BRIEF REPORT

Structural origins of hemostasis and adaptive immunity

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Abstract

Background: Adaptive immunity in jawless fishes is performed by a unique set of proteins termed variable lymphocyte receptors (VLRs). Here we compare the crystallographic structures of VLRs and the human primary hemostasis receptor, glycoprotein (GP) Ib. It has been estimated jawless fish vertebrates diverged from jawed vertebrates 500 million years ago. Identifying structural similarities provides insights into the origins of primary hemostasis and the unique adaptive immunity of jawless fishes.

Methods: Three-dimensional structures obtained from crystallographic data and primary sequences alignments are compared. The results focus on overall domain arrangement to include the structural roles of leucine-rich repeats (LRRs), disulfide bond, and disulfide loop arrangements.

Results: The crystal structures of human GPIb (GPIbαN) and jawless fish VLRs are made up of three common segments each. The N-terminal cap and the C-terminal cap are characterized by disulfide bonds conserved in both GPIbαN and VLRs. The body of each molecule consists of LRRs which varies depending on the number of LRRs present in each molecule. The stacking of the LRRs results in the formation of a concave surface which serves as a motif to build ligand-binding specificity with the flanking regions.

Conclusion: A comparison of VLR and GPIb structures reveals a phylogenetic trail of cellular differentiation contributing to mammalian hemostasis and jawless fish adaptive immunity. The results provide a structural basis to explain some of the interrelationships between hemostasis and immunity in vertebrates and potentially identifies a common ancestral motif linking hemostasis and immunity.

KEYWORDS
adaptive immunity, blood platelets, hemostasis, phylogeny, Platelet Glycoprotein GPIb-IX Complex

Essentials

- Structural similarities between platelet GPIb and immune proteins of jawless fishes are profiled.
- VLRs from lamprey eels and hagfish share a conserved domain arrangement with platelet GPIb.
- Leucine-rich repeats flanked by disulfide loops are common between glycoprotein Ib and VLRs.
- Ancestors of the vertebrate lineage likely contain an ancient domain for hemostasis and immunity.
1 | INTRODUCTION

Mammalian hemostasis is controlled by a highly evolved arrangement of receptors, ligands, enzymes, and cofactors that all coordinate to prevent blood loss and support normal wound repair following injury. In the human situation a designation of primary (platelet phase) versus secondary (fluid phase) hemostasis provides an early differential diagnosis for many of the different bleeding disorders. At the center of mammalian primary hemostasis is the platelet adhesion receptor, glycoprotein (GP)Ib-IX, containing a well characterized binding site for von Willebrand factor (VWF). The platelet GPib-IX complex and the genes supporting its expression are quite unique with the most closely related mammalian structure or gene being GPV which can be purified as part of the same, albeit larger, platelet receptor complex, the GPib-IX-V complex resides in the extracellular N-terminus of α-subunit of the GPib (GPibN) expressed on the surface of circulating platelets.

Here, we highlight structural similarities derived from crystallographic analyses between GPibN and variable lymphocyte receptors (VLRs) of the jawless fishes, the lamprey eel and hagfish. The VLRs are a unique adaptive immune system, completely different from the V, D, and J immunoglobulin genes responsible for vertebrate adaptive immunity. Lamprey VLR germline genes undergo somatic rearrangements to create a repertoire of mature VLR genes each providing unique diversity for immune function. Individual variation does exist among the VLR proteins, while preserving a general domain organization. It is estimated the jawless fishes diverged in vertebrate phylogeny more than 500 million years ago. Thus, the jawless fishes present an opportunity to study the functionally important mammalian proteins within an ancient lineage. Here, the common domain organization between GPibN and VLRs will highlight a structural motif with ancestral roots in both hemostasis and immunity.

2 | METHODS

We searched the RCSB protein data bank (www.rcsb.org) for structures similar to GPibN, using DALI server. Close structural neighbors of GPibN were VLRs of the jawless fishes, the lamprey eel and hagfish. Analysis focused on an N-terminal cap (NT), LRRs, and a C-terminal cap (CT). Both the NT and CT have conserved disulfide bonds. GPibN has 8 LRRs, while the VLRs discussed here vary from 6 to 8. Therefore, we employed the following strategy for structural comparisons. We split the VLR into two parts, the first comprising of NT and LRRs and the second being CT. These parts were separately superposed with the corresponding regions of GPibN using the program COOT. The figures were generated using the program PyMOL (PyMOL Molecular Graphics System, Version 1.3 Schrödinger, LLC, Cambridge, MA, USA).

3 | RESULTS AND DISCUSSION

Highlighting the structural similarities are a superposition of GPibN with the VLRAs of sea lamprey (VLRAlamp) and hagfish (VLRAhag), and hagfish VLRB (VLRBhag) (Figure 1A–C). The most striking unifying structure between GPibN and the VLRs are the LRR modules that vary in number for the individual VLRs compared to the 8 LRRs found in human GPibN (Figure 2). The LRRs in all of these proteins are

![Figure 1](image-url)
flanked by motifs containing disulfide bridges (Figure 1D, E). GPIbα has a single amino-terminal intramolecular disulfide bridge between Cys^5^-Cys^17. VLRA_\text{lamp} has two amino-terminal disulfide loops between residues Val^227^-Ser^241 in VLRAlamp, and similarly in VLRA_hag and VLRB_hag. However, the loop is not a universal requirement as evidenced by the crystal structure (Figure 3B). Thus, the cysteines and disulfide bridges are similarly arranged while functioning in either hemostasis or immunity.

As shown by the crystal structure of the GPIbα/VWF complex, residues Val^227^-Ser^241 form a large loop that undergoes a conformational change to interact with VWF. In complex with VWF, these residues adopt a β-hairpin structure and form an extension of the central β-sheet of the VWF A1 domain through strong hydrogen bond interactions (Figure 4A). VLRA_\text{lamp} contains a similarly positioned loop, albeit shorter, formed by Asn^211^-Ser^220 (Figure 1E). Interestingly, VLRA_\text{lamp} crystal structures with antigens, hen egg white lysozyme, or H-antigen trisaccharide, demonstrate the primary binding region of VLRA_\text{lamp} is within the similar loop supporting the GPIb/VWF interaction (Figure 4A–C). Thus, an overall conservation of domain structure likely facilitates the intermolecular interactions involving the Val^227^-Ser^241 loop of GPIbα and the similar loops within VLRA_\text{lamp} and VLRA_\text{hag}. However, the loop is not a universal requirement as evident by its absence in VLRB_\text{hag} (Figure 1B). Thus, the role of the loop varies in the antigen binding properties for individual VLRs while being essential to the GPIbα/VWF interaction.

Based on gene arrangement, others have proposed the VLR genes arose from some primitive GPIb-IX complex in the vertebrate lineage. Conserved positions of the intron sequences within 5′ untranslated sequences, intron-less coding sequences for leucine-rich repeats (LRRs), and coding sequences for a connecting peptide and a threonine-proline-rich stalk are shared between the GPIb-IX genes. Based on sequence comparisons, Rogozin et al. have suggested that GPIbα and VLRs may share a common evolutionary origin. Other investigators have shown that parts of VLRA superpose well with parts of GPIbα, but these comparisons focused primarily on the LRRs.

### Figure 2: Sequence of alignment of leucine-rich repeats.

Comparative alignment of leucine-rich repeats (LRRs) among 3 representative VLR sequences illustrated in Figure 1 along with the extracellular glycoprotein Ibx domain of the human platelet receptor. The LRR numbers vary among the lamprey and hagfish VLR between 4 and 8. Leucine alignments are highlighted in yellow. Other similarities where 3 out of 4 residues match are highlighted in gray. The LRR numbers vary among the lamprey and hagfish VLR between 4 and 8. Leucine alignments are highlighted in yellow. Other similarities where 3 out of 4 residues match are highlighted in gray.

| VLRAlamp | VLRB_hag | Figure 2 |
|----------|----------|----------|
| PK-GFFGSHLLPFALFLHGPNW | PEGAFDSELKLKMQLQENPND | |
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Cys^3^-Cys^11 and Cys^9^-Cys^20 with the latter forming a disulfide linked β-hairpin similar to the hairpin loop in GPIbα (Figure 1D). Both VLRA_\text{hag} and VLRA_\text{lamp} have the amino-terminal disulfide bridges similar to those found in VLRA_\text{lamp} (Figure 3A). Carboxy-terminal to the LRRs are a pair intramolecular disulfide bridges found in VLRA_\text{lamp}, VLRA_\text{hag}, and GPIbα (Figures 1E and 3B). The GPIbα disulfide intramolecular bridges form between Cys^1^->^2^ and Cys^1^->^2^, whereas VLRA_\text{lamp} disulfide loops are formed between Cys^192^-Cys^228^ and Cys^192^-Cys^246^ (Figure 1E). VLRA_\text{hag} disulfide loops are formed between Cys^185^-Cys^218^ and Cys^187^-Cys^230^ (Figure 3B). VLRA_\text{hag} has only a single Cys^241^-Cys^247^ bond with two remaining residues, Cys^243^ and Cys^248^, unpaired in the crystal structure (Figure 3B). Thus, the cysteines and disulfide bridges are similarly arranged while functioning in either hemostasis or immunity.

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They served as anchoring points around which changes took place. The similar gene arrangements along similar protein domain conservation seems to discount the structures being the result of convergent evolution, but does not unequivocally eliminate the possibility of a convergent evolution.

Striking in the structural similarities between human GPIbα and the jawless fish immune system are implications for a common structural origin for primary hemostasis and the unique immune system of the jawless fishes. The subphylum vertebrata (phylum Chordata) is divided into agnatha (jawless fish) and gnathostomata (jawed fish) with the former containing only the lamprey eel and the hagfish lineages (Figure 4D). Based on fossil records, the agnatha lineage is estimated to have diverged from gnathostomata more than 500 million years ago during the Cambrian period. The gnathostamata group contains more than 60,000 species of vertebrates including all mammals.
birds, reptiles, amphibians, and fishes. Interestingly, the protochordates which comprise the remaining two subphyla of chordata lack a plasma-based coagulation system based on the apparent absence of a thrombin ortholog. Thus, if one attempts to trace the origins of primary and secondary hemostasis, the subphylum vertebrata is a logical starting place where elements of both primary and secondary hemostasis can be identified.

These data may reflect a phylogenetic trail of cellular differentiation and the origins of hemostasis. In non-mammalian vertebrates, the role of the platelet is performed by a circulating nucleated cell, termed the thrombocyte. Interestingly, in more primitive animals an overlap exists where the same cell can support both hemostasis and immunity. Indeed, invertebrate rudimentary hemostasis and immune function are performed by a single cell type found in blood (hemolymph), termed the hemocyte. In the case of mammalian platelets there is emerging evidence for their participation in immune function, as well. Thus, when viewed in the overall phylogenetic trail the need for mechanisms to limit blood loss (hemostasis) and protect against foreign invaders (infection) can be traced to an ancestral protein whose remnants are now highly specialized.

When considering the presence of an even more ancient gene/protein present in the ancestors of the vertebrate lineage, did this molecule have both hemostatic and immune functions? If so, it suggests an ancestral homologous motif that has evolved to become more dedicated to either the prevention of blood loss or a defense mechanism. Interestingly, the highly specialized mammalian GPIb-IX complex, and other platelet receptors as well, are now being recognized for their ability to participate in inflammatory processes. Still to be defined, is the molecular basis of hemostasis in the jawless fishes. However, the possibility for residual ancient homologous functions linking primary hemostasis to inflammation exists and this is likely to provide some unexpected and exciting future discoveries.

AUTHOR CONTRIBUTIONS

Jerry Ware and KI Varughese directed the study, discussed the results and conclusions, and co-wrote the manuscript.

RELATIONSHIP DISCLOSURE

The authors declare no competing financial interests.

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