be determined by an apoptotic mechanism.[23] All organelles present in the mature platelet develop during megakaryocyte maturation. Platelets contain a number of distinguishable structural elements including a delimited surface membrane; invaginations of the surface membrane that form the open canalicular system; a separate, membrane-delimited dense tubular system; a cytoskeletal network; a peripheral band of microtubules; and numerous specialized organelles including α- and dense granules, lysosomes, microperoxisomes, and mitochondria.[24-27] Although platelets do not contain a nucleus and therefore host no DNA, platelets do inherit a genome in the form of messenger RNA (mRNA) from their megakaryocyte progenitor cells.[28,29] In addition, platelets have all of the necessary molecular tools and pathways necessary to translate mRNA into protein.[29-40] Platelets play an essential role in preserving vascular integrity and in maintaining hemostasis. In the case of an injury to the endothelial cell layer of the blood vessel wall, platelets will adhere to the injury site through

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0887-7963/08/$ - see front matter
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doi:10.1016/j.tmrv.2008.05.004

interactions with von Willebrand factor, aggregate with other platelets, release compounds that stimulate further aggregation, and form a loose platelet plug mediated by the formation of fibrin strands that are further cross-linked to form a fibrin net.[41,42] At the same time, platelets become activated by the transmission of a number of intracellular signals, resulting in the secretion of biologically active proteins necessary to trigger processes such as cellular chemotaxis, proliferation, and differentiation; removal of tissue debris; angiogenesis; the laying down of extracellular matrix; and the regeneration of the appropriate type of tissue.[43,44]

Platelet transfusions are routinely used as life-saving procedures during surgery, during chemotherapy, and for patients with particular bleeding disorders. Unfortunately, platelet storage is limited because of the risk of bacterial contamination and decreased functionality over time, the latter often referred to as the *platelet storage lesion* (PSL). This article briefly reviews the application of specific assays to determine platelet function and summarizes the results of recent proteomic approaches that assess protein changes during platelet storage to uncover a more mechanistic understanding of the storage lesion.

TRANSFUSION MEDICINE: LIMITATIONS OF PLATELET STORAGE

Given their role in mediating hemostasis and thrombosis, it is not surprising that transfusion of platelets has become a central part of disease treatment. The first demonstration of the effectiveness of transfusions of platelets was described in 1910, but it was not until the development of plastic polymer platelet storage containers in the 1960s and 1970s that platelet transfusions became standard treatment for bleeding thrombocytopenic patients with bone marrow failure.[45] Studies demonstrated the benefit of prophylactic platelet transfusions to prevent bleeding as opposed to the use of platelet transfusions solely as a therapeutic strategy aimed at treating bleeding once it had occurred.[46,47] Ever since platelet transfusions were shown to reduce mortality from hemorrhage in patients with acute leukemia in the 1950s, the use of this therapy has steadily grown to become an essential part of the treatment of cancer, hematologic malignancies, marrow failure, and hematopoietic stem cell transplantation. Platelet concentrates were most frequently transfused into thrombocytopenic recipients to maintain primary hemostasis, in patients with reduced numbers of platelets, or in those whose platelets are not fully functional, either because of a specific platelet disorder or, in some patients, after taking medication such as acetylsalicylic acid.[48,49] Defects that impair function can affect platelet receptors, secretory responses, or intracellular signaling pathways. Examples of qualitative platelet disorders include Glanzmann's thrombasthenia and Bernard-Soulier syndrome.[49,50] The treatment of platelet disorders is primarily by transfusion of platelet concentrates when clinically necessary, Today, in Canada, a mix of more than 300,000 whole-blood–derived and apheresis platelet products (2 million products in the United States and 2.5 million products in Europe) is manufactured annually to meet this transfusion need.

Compared with other blood products, platelets have a markedly short shelf life owing to the deterioration of the quality of the platelets stored at 22°C. Although the development of second-generation storage containers in 1984 led to the extension of platelet storage times from 5 to 7 days,[51] it was clear by 1986 that the bacterial contamination of platelet products during collection resulted in a significant increase in the reported cases of clinical sepsis with the transfusion of 7-day–old platelets as compared with the 5-day storage product.[52,53] As a result, the storage of platelet products was subsequently restricted to 5 days. Whereas the risk of transfusion-related transmission of viral diseases has steadily decreased over the last 40 years, the risk of transmission of bacteria remained about the same until the recent emphasis on bacterial risk reduction strategies for platelet products including the diversion of the initial 10 to 30 mL of donor blood that contains the skin plug as well as the culture of platelet products before use.[54-57] Although the aforementioned strategies result in a general reduction of bacterial risk, they do not prevent such events from occurring, as platelet contamination with bacteria that evade detection by quality control culture remains a significant residual transfusion risk.[58,59] The only approach that is likely to achieve near-absolute bacteriologic safety is the inactivation of bacteria by pathogen reduction technologies. However, it is clear that these treatments have some effects on the platelets, too, and may exacerbate the development of the storage lesion.[58,60-63]

PSL: MONITORING IN VITRO FUNCTIONALITY

The *PSL* is best defined as the sum of all the deleterious changes in platelet structure and function that arise from the time the blood is withdrawn from the donor to the time the platelets are transfused to the recipient typified by altered morphologic features and increased release of platelet α-granules and cytosolic proteins. Although platelets that are stored over a period of 7 days are generally still viable, studies have suggested an overall reduction in their therapeutic efficacy that is associated with morphologic, biochemical and functional changes.[64,65] Included with these are reports of the development of abnormal forms,[66] loss of disc shape,[67] decreased mean platelet volume,[68,69] increased volume and density heterogeneity,[70] increased release of platelet α-granules and cytosolic proteins,[71,72] increased procoagulant activity,[73] and altered glycoprotein (GP) expression, all of which are characteristic of platelet activation.[68,72,74-77]

This quality of aging has traditionally been quantified by in vitro measures[78] resulting in a decrease in platelet morphology determined by the Kunicki morphology score and response to agonist monitored by the extent of shape change (ESC), together with an increase in hypotonic shock response (HSR), with ESC and HSR assessed by light-scattering techniques. Both the morphology score and HSR are considered to have appropriate sensitivity to platelet changes during storage.[79] Flow cytometry permits the rapid analysis of large numbers of platelets within relatively small quantities of sample assessing changes on the platelet surface such as GP expression (GPIb, GPIIb, GPIIIa), the generation of platelet activation markers (CD40L, CD62P, CD63), and the exposure of negatively charged phospholipids as determined by annexin V binding. Monitoring of GP expression under stimulation with agonists such as adenosine diphosphate or thrombin revealed reduced responsiveness during storage.[80] Using clinical chemistry analyzers, the solution pH[64,65,81] paralleled with determination of the blood gas pO_2 and pCO_2 as well as glucose and lactose concentration in the storage bag can be readily determined. Nevertheless, most of these measures are not currently used in hospitals for standardized quality assessment of platelets before transfusion, but are instead restricted to research applications. To be of clinical value, such measures should reflect one or more of the physiologic functions of platelets and should be simple, be practical, and fit into the transfusion laboratory setting.[76]

Unfortunately, reliable in vivo performance measures of allogeneic platelets after transfusion by in vitro approaches remains a work in progress. Measurements of platelet recovery and survival after autologous transfusion of radiolabeled platelets[82] into normal volunteers have resulted in a reduction of at least 25% in reinfusion studies.[64,65,83-87] Although these data appear to correlate very well with in vitro measures analyzing ESC and determination of lactose production,[82] we still lack a thorough understanding of how in vitro results in platelet concentrates predict platelet function in vivo after transfusion.[78] Indeed, methods of platelet preparation may alter the recovery and survival characteristics of platelets after transfusion. A recent study by Arnold et al[88] suggests that platelet viability is better preserved in TRIMA apheresis platelets than in leukoreduced platelet-rich plasma (PRP) platelets. However, the relative clinical efficacy of these platelet products for bleeding prevention and treatment has yet to be determined and will need to be addressed in well-designed randomized clinical trials. Comparison of platelets derived from PRP, buffy coat, or apheresis technologies has also demonstrated differences in terms of in vitro functional activity. This observation is believed to be related to the existence of heterogeneous subpopulations of platelets,[89] although further studies are required to substantiate this hypothesis.

APPLICATION OF PROTEOMICS TO THE PSL

Understanding the mechanisms that lead to the development of storage lesion has been of longstanding interest. Storage-related changes in the pattern of cytosolic and membrane proteins were first noticed in 1987 by Snyder and colleagues[90] through the use of 2-dimensional (2D) gel electrophoresis. Unfortunately, they were only able to identify 2 actin fragments as significantly accumulating in platelets during the first 7 days of storage because of limits in genome sequencing and bioinformatics at that time. The potential of proteomics as a viable tool for the identification of the PSL has since increased dramatically with the development of mass spectrometry[4] and has required the development of quantitative proteomic techniques such as differential gel electrophoresis (DIGE),

isotope-coded affinity tagging (ICAT), and isotope tagging for relative and absolute quantitation (iTRAQ) (for a review, see Ong et al[91]). Thiele et al[92] recently used one such technique (DIGE) to comprehensively assess the impact of storage on the global proteome profile of therapeutic platelet concentrates (PC). Although they were unable to represent membrane proteins because of their high hydrophobicity (a frequent shortcoming of gel-based proteomic approaches), this group found that roughly 3% of the cytosolic platelet proteome displays a change in relative intensity over a storage period of 9 days.[92] Of these, septin 2, *β*-actin, and gelsolin were found to increase significantly in concentration during this time and may be related to apoptosis.[92-94]

In yet another gel-based study, this one focused on storage-induced changes in the PC supernatant, Glenister et al[95] identified modifications to platelet proteins trem-like transcript 1 and integrin-linked kinase, which they suggest may influence platelet-endothelium interactions. Moreover, the concentrations of the platelet-derived cytokines CXCL7, epidermal growth factor, platelet-derived growth factor, brain-derived neurotrophic factor, and CCL5 were all found to increase during a 7-day storage period, the latter 3 showing significant increases in their relative concentrations between days 5 and 7.[95] Greening et al have since performed a comparison of human membrane-cytoskeletal proteins with the plasma proteome (online prepublication, DOI: 10.1002/prca.200780067). This correlation sets the basis for the identification and classification of proteins that are selectively acquired from plasma by platelets (such as L-lactate dehydrogenase, serum albumin, fibrinogen, carbonic anhydrase, endoplasmin, and multimerin 1) from those that are endogenous to platelets (such as actin, actinin, filamin, tropomyosin, thrombospondin-1, platelet basic protein, platelet factor 4, and stomatin) and are potentially released into the circulation or made available for concentrated and focal release at vascular sites of injury.

These observations made in these 2 articles[92,95] were corroborated in a complementary proteomic

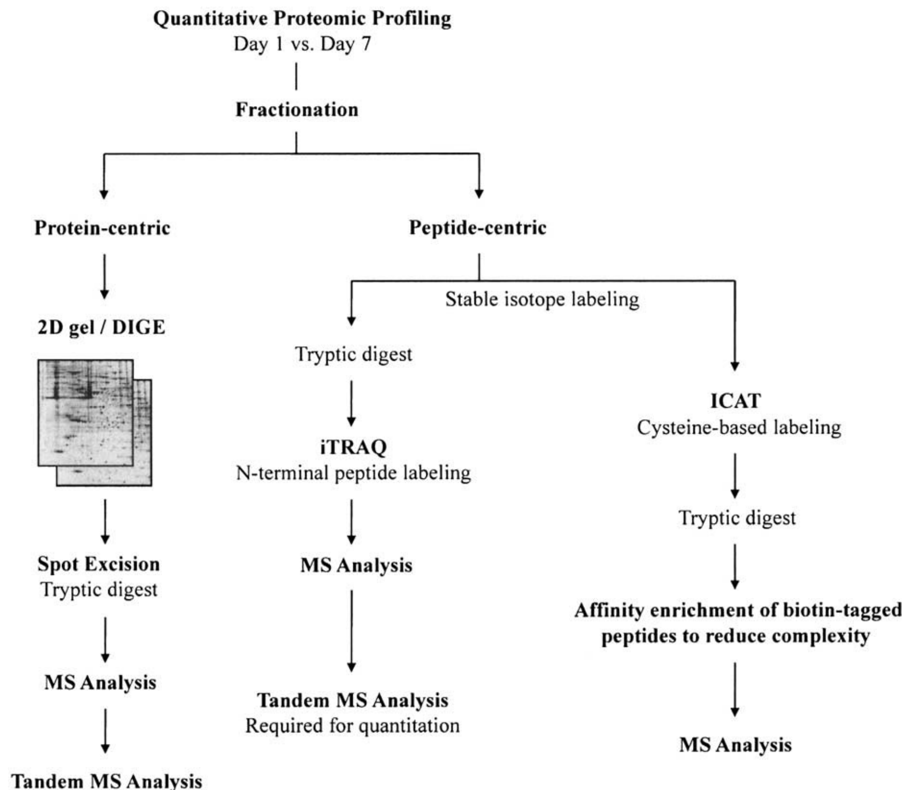

Fig 1. A schematic of the experimental setup and workflow for a complementary proteomic assessment of changes occurring in a platelet unit during storage. Abbreviation: MS, mass spectrometry.

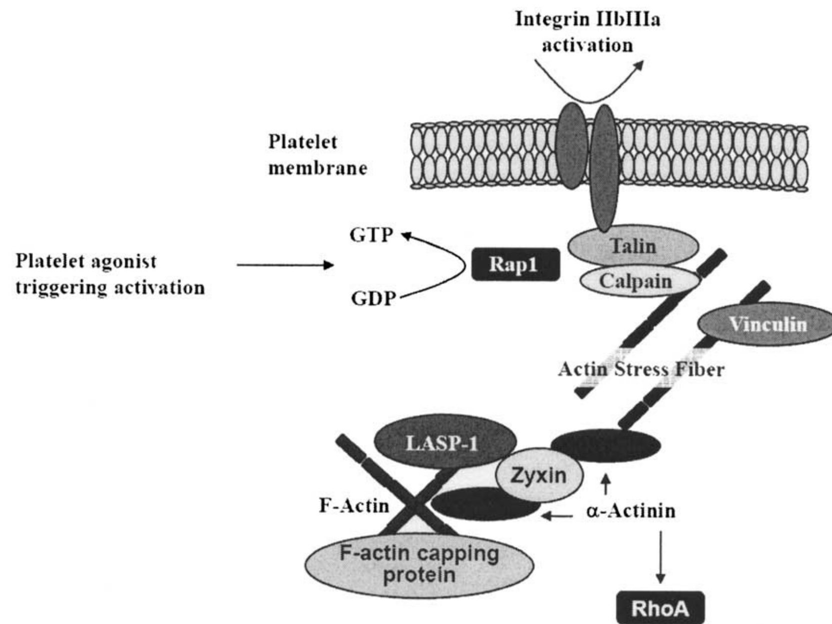

Fig 2. Model of the integrin signaling pathway mediated by the GPIIb/IIIa. All proteins displayed are identified to be changing during platelet storage by proteomic approaches, leading to the potential involvement of this pathway to the storage lesion. Abbreviations: GDP, guanosine diphosphate; GTP, guanosine triphosphate; LASP-1, LIM and SH3 domain protein 1.

study of our own design that addressed the relative differences among DIGE, ICAT, and iTRAQ in the analysis of the PSL (Fig 1) and further identified platelet proteins α-actinin 1, ARP2/3 complex 16-kd subunit, cofilin, GPIIb α-chain precursor, myosin heavy chain, Rapl, talin 1, 14-3-3 protein ζ/δ (also identified by Thiele et al), thrombospondin 1, and the tubulin β5 chain as changing significantly in relative concentration over a 7-day storage period.[96] Integrin-linked kinase and CXCL7 also showed significant changes in their relative concentrations during storage; however, both presented lower levels in platelets on day 7 vs day 1. It is therefore likely that these cytokines are released by platelets into the surrounding plasma during storage and so account for their elevated levels in the PC supernatant.[95] Further analysis of the integrin GPIIb/IIIa complex established that GPIIIa increases in both relative concentration and surface expression within the first 7 days of storage and demonstrated that platelets are capable of translating both GPIIb and GPIIIa over a period of 10 days.[97] This is not altogether surprising because platelets are known to contain all of the molecular tools necessary for protein biosynthesis from cytoplasmic mRNA and have been shown to synthesize membrane GPs Ib, IIb, IIIa; fibrinogen; thrombospondin; and von Willebrand factor.[30,37,38]

It is interesting to observe how all of these proteins are related. Actin and tubulin are both components of the cytoskeleton and are known to interact directly with actinin, ARP2/3, cofilin, myosin, talin, proteins 14-3-3 and Rap1 (some of which are involved in cell signaling via GPIIb/IIIa, Fig 2), and thrombospondin (which has been shown to act on a subpopulation of platelets in response to simultaneous activation with collagen and thrombin[98] to induce the formation of focal adhesions[99]). Septin 2 is a member of an evolutionary conserved family of guanosine triphosphate–binding proteins[100] that interact with the actin cytoskeleton and have been implicated in cytokinesis, cellular morphogenesis, and vesicle trafficking.[101,102] Fibrinogen, pleckstrin, and the 78-kd glucose-dependent protein were also identified as changing in relative concentration during storage in both studies and, along with the aforementioned list, support reports of platelet activation during storage.[103] Moreover, the increase in relative concentration of GPIIb/IIIa —along with a remarkable number of proteins known to participate in integrin signaling—indicates a possible link between this pathway and that of the storage lesion.

Table 1. Examples of Results From Platelet Quality In Vitro Measures Correlating With Results From Proteomic Approaches

Platelet function	Impact on storage on in vitro measures	Information gained from proteomic approaches
Activation	Increase in CD62P expression due to α-granule release	Some proteins found to be changing during storage agree with changes observed in proteomic studies analyzing platelet activation[13]; examples include thrombospondin, clusterin, and cyclophilin A, which decrease in concentration and are known to be released from the platelet[95,111]
Morphology	Shape change from discoid to spheroid monitored by ESC	Appearance of actin isoforms as well as changes in actin binding proteins, eg, cofilin, gelsolin, or proteins of the ARP2/3 complex most likely involved in cytoskeletal rearrangement[96]
GP expression	Increased expression of CD41 and CD61	Several proteins increase in their amount most likely because of protein synthesis as shown for GPIIIa[97]
Metabolic activity	Increase in pO_2 and lactate; decrease in pCO_2 and glucose	Changes in metabolic pathway proteins such as pyruvate kinases and acyl-protein thioesterase[96]
Signaling	Slight decrease in vWF binding	Amounts of subunits of the GPIb/IX/V complexes remain almost constant; proteins involved in the signaling pathway mediated by this pathway such as 14-3-3 ζ/δ and filamin are observed to change spot positions most likely because of alterations in posttranslational modifications[96]
	Reduced calcium ion flux	Decrease in the protein amount of calmodulin and changes in its associated protein caldesmon[96]
	Increase in GPIIb/IIIa activation monitored by binding of the antibody Pac-1	Proteins involved in the integrin αIIbβ3 pathway are observed to change spot positions because of alterations of posttranslational modifications[96]
Adhesion	Decrease in fibrinogen binding	Reduced protein amounts of different fibrinogen chains[96]
Coagulation	Increase in phosphatidyl-serine exposure monitored by annexin V binding	Changes in coagulation factors V and XII[96]

Unfortunately, despite significant strides in the field of platelet proteomics, variation in individual protein concentration (donor-donor variability) continues to represent an important limiting factor in the study of the PSL. This observation is complicated by clear differences in the types and number of proteins identified by the different proteomics tools that are currently available.[104] As a result, careful attention must be paid to determining which technology yields the most appropriate information.[104,105] A combination of both protein- and peptide-centric approaches should generally be considered when analyzing the platelet proteome, as using any single proteomics method to study platelet storage changes may give insufficient information.[96] High-abundance proteins such as actin can also represent an important limiting factor in the study of platelet proteomes. Proteomic tools such as 2D gels and iTRAQ often fall short for low-abundance proteins. To overcome this problem, many prefractionation techniques have been proposed over the years, the most common being the use of multiple affinity columns, and should be considered.[106] Lastly, it is important to keep in mind the extent to which we can rely on the proteomic information collected. In this regard, researchers should adhere to standards proposed by the International Society of Thrombosis and Haemostasis.[107] This requires that investigators

minimize the degree of platelet activation during blood collection and cell isolation to limit activation-dependent changes in posttranslation modification; stop experimental reactions as quickly as possible to minimize postactivation changes in proteins, notably degradation; ensure that the degree of contamination of the platelet sample with other cell types and plasma is kept to an absolute minimum; remain conscious of factors that might influence the uptake of proteins and protein binding by platelets (eg, buffer) and that may alter the composition of the platelet proteome; recognize that there is a potential for error in peptide sequencing that is governed by the criteria used for acceptance of the predicted sequence and the potential of splice variants, which cannot be predicted from genomic databases; and appreciate that there is a potential for error in searching protein databases such as the National Center for Biotechnology Information, Swiss-Prot, and TrEMBL and that the predictive value of sequenced peptides does not take into account factors such as alternative splicing, polymorphisms, and posttranslational modifications.

A comparison of the 2 most recent studies analyzing the PSL using different proteomic approaches[92,96] revealed excellent agreement in protein changes as seen for talin, tubulin, and thrombospondin. On the other hand, various disagreements in the proteins identified between these approaches might be due to the technologies used, different protocols (laboratory-to-laboratory variation),[108] effects of undiscovered changes in posttranslational modifications, or the lack of specific protein detection due to its low abundance. Furthermore, as mentioned above, platelets undergo changes in shape during storage and other signs of activation. This is supported by the observation of increasing expression of the platelet activation marker CD62P on the surface, however, to a moderate level compared with agonist activation.[109] The recent proteomic approaches reveal that some features monitored during storage were also observed in a study analyzing the changes in the platelet proteome during activation.[108] Proteins such as fibrinogen, 78-kd glucose-regulated protein, and 14-3-3 ζ/δ change in agreement with and foster the activation hypothesis of early investigations.[110] Moreover, the decrease in thrombospondin, SPARC (osteonectin), and pleckstrin that is observed during storage may be explained by their release from the platelet. Most recently, a proteomic study of proteins released during storage has supported this explanation,[95] providing further evidence of platelet activation during storage. These examples demonstrate the complementarity of different proteomic approaches to achieve a complete working model. On this basis, it is now possible to design biochemical and physiologic experiments to understand the meaning of these proteomic findings. These first proteomic studies on the storage lesion also enable one to correlate in vitro measures used to assess platelet functionality with proteomics results with some examples listed in Table 1. As mentioned above, the increased expression of the activation marker CD62P is in agreement with signs of activation during storage when compared with studies from activated platelets. The increased expression of CD61 is explained by translation of GPIIIa during storage.[97] Changes in shape change are consistent with alteration of actin isoforms (unpublished data), and the metabolic activity is linked to changes in mitochondrial proteins. To confirm these links and improve our understanding of how in vitro measures relate to platelet viability and function in vivo, future work should include platelet fractionation and proteomic analysis of the membrane, cytosol, cytoskeleton, and the different platelet granules during storage and upon platelet activation. Signaling, adhesion, and coagulation factors will need to be examined closely by both proteomic and classic in vitro measures and related to metabolic and translational changes that have been observed in platelets during storage.

APPLICATIONS IN TRANSFUSION MEDICINE

Applications of proteomic "fingerprinting" for the purposes of identifying factors responsible for declining recovery and survival of stored platelets can be separated into the categories of basic research and process assessment.

The cold storage lesion is a prime example of the first, as platelets stored at temperatures less than 15°C have been shown to undergo extensive morphologic changes that are consistent with signal activation, and are not currently licensed for transfusion.[112,113] Hoffmeister et al[114] revisited this issue in a murine model and were able to show that poor survival of cold-stored platelet units is associated with a virtually irreversible clustering of α-subunits of GPIb on the platelet surface. In the case of cold-stored platelets, it seems that rather than modifying the storage medium to improve and

extend platelet storage, the platelets themselves should be treated such that they can be stored under refrigeration without a subsequent loss of viability.[115] However, the modification of platelets to prevent cold-induced clearance has not proven effective for long-term storage.[116] Proteomics offers the power to characterize protein mixtures in such systems, determine relationships between proteins, resolve their function, and identify protein-protein interactions of interest in the PSL process. In this regard, 2D gel electrophoresis, DIGE, iTRAQ, and ICAT can be used to identify protein isoforms that may enable platelets to be stored longer, and resolve conditions under which such platelets store better. As many differential effects on proteins themselves come from post-translational modifications such as phosphorylation or glycosylation, monitoring these will contribute to a better understanding of how platelets function under various storage conditions. In addition to potentially allowing for extended storage, platelet additive solutions (PAS) and refrigeration would almost certainly reduce the risk of bacterial growth in contaminated units and may help in the development of platelet substitutes.

In addition to helping answer basic research questions, proteomics is an attractive tool by which to assess established processes such as the use of PAS and pathogen inactivation strategies. Generally, PAS require plasma concentrations of 20% to 30% to maintain platelet metabolism. Acetate is often added as a second metabolic fuel and buffer agent along with electrolytes such as magnesium and potassium, which are commonly used to inhibit platelet activation and aggregation during storage.[117] Because additive solutions replace 70% to 80% of the plasma in the original platelet unit, they are thought to promote reduced allergic and febrile transfusion reactions,[118] decreased transfusion of blood type antigen and human leukocyte antigen antibodies, and increased plasma availability for fractionation.[117] Unfortunately, the value of in vitro platelet quality markers such as pH, glucose consumption, and lactate production is limited in the prediction of in vivo function. Storage indices can only take one so far as monitoring criteria that have been correlated to platelet viability and function. Without a clear understanding of the factors involved in the initiation or exacerbation of the storage lesion, and the molecular mechanisms by which they function to promote platelet activation during storage, it is impossible to ameliorate the storage lesion by targeted approaches; and we are left with empirical approaches that have thus far been unsuccessful.

A second major area of platelet research, in terms of quality monitoring, includes pathogen inactivation strategies including photoactive psoralen compound S59[119] (Cerus, Concord, CA), riboflavin treatment,[120,121] and gamma irradiation,[122] which are among the most developed approaches. Psoralen works by intercalating into the nucleic acids of treated cells that, upon exposure to UV light, becomes activated and binds the nucleic acid core, preventing subsequent DNA/RNA replication. Riboflavin works in much the same way, intercalating between the bases of DNA or RNA. On exposure to light (UV or visible), riboflavin becomes activated and oxidizes guanine, thus preventing replication of the pathogen's genome. Similarly, gamma irradiation of platelet components eliminates the proliferative capacity of leukocytes before administration and has been shown to successfully reduce the risk of transfusion associated graft-vs-host disease in immunocompromised patients. External stimuli such as drug intervention for therapeutic studies can alter protein expression levels in cells. As platelets have been shown to translate functionally significant proteins during storage, RNA cross-linking by UV and gamma irradiation should inhibit this process; and its effect on the storage lesion will certainly need to be investigated. Proteomic tools such as iTRAQ and ICAT can be used to study phenomena such as phosphorylation and signal transduction pathways when looking for protein markers as potential drug targets. By providing a snapshot of the multifaceted response of human blood platelets to storage, inhibitors can then be focused to identify and asses the effect of specific proteins, signaling pathways. and translational machinery governing platelet activation during storage. This represents an important step toward designing targeted interventions that can extend the storage of platelets beyond 7 days.

In each of these examples, proteomics techniques can be used to provide a rapid, comprehensive diagnostic of proteolytic events and posttranslational modifications related with improved platelet fitness. Indeed, the use of proteomics in conjunction with an understanding of the activity, interactions, regulation, and localization of key proteins under a combination of processing conditions, PAS/

plasma ratios, and UVC/gamma irradiation may provide an excellent starting point for examining the in vitro characteristics of these varied elements on platelet viability and function. Description of the platelet proteome will enable the rapid detection of protein expression and abundance profiles that may be used as a measure of platelet health, to which PAS and different storage conditions can be compared. Definition of the platelet proteome during storage and the elucidation of biologically meaningful relationships between platelet storage conditions and the PSL will undoubtedly yield a better, longer-lasting product.

CONCLUSION

Bacterial risk receives and deserves a lot of attention when it comes to extension of platelet storage; however, determining the quality of platelets during storage needs to be treated with equal importance. Because the platelet life span is 7 to 10 days, extension of the platelet shelf life by 2 to 3 days would improve platelet inventory and efforts of donor recruitment tremendously, as well as the overall cost of provision of this blood product to patients. Proteomic studies on the PSL are aiming at understanding the changes occurring within the platelet proteome and enabling the design of biochemical and physiologic experiments to understand the meaning of these proteomic findings. Proteomics thus provides an excellent tool to decode complex processes by identifying novel platelet-expressed proteins, dissecting mechanisms of signaling or metabolic pathways, and analyzing functional changes of the platelet proteome. Taken together, the articles that we have reviewed offer a potential correlation to in vitro observations during storage and demonstrate the complementarity of different proteomic approaches to achieve a complete working model of the storage lesion. However, one has to keep in mind that proteomic techniques are limited in sensitivity as well as in the dynamic range and are especially dependent on the protein separation method used. Therefore, translation of proteomic results into platelet biochemistry and physiology is necessary to unravel mechanisms of PSL in time and space toward more successful platelet transfusion.

ACKNOWLEDGMENTS

This work is supported by the Canadian Blood Services, the Michael Smith Foundation for Health Research, the Canadian Institutes of Health Research, and the Canada Foundation for Innovation. This is a fast-growing field with an ever-expanding literature. As such, we regret that we may have not acknowledged all recent contributions because of publication timing.

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