Structural basis for platelet collagen responses by the immune-type receptor glycoprotein VI

Katsunori Horii, Mark L. Kahn, and Andrew B. Herr

Activation of circulating platelets by exposed vessel wall collagen is a primary step in the pathogenesis of heart attack and stroke, and drugs to block platelet activation have successfully reduced cardiovascular morbidity and mortality. In humans and mice, collagen activation of platelets is mediated by glycoprotein VI (GPVI), a receptor that is homologous to immune receptors but bears little sequence similarity to known matrix protein adhesion receptors. Here we present the crystal structure of the collagen-binding domain of human GPVI and characterize its interaction with a collagen-related peptide. Like related immune receptors, GPVI contains 2 immunoglobulin-like domains arranged in a perpendicular orientation. Significantly, GPVI forms a back-to-back dimer in the crystal, an arrangement that could explain data previously obtained from cell-surface GPVI inhibition studies. Docking algorithms identify 2 parallel grooves on the GPVI dimer surface as collagen-binding sites, and the orientation and spacing of these grooves precisely match the dimensions of an intact collagen fiber. These findings provide a structural basis for the ability of an immune-type receptor to generate signaling responses to collagen and for the development of GPVI inhibitors as new therapies for human cardiovascular disease. (Blood. 2006; 108:936-942)

© 2006 by The American Society of Hematology

Introduction

Thrombus formation in the arterial vasculature is a process initiated by the interaction of several platelet receptors with collagen and collagen-associated proteins at the site of vascular injury. Initially, platelets are tethered transiently to exposed collagen when the receptor GPIbα interacts with collagen-bound von Willebrand factor (VWF). For stable platelet adhesion to occur, the immunoglobulin (Ig)-like receptor GPVI must bind to collagen, triggering the activation of a signaling cascade. GPVI signaling leads to inside-out activation of the platelet integrins αIIbβ3 and αIbβ3. Activated αIIbβ3 binds tightly to a specific sequence in collagen to allow firm adhesion of the platelets to the site of injury, and activated αIbβ3 mediates platelet aggregation. In addition, GPVI signaling stimulates secretion of platelet granule contents to activate nearby circulating platelets and propagate thrombus formation. In humans, GPVI deficiency causes a loss of platelet activation in response to collagen, and GPVI polymorphisms have been linked to increased risk of myocardial infarction. Remarkably, loss or inhibition of GPVI prevents arterial thrombus formation in animal models but causes only mildly prolonged bleeding times in mice and humans, suggesting that GPVI could be a prime therapeutic target for prevention of arterial thrombotic diseases such as heart attack and stroke.

The gene for GPVI is found in the leukocyte receptor cluster (LRC) on human chromosome 19. The sequence of the GPVI ectodomain was predicted to form 2 Ig-like domains comprising the collagen-binding domain followed by a heavily O-glycosylated stalk. Like other LRC receptors, GPVI associates with the FcR γ-chain coreceptor, and signaling is mediated both indirectly through the γ-chain and directly through the GPVI cytoplasmic domain. The quaternary structure of fibrous collagen is required for GPVI activation, although GPVI can also be activated by a synthetic collagen-related peptide (CRP) containing cross-linked strands of the repeating tripeptide (POG)n, where P is proline, O is hydroxyproline, and G is glycine. Recently, GPVI has been shown to interact with the ectodomain of GPIbα on the surface of platelets and to bind to laminin, a matrix protein exposed at sites of vascular injury. Multimeric snake venom proteins such as convulxin can also strongly activate GPVI, suggesting that clustering of GPVI receptors through multiple binding events leads to activation. To better understand the molecular basis for collagen activation of platelets by GPVI, we have determined the crystal structure of the collagen-binding domain (CBD) of human GPVI and have characterized its interaction with CRP by experimental and computational methods.

Materials and methods

Cloning, expression, refolding, and purification of GPVI

Human GPVI cDNA was prepared as described. The DNA sequence encoding the CBD (residues Q1-T183) was amplified by polymerase chain

From the Department of Molecular Genetics, Biochemistry & Microbiology, University of Cincinnati College of Medicine, Cincinnati, OH; and the Department of Medicine, Division of Cardiology, University of Pennsylvania, Philadelphia, PA.


Supported in part by funds from the State of Ohio Eminent Scholar Program and an Award from the American Heart Association to A.B.H.

K.H. and A.B.H. designed research, performed research, analyzed data, and wrote the paper; and M.L.K. contributed vital reagents and wrote the paper.

The online version of this article contains a data supplement.

Reprints: Andrew B. Herr, Department of Molecular Genetics, Biochemistry & Microbiology, University of Cincinnati College of Medicine, 231 Albert Sabin Way, Cincinnati, OH 45267-0524; e-mail: andrew.herr@uc.edu.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 U.S.C. section 1734.

© 2006 by The American Society of Hematology
Cultures were grown to an Escherichia coli transformed into which yielded 2 clear solutions. All crystallographic refinements were with 0.1 mM isopropyl CBD was solubilized from inclusion bodies using denaturant buffer (6 M guanidine hydrochloride, 20 mM NaHPO₄, 10 mM imidazole, 1 mM dithiothreitol, pH 7.3) and purified under denaturing conditions by immobilized-metal affinity chromatography (IMAC) using Ni-NTA agarose (QIAGEN, Valencia, CA).

The denatured and reduced protein was refolded by rapid dilution with vigorous stirring in refolding buffer (1 M l-arginine, 2 mM EDTA, 5 mM Tris-HCl, pH 8.0), cleaved overnight at room temperature, and further purified by IMAC and size exclusion chromatography using a HiLoad 26/60 Superdex 75 column (Amersham Biosciences, Piscataway, NJ) equilibrated with TBS buffer (150 mM NaCl, 20 mM Tris-HCl, pH 7.4). The recombinant GPVI was judged to be more than 90% pure by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) with a yield of 0.5 to 1.0 mg per 1 L LB. CRP used for binding experiments was purchased from Peptide International (Louisville, Kentucky) as a non–cross-linked (POG)₁₀ polypeptide.

**Crystallization and structure determination**

Purified GPVI was crystallized by mixing 0.4 μL GPVI (5 mg/mL in TBS) with 0.4 μL crystallization buffer (1 M ammonium sulfate and 5% MPD) in sitting drop crystallization plates. Small needle-shaped crystals appeared in 3 days and were improved by microseeding and macroseeding techniques. For seeding, 2 μL protein solution (10 mg/mL in TBS) was mixed with 2 μL crystallization buffer (0.9 M ammonium sulfate, 8% MPD, and 20% glycerol). After seeding, diamond-shaped platelike crystals grew to a maximum size of approximately 150 × 150 × 20 μm in one month. The crystals belong to the space group P2₁₂₁₂ with 2 GPVI molecules in the asymmetric unit. Data were collected at 100 K with an R-AXIS IV++ image-plate detector using CuKα radiation generated by a Micromax-007 rotating anode generator (Rigaku MSC, The Woodlands, TX). A complete data set was collected to 0.24 nm (2.4 Å) resolution from 176 images (0° oscillation with 5-minute exposure time), processed by Mosflm, and scaled by SCALA from the CCP4 suite. The structure was solved by molecular replacement using Phaser1.3.1.¹⁶ The search model used was LIR-1 (pdb 1G0X) after truncating loops, E loop and manual model rebuilding. Both 2Fᵣ- Fc and Fc- F₀ electron density maps were used to manually rebuild the model with XtalView.¹⁵ When the value of the R-factor dropped to 24%, solvent molecules and ions were gradually included. The electron density was well defined for the overall structure except for 2 residues of the N-terminus for molecule A and residues 99 to 107 and 130 to 137 for molecule B. The final model contained 182 and 167 residues for molecules A and B, respectively. Criteria for inclusion of solvent and ion molecules included height and shape of the electron density peaks and appropriate coordination by GPVI residues. Data collection and refinement statistics are reported in Table 1.

**Docking of CRP to GPVI**

We used the PatchDock server²³ (http://bioinfo3d.cs.tau.ac.il/PatchDock) to predict the binding orientation of CRP on GPVI. A truncated CRP with the sequence (POG)₅ was created from the crystal structure of (POG)₅POA(POG)₅ (pdb 1CAG)²⁴ and used as the ligand, with GPVI1D₁ as the receptor. Six of the top 10 solutions showed CRP bound in a putative binding groove adjacent to the C’E loop and neighboring several residues implicated in collagen and CRP binding (K41, K59, R60, and R166)²⁵,²⁶. Three of these solutions bound in one orientation (i.e., with the N-termini of CRP closer to GPVI residue F54), and the other 3 solutions bound in one orientation (ie, with the C-termini closer to F54), consistent with the pseudo-2-fold symmetry within CRP.

We also used the FTDock program from the 3D-Dock software package to dock CRP to GPVI.²⁷ Because FTDock does not recognize hydroxyproline, a modified CRP with the sequence (PGP)₉ was created from the crystal structure of (PGP)₁₀ (pdb 1K6F).²⁸ The grid-based shape complementarity

---

**Table 1. Crystallographic data processing and refinement statistics**

<table>
<thead>
<tr>
<th>Data collected</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength, nm [Å]</td>
<td>0.15418 [1.5418]</td>
</tr>
<tr>
<td>Space group</td>
<td>P2₁₂₁₂</td>
</tr>
<tr>
<td></td>
<td>[45.29]; c = 7.513 [75.13]</td>
</tr>
<tr>
<td>Resolution range, nm* [Å]</td>
<td>2.00-0.24 [20-2.4]</td>
</tr>
<tr>
<td></td>
<td>(0.252-0.240 [2.52-2.40])</td>
</tr>
<tr>
<td>Measured reflections</td>
<td>50 335</td>
</tr>
<tr>
<td>Unique reflections</td>
<td>15 739</td>
</tr>
<tr>
<td>Average l for t (l)</td>
<td>6.6 (2.1)</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>99.3 (97.4)</td>
</tr>
<tr>
<td>Rsym, %</td>
<td>7.9 (34.8)</td>
</tr>
</tbody>
</table>

**Refinement statistics**

| Resolution range used in refinement, nm [Å] | 1.0-0.24 [10-2.4] |
| R factor, %                               | 22.3              |
| Rfree, %                                 | 27.6              |
| No. of heavy atoms                        | 1 412             |
| Molecule A (residues: 2-183)             | 1 309             |
| Molecule B (residues: 98-108-129, 138-183)| 177               |
| Water molecules                          |                   |
| Glycerol (2), SO₄(2-), Cl⁻ (1)            | 23                |
| Average B factor, nm² [Å²]               |                  |
| Molecule A                                | 0.443 [44.3]      |
| Molecule B                                | 0.509 [50.9]      |
| Solvent                                   | 0.513 [51.3]      |

**Ramachandran plot**

| No. residues in most favored             | 252               |
| No. residues in additional allowed      | 33                |
| No. residues in generously allowed      | 1                 |
| No. residues in disallowed              | 0                 |
| rms deviations from ideal               |                  |
| Bond lengths, nm [Å]                    | 0.0006 [0.006]    |
| Bond angles, degree                     | 1.50              |

---

*Values in parentheses refer to the highest resolution shell.

Rᵣ = Σ|Fᵣ - |Fᵣ| |/Σ|Fᵣ|, where h specifies unique reflection indices, and i indicates symmetry equivalent observations of h.

R factor = |Σ|Fᵣ – |Fᵣ| |/Σ|Fᵣ|, where |Fᵣ| and |Fᵣ| are the observed and calculated structure factor amplitudes, respectively.

Rfree is the same as R factor, but for a 5% subset of all reflections.

CRP were spun at 48 000 rpm. Sedimentation coefficients were determined using the program SEDFIT.²¹ For sedimentation equilibrium experiments, mixtures of 10 μM CRP and 10, 40, and 80 μM GPVI were spun at speeds of 16 000, 19 000, 29 000, 35 000, and 48 000 rpm. Data files were trimmed and analyzed by global fitting using the programs WinREEDIT and WinNOLIN (Jeff Lary, University of Connecticut, Storrs, CT). Values of sa, the weight-average sedimentation coefficient determined by SEDFIT, were fitted to a single-site binding isotherm using SEDPHAT.²²
search was performed using 148 grid units in each dimension (grid point spacing: 0.07 nm [0.7 Å]). The docking solutions were sorted by surface complementarity and the top solution was also found in the same surface groove in D1 identified by PatchDock. Furthermore, filtering the solutions by proximity to K59 reveals a second solution within the binding groove, but in the opposite orientation.

Calculation of estimated 2D \( K_d \) at plateau surface

Correlating the reported GPVI receptor density on the platelet surface\(^ \text{29} \) with an estimated \( K_d \) for dimerization of soluble GPVI CBD was carried out according to the approach of Dustin et al.\(^ \text{30,31} \) The interaction of 2 membrane-embedded receptors is estimated to occur with a loss of 3 degrees of freedom (compared with their interaction as soluble receptors), due to their restriction to 2-dimensional diffusion within the plane of a lipid bilayer.\(^ \text{32} \) The 3D \( K_d \) was converted into \( \Delta G \) and corrected by a factor of \(-1.5 \text{ RT} \) to account for the 2D restriction of the receptors. The corrected 3D \( K_d^* \) was then converted from molar units to receptors per cubic micrometer. Finally, the 2D \( K_d \) was estimated based on the equation 2D \( K_d = \sigma (3D \ K_d^*) \), where \( \sigma \) is the confinement region corresponding to the distance the receptor extends outward from the membrane. For GPVI, the confinement region was calculated to be 22 nm by adding the height of the CBD (5.2 nm) and the estimated height of the 65-residue O-glycosylated stalk (16.9 nm, based on the reported dimensions of 0.26 nm per residue for the O-glycosylated stalk of CD83\(^ \text{3})\)).

Other computational methods

Generation of collagen fiber models was carried out by applying crystallographic symmetry to the CRP structures (pdb 1CAG, 1CGD, and 1BKV) using the program O.\(^ \text{34} \) Analysis of buried surface areas and interdomain angles were calculated using Areaimol from the CCP4 suite\(^ \text{15} \) and Dom_Angle, respectively.\(^ \text{35} \) Domain boundaries were defined as residues 0 to 89 for D1 and residues 90 to 183 for D2. Figures were generated with PyMOL (DeLano Scientific, San Francisco, CA),\(^ \text{36} \) Molscript,\(^ \text{37} \) and Raster3D.\(^ \text{38} \)

Results

Crystal structure of the collagen-binding domain of GPVI

The crystal structure of the GPVI CBD was solved by molecular replacement using data to a resolution of 0.24 nm (2.4 Å). The CBD is composed of 2 Ig-like domains oriented 90° apart, similar to other LRC receptors such as FcRεI and the leukocyte Ig-like (LILR or LIR) and killer-cell Ig-like (KIR) receptor families\(^ \text{17,39-41} \) (Figure 1A,C; for a sequence alignment see Figure S1, available on the Blood website by clicking on the Supplemental Figure link at the top of the online article). Both the N-terminal and C-terminal domains (D1 and D2, respectively) of GPVI most closely resemble the domains of p58 KIR,\(^ \text{40} \) with rms deviations of 0.17 and 0.16 nm (1.7 and 1.6 Å) over 83 and 82 residues, respectively. There were 2 GPVI molecules per asymmetric unit, with D1-D2 interdomain angles ranging from 90° to 92° and with D1-D2 interfaces that buried 8.55 to 8.77 nm\(^ 2 \) (855-877 Å\(^ 2 \)).\(^ \text{2} \) These interdomain angles and interface areas are comparable with those observed for FcRεI, LIR-1, and p58 KIR, although the interface area for GPVI is somewhat less extensive. As in many LRC receptors, a conserved cis proline in D1 (P14) introduces a bend after the first β strand, creating a distinct A′ strand that forms hydrogen bonds with the G strand. As a result, the Ig fold of D1 is I-type, formed by 2 β sheets composed of the ABE and A′GFCC′ strands. D1 also contains a short 310 helix and 2 stretches of polyproline type II helix. Within D1, the most significant differences between GPVI and other LRC receptors occur in the C′ and E strands and intervening C′E loop, which adopt unusual conformations due to an 11-residue deletion in GPVI (Figure 1B,D). This deletion creates a shallow, primarily

Figure 1. Structure of the GPVI CBD. (A) Ribbon diagram of GPVI. The N-terminal domain is labeled D1, and the C-terminal domain is D2. The predicted N-glycosylation site at NT2 is shown by a gold ball. (B) Simulated annealing electron density omit map showing the C′E region of the CBD that differs from related immune receptors, with a ball-and-stick model of the deleted region superimposed. (C) Superposition of GPVI (blue), p58 KIR\(^ \text{40} \) (green), and FcRεI\(^ \text{41} \) (yellow). The orientation is similar to that shown in panel A. (D) Superposition of D1 of GPVI (blue) and p58 KIR\(^ \text{40} \) (green) shown in 2 orientations, highlighting the unusual C′E region in GPVI (blue arrows). (E) Superposition of D2 of GPVI (blue) and p58 KIR\(^ \text{40} \) (green) in 2 orientations, highlighting the lack of an A′ strand and the extended CC′ hairpin in GPVI (note blue/green arrows).
The asymmetric unit of the crystal contains a parallel, back-to-back dimer formed by the D2 domains of the 2 GPVI molecules (Figure 2A). The G strands of the D2 domains interact to create a continuous β sheet across the dimer interface. The unusual D2 architecture lacking an A’ strand is necessary for dimer formation, since the presence of A’ strands would sterically block the G strands from forming a continuous β sheet. The dimer interface buries 10.46 nm$^2$ (1046 Å$^2$) of accessible surface area, which is within the range expected for dimeric proteins, although lower than average for a protein of this size. The dimer interface shows excellent surface complementarity, with a shape complementarity index of 0.73, comparable with oligomeric proteins ($S_c = 0.70-0.74$) and protease-inhibitor complexes ($S_c = 0.71-0.76$). The interface is dominated by hydrophobic interactions along with hydrogen bonds contributed by the peptide backbone in the parallel G strands of both D2 domains (Figure 2B). Of interest, the GPVI dimerization mode is essentially identical to that observed for CD3ε and CD3δ receptor heterodimers in the T-cell receptor (TCR) complex. Like GPVI, CD3ε and CD3δ form back-to-back dimers with the G strands forming a continuous β sheet (Figure 2C). Indeed, the CD3ε δ dimer$^{45}$ superimposes on the GPVI D2 dimer with an rms deviation of only 0.2 nm (2.0 Å) over 55 residues, which is remarkable given the low sequence identity between GPVI and the CD3 receptors (20% or 14% identity between GPVI and CD3ε or γ, respectively).

**Interaction of GPVI CBD and collagen-related peptide in solution**

To understand how GPVI associates with the macromolecule collagen, we next studied the interaction between the GPVI CBD and CRP, which functionally mimics collagen in biologic assays. To analyze the affinity of the interaction under conditions favoring a 1:1 complex, sedimentation velocity analytical ultracentrifugation experiments were carried out by titrating GPVI with CRP (a non–cross-linked (POG)$_3$ triple helix) at up to 35-fold molar excess (Figure 3A). The weight-averaged sedimentation coefficient ($s_w$) of each dataset was plotted as a function of CRP concentration and fitted to a single-site binding isotherm, yielding a $K_d$ of 5 μM (Figure 3A inset). This affinity is significantly tighter than that determined by surface plasmon resonance (SPR)$^{25}$; however, the SPR experiment measured binding of GPVI to immobilized, cross-linked CRP with a different sequence than the non–cross-linked CRP described here.

Additional sedimentation velocity and sedimentation equilibrium experiments conducted using 1:1, 4:1, or 8:1 molar ratio mixtures of GPVI/CRP indicated that multiple GPVI molecules can bind to a single CRP triple helix, consistent with the presence of...
How does the GPVI dimer recognize CRP and fibrous collagen? Unfortunately, complexes of GPVI with CRP were found to be resistant to crystallization, most likely due to excessive heterogeneity of the complexes, which results from the ability of GPVI to bind at multiple overlapping sites along the triple helix. In order to identify collagen-binding sites on GPVI, we therefore used 2 different computational algorithms, PatchDock\textsuperscript{23} and FTDock,\textsuperscript{27} to dock CRP onto GPVI. Both docking programs positioned CRP within the shallow groove on D1 adjacent to the C′E loop (Figure 4A-B). The floor of the putative binding groove is formed by several hydrophobic residues (L53, F54, P56, L62, and Y66, and the aliphatic portion of K41), with several polar (S43, S44, Q48, Q50, S61) and basic (K41, R46, K59, R166) residues around the periphery (Figure 4B-D). This groove is unique to GPVI among LRC receptors, as it results from the 11-residue deletion in GPVI relative to other collagen fibers. The geometric compatibility of the binding grooves with collagen helices would allow the GPVI dimer to bind simultaneously to 2 helices within a collagen fiber.

Discussion

The receptor GPVI is central to the process of collagen-mediated platelet activation and subsequent thrombus formation. The atomic structure of GPVI is therefore of interest in terms of understanding how an immune-type receptor can recognize fibrous collagen. Furthermore, the structure allows the identification of potential regions responsible for interacting with collagen, which may serve as desirable targets for inhibitory drugs. The crystallographic data presented here reveal that the GPVI CBD adopts a fold previously seen in related immune receptors of the leukocyte receptor cluster, but an 11-residue deletion in the sequence of GPVI relative to other LRC receptors creates a shallow groove on the surface of D1 that...
forms a putative collagen-binding site, based on docking algorithms and mutagenesis data. The CBD forms a back-to-back dimer in the crystal in which the 2 putative collagen-binding grooves are nearly parallel and separated by 5.5 nm (55 Å), a configuration that matches the orientation and dimensions of triple helices within fibrous collagen.

The dimeric GPVI conformation observed in the crystal is intriguing and may well represent the physiologically relevant form of GPVI on the platelet surface. Previous studies have shown that soluble GPVI-Fc fusions, but not monomeric soluble GPVI, inhibited platelet activation, suggesting that either a dimeric conformation or the higher avidity conferred by the Fc fusion was required to effectively compete with cell-surface GPVI for binding to collagen. This was further supported by surface plasmon resonance assays showing that the GPVI-Fc fusion bound collagen nearly 200-fold more tightly than monomeric GPVI did.

The data presented here suggest that GPVI dimerization is a rather weak interaction that nonetheless could occur on the platelet surface. Analytical ultracentrifugation experiments indicated that the soluble GPVI CBD construct used for crystallization remained monomeric in solution at up to 100 μM (Figure 3A and data not shown). However, the construct we crystallized lacks the stalk region, which could help stabilize a dimeric conformation, as seen for CD94. Furthermore, the high density of GPVI at the platelet surface would favor dimer formation. It is well established that weak protein-protein interactions in solution occur to a significant extent when the components are restricted to 2-dimensional diffusion in a lipid bilayer. For example, if the Kd for GPVI dimerization in solution were 420 μM (~ 8.5 mg/mL), this would correspond to a 2D Kd of 1260 receptors per square micrometer, which is equivalent to the GPVI density on platelets. Thus even very weak dimerization of the CBD in solution would be sufficient to allow significant dimerization of GPVI at the platelet surface. Of interest, activation of GPVI signaling by collagen is critically dependent on GPVI surface density; RBL cells transfected with GPVI were unresponsive to collagen unless expressed at a surface density approximating that observed on platelets.

The GPVI structural data provide a framework for understanding the interaction between GPVI and collagen or CRP by allowing accurate mapping of mutagenesis results onto the surface of the GPVI dimer. The residues implicated in collagen or CRP binding fall into 2 clusters: the primary region includes basic residues on the surface of D1 including K41, K59, R60, and R166; the secondary residues are positioned such that they could interact with adjacent triple helices within an intact collagen fibril. The mutagenesis results are therefore consistent with the mode of interaction between GPVI and a collagen fibril illustrated in Figure 4B, in which a GPVI dimer binds simultaneously to several triple helices within the collagen fibril. This binding mode also suggests a model for the initiation of signal transduction triggered by receptor clustering that accompanies collagen binding (Figure 5).

These studies establish a structural basis for the ability of platelets to recognize and be activated by the vessel wall matrix protein collagen. Platelet activation is a critical step in the pathogenesis of human vascular diseases and new antiplatelet agents have revolutionized the immediate treatment of myocardial infarction. The early role of GPVI in arterial thrombus formation and the relative lack of bleeding associated with human GPVI-deficiency states suggest that new therapies aimed at inhibiting GPVI function might provide an ideal long-term treatment approach to these diseases. A structural understanding of collagen recognition by GPVI will provide a foundation for the development of such novel therapeutic agents.

Acknowledgments

We thank Drs Rhett Kovall, Tom Thompson, and Jeff Wilson for helpful advice, and Dr Pamela Bjorkman and Deb Conrady for comments on the article.

Coordinates have been deposited at the RCSB protein data bank (PDB ID 2G17).
References

Structural basis for platelet collagen responses by the immune-type receptor glycoprotein VI

Katsunori Horii, Mark L. Kahn and Andrew B. Herr