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Isolation, Characterization and Sequence Analysis of Five IgG Monoclonal Anti-β_2-Glycoprotein-1 and Anti-Prothrombin Antigen-Binding Fragments Generated by Phage Display[1]

Reginald U. Chukwuocha,[2]* Elie T. Hsiao,† Peter Shaw,‡ Joseph L. Witztum,‡ and Pojen P. Chen*

We have isolated five monoclonal IgG anti-β_2-glycoprotein-1 (anti-β_2G-1) and anti-prothrombin Fab from a patient with autoantibodies to oxidized low-density lipoproteins by phage display method. Analysis of their binding specificity revealed that all three β_2GP-1-enriched mAbs (B14, B22, B27) reacted with β_2GP-1 while both prothrombin-isolated mAbs (P11 and P13) reacted with prothrombin. Intriguingly, mAb P11 reacted with β_2GP-1 and prothrombin and showed comparable binding affinity to both Ags, with K_d values of 1.6×10^{-6} M for β_2GP-1 vs 3.2×10^{-6} M for prothrombin. This clone may thus, define a hitherto unknown shared epitope between β_2GP-1 and prothrombin. Sequence analysis of all five clones showed significant mutations of the expressed genes. One rearranged V-D-J segment was repeatedly employed by three clones (mAbs B22, B27, and P13). However, all three clones used different L chains. Of note, the pairing of VH6-D-J with the L5-Vk1 L chain in mAb P13 resulted in the loss of binding to β_2GP-1 and specific reactivity to prothrombin. Together, these data suggest that while the VH6-D-J chain may be important in the binding to β_2GP-1, pairing with certain L chains may influence this binding. These data are the first human IgG anti-β_2GP-1 and anti-prothrombin sequences reported; both represent the major subsets of antiphospholipid Abs present in antiphospholipid syndrome patients. *The Journal of Immunology,* **1999, 163: 4604–4611.**

Antiphospholipid Abs (aPL),[3] which include anticardiolipin Abs (aCL) detected by ELISA and lupus anticoagulant (LAC) Abs detected by in vitro blood clotting assays, are associated with thrombosis, recurrent fetal loss, and thrombocytopenia in patients with antiphospholipid syndrome (APS) (1–3). It is generally considered that the binding targets of aPLs include negatively charged phospholipids (PLs), various plasma proteins or complexes formed by PLs, and plasma proteins (4–7). These plasma proteins include β_2-glycoprotein-1 (β_2GP-1), prothrombin, annexin V, protein C, and its cofactor protein S (5, 8, 9). In 1990, two studies showed that the binding of aPL to cardiolipin (CL) was enhanced by β_2GP-1, suggesting that aPLs recognized a complex of CL and β_2GP-1 (7, 10). Other studies have reported that aPL reacted with β_2GP-1 alone (5, 11, 12). Over the last few years, the consensus is that anti-β_2GP-1 Abs make up a significant percentage of aPL found in APS patients (5, 7, 12–14). In addition, recent studies show that anti-β_2GP-1 Abs are more closely associated with APS thrombosis (5, 12, 15).

In contrast, increasing attention is being paid to anti-prothrombin Abs and the role they may play in thrombosis in APS patients. The prevalence of these Abs in patients varies greatly, ranging from 20 to 60% when detected via ELISA using immobilized human prothrombin on activated poly(vinyl chloride) plates (8, 16). Importantly, it was reported that affinity-purified IgG anti-prothrombin Abs bound to immobilized phosphatidylserine (PS) in the presence of Ca^{2+} and prothrombin, suggesting that IgG anti-prothrombin Abs bound to prothrombin and then is transported onto PS as a "passenger" upon prothrombin binding to PS (17, 18). In this context, it is conceivable that anti-prothrombin IgG may cross-link prothrombin molecules and thus increase the valency of interactions between prothrombin and PS. This implies that the anti-prothrombin IgG may enhance the binding of prothrombin to PS and to PL surface on endothelial cells and thus increase thrombin generation and promote thrombosis.

The oxidative modification of low-density lipoproteins (Ox-LDL) is thought to play an important role in various disease states including atherosclerosis (19, 20). Studies have shown that PLs are structurally similar to LDL and circulating lipoproteins contain various amounts of PLs and β_2GP-1 (21). In addition, aPL may be directed against epitopes of oxidized PLs and cross-react with Ox-LDL (22, 23). Together, these data suggest that there is an overlap between aPL and anti-Ox-LDL Abs.

Although significant progress has been made in understanding the binding specificities of aPL, little is known about the structures and genetic basis of these potentially pathogenic autoantibodies. For reasons that are connected with the low efficiency of generating IgG Abs by conventional methods, few IgG aPL have been generated and studied. As a result, structure analysis of the potentially pathogenic Abs in APS has been difficult. Here we describe the isolation of five IgG monoclonal anti-β_2GP-1 and anti-

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[3] Abbreviations used in this paper: aPL, antiphospholipid Abs; aCL, anti-cardiolipin Abs; APS, antiphospholipid syndrome; LAC, lupus anticoagulant;; SLE, systemic lupus erythematosus; β_2GP-1, β_2-glycoprotein-1; CL, cardiolipin; LDL, low density lipoprotein; Ox-LDL, oxidized low density lipoprotein; PL, phospholipid; PS, phosphatidylserine; BBS, borate-buffered saline; FR, framework region; R/S, replacement to silent; CDR, complementarity-determining region.

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prothrombin Abs by phage display method from a patient with anti-Ox-LDL Abs. We report a detailed characterization of their binding specificities and sequence analysis.

Materials and Methods

Patient

The patient is a 72-year-old male who underwent coronary artery bypass surgery despite having low total plasma cholesterol levels for many years. His plasma was screened for the presence of autoantibodies to epitopes of Ox-LDL as part of a study being conducted in patients seen in the Lipid Research Clinic at the University of California at San Diego. Because anti-Ox-LDL Abs overlap substantially with aPL, we analyzed his sera for aCL and reactivities with β_2GP-1 and prothrombin. The results showed that he had significant titers of anti-β_2GP-1 Abs at one in one hundred (1:100, in borate-buffered saline (BBS), 0.2 M boric acid, 0.15 M NaCl, pH 8.2, containing 0.25% gelatin) and anti-prothrombin Abs (1:50, in calcium buffer, 50 mM Tris-HCl, 150 mM NaCL, 50 mM $CaCl_2$, pH 7.5, containing 0.25% gelatin) (data not shown). Accordingly, his monocytes were isolated and used to prepare a combinatorial library.

The control library was prepared from a normal individual whose plasma did not contain anti-DNA, anti-β_2GP-1, and anti-prothrombin Abs (data not shown).

Construction of combinatorial libraries

An IgG1 κ and λ libraries were constructed according to published protocols (24, 25). Briefly, lymphocytes from the patient and control subjects were isolated and used as the source of total RNA for the phage library construction. PCR was then used to amplify and clone Fab genes from isolated cells into the phage display vector, pComb3H. Phage Fab clones are then selected based on Ab-binding specificity on β_2GP-1 or prothrombin-coated plates. We obtained a library of 10^8 members with an insert frequency of >80% as determined by restriction endonuclease analysis.

Selection of aPL Fab clones and initial characterization of the isolated clones

The selection of aPL clones was performed as previously described (24). Briefly, microtiter plates (3690; Costar, Cambridge, MA) were coated overnight at 4°C with either β_2GP-1 at 10 μg/ml in BBS or prothrombin (Enzyme Research Laboratories, South Bend, IN) at 10 μg/ml in calcium buffer (50 mM Tris, 150 mM NaCl, and 50 mM calcium chloride, pH 7.5). After washing, β_2GP-1-coated plates were blocked with 3% of BSA in BBS while prothrombin-coated plates were blocked with 0.25% gelatin for 1 h at room temperature. Then, freshly prepared phage particles (10^{12} phage particles) were added and incubated for 2 h at room temperature. Thereafter, wells were washed extensively with TBS containing 0.5% Tween 20 (TBST) and bound phage particles were eluted with 50 μl of 0.1 M HCl/glycine (pH 2.2)/0.1% BSA. Following the third round of panning, phagemid DNA was recovered and used to generate soluble Fab as previously described (24).

Then, each isolated clone was lysed and analyzed for soluble Fab by ELISA. Wells were coated with goat anti-human IgG Fab mAb (Cappel Research Products, Durham, NC) overnight at 4°C and blocked with 0.25% BSA. Bacterial lysates containing soluble Fab were added to wells in duplicates, and the bound Fab were detected with enzyme-labeled goat anti-human IgG. Fab from each positive clone were affinity purified with the goat anti-human Fab column and analyzed for their binding property.

Ab-binding ELISA

The binding specificity of Fab clones were determined as previously described for anti-β_2GP-1 and anti-prothrombin Abs (24). For anti-β_2GP-1, microtiter plates were precoated with 10 μg/ml β_2GP-1 in BBS overnight at 4°C. After blocking with 0.25% BSA, serial dilutions of purified Fab were distributed to wells in duplicates, and plates were incubated for 2 h at room temperature. The plates were then washed four times with BBS and incubated for 1 h at room temperature with affinity-purified enzyme-labeled goat anti-human IgG.

ELISA for the detection of anti-prothrombin activity was similar to the anti-β_2GP-1 ELISA assay with some modification. Briefly, wells were

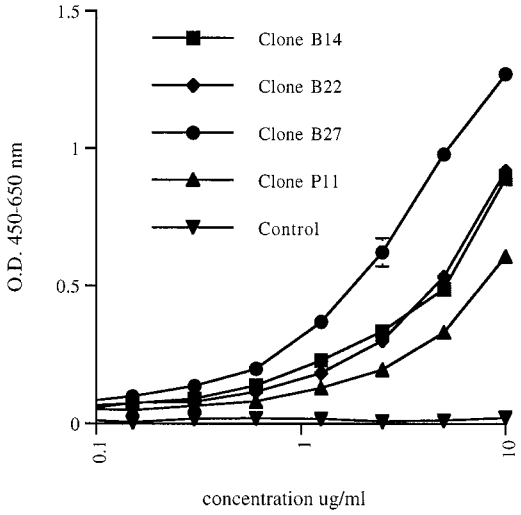

FIGURE 1. Binding specificity of affinity-purified monoclonal Fab mAbs B14, B22, B27, and P11. mAbs were analyzed against β_2GP-1. Bars represent mean net OD ± SD readings of duplicate samples.

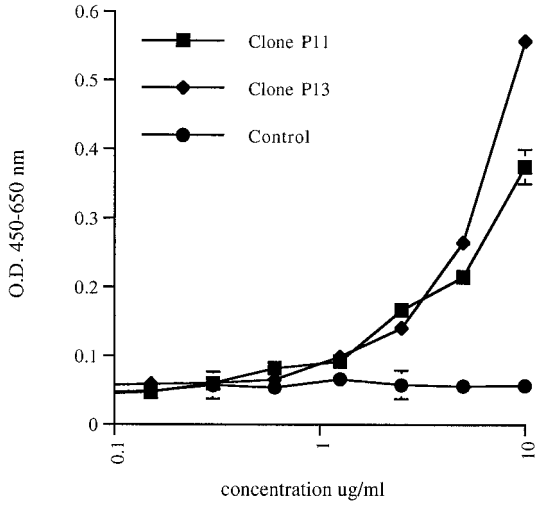

FIGURE 2. Binding specificity of affinity-purified monoclonal Fab P11 and P13. mAbs were analyzed against prothrombin. Bars represent mean net OD ± SD readings of duplicate samples.

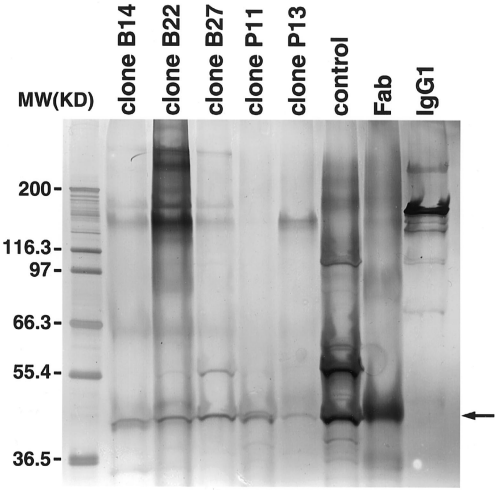

FIGURE 3. SDS-PAGE of mAbs, in silver staining. *Lane 1*, m.w. marker; *lane 2–7*, nonreduced mAbs B14, B22, B27, P11, P13, and a control Fab clone isolated from a normal human lymphocyte library; *lane 8–9*, nonreduced human Fab and IgG1 standard used as positive controls. Arrow indicates the position of the 47-kDa Fab band.

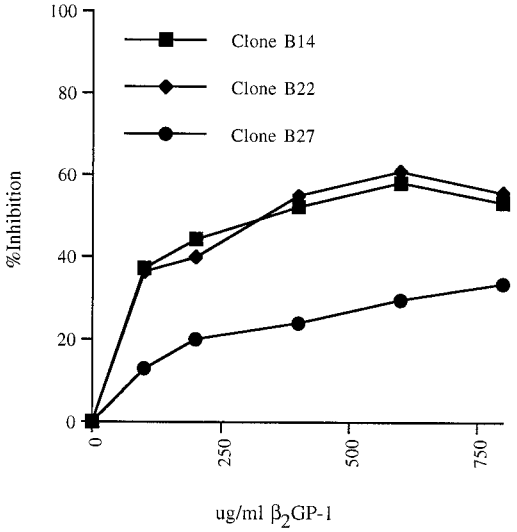

FIGURE 4. Competitive inhibition of binding of mAbs B14, B22, and B27 to β_2GP-1 and representative mean OD readings of duplicate samples.

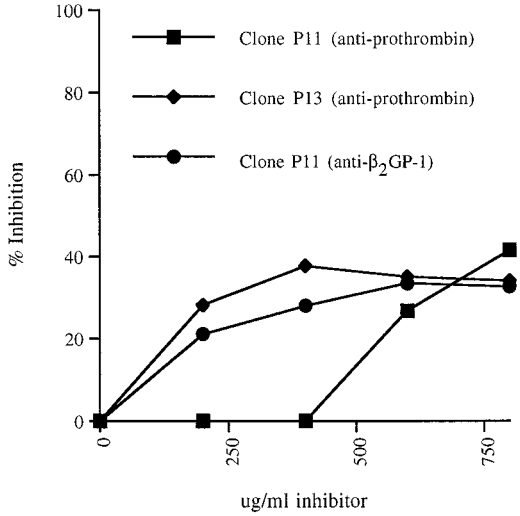

FIGURE 5. Competitive inhibition of binding of mAbs P11 and P13 to prothrombin and to β_2GP-1 (mAb P11) and representative mean OD readings of duplicate samples.

coated with 10 μg/ml prothrombin (Enzyme Research Laboratories) in calcium buffer and blocked with 0.25% gelatin in calcium buffer.

The purified Fab were also used to study the binding specificities of the mAbs to a panel of unrelated Ags, including: chicken OVA, collagen, and ssDNA. All Ags were used at 10 μg/ml, and ELISA was performed as described for β_2GP-1 and prothrombin.

SDS-PAGE analysis of Fab

Purified mAb Fab and control proteins (150 ng except for mAb P13, which was 100 ng) were loaded into 7% Tris-acetate gel for nonreducing SDS-PAGE analysis (NOVEX, San Diego, CA). After electrophoresis, the gel was analyzed by silver staining for the detection of Fab protein bands according to the manufacturer's instructions (Pierce, Rockford, IL).

Determination of Fab-binding affinity

The binding affinity of each mAb was determined by Ag inhibition, with each mAb used at a concentration that gave 50% maximal binding. Diluted mAbs (mAbs P11, 20 μg/ml; B14, 10 μg/ml; B22, 20 μg/ml; B27, 10 μg/ml; P13, 30 μg/ml) were then preincubated for 2 h at room temperature with an equal volume of buffer or increasing concentration of β_2GP-1 or prothrombin (100, 200, 400, 600, and 800 μg/ml). The amount of free mAb in the Ab inhibitor mixtures were then measured in an anti-β_2GP-1 or anti-prothrombin ELISA using Ag-precoated plates. The average mAb affinity was calculated according to previously described method (26).

Sequencing of Fab V region genes

Sequencing was performed on purified dsDNA by using previously published sequencing primers (25). Sequence data were compiled and analyzed using the Basic Local Alignment Search Tool (BLAST) (27).

Results

Panning of the aPL phage clones

After panning against β_2GP-1, three clones were isolated from the κ library while none was isolated from the λ library. For prothrombin, two clones with anti-prothrombin activity were recovered from the κ library alone. Restriction analysis of their DNA revealed that all contained both the H and L chain inserts.

Characterization of the anti-β_2GP-1 and anti-prothrombin Fab

To study the binding property of these clones, affinity-purified Fab were analyzed against β_2GP-1, prothrombin, and other unrelated Ag. As shown in Fig. 1, all three β_2GP-1-enriched clones (termed mAbs B14, B22, B27) reacted with β_2GP-1. Of these, two (mAbs B14 and B22) reacted with CL when complexed with β_2GP-1 (data not shown). All three clones did not react with four unrelated Ags, including collagen, OVA, prothrombin, and ssDNA (data not shown). In Fig. 2, both prothrombin-selected clones (termed mAb P13 and P11) reacted with prothrombin. When tested against four unrelated Ags (β_2GP-1, collagen, OVA, and ssDNA), P11 reacted strongly with β_2GP-1 (Fig. 1), weakly with OVA and ssDNA, but not at all with collagen (data not shown). Fig. 3 shows that each of the mAb Fab displayed the expected 47-kDa Fab band on silver staining of SDS-PAGE gel.

Table I. *Ig gene usage of five aPL monoclonal Fab from a patient with Ox-LDL*

		VH germline				VK germline		
mAb	K_d	Putative	Homology (%)	D	JH	Putative	Homology (%)	JK
B14	7.0×10^{-5} M	VH-26 (VH3)	92	21/07	1	A20 (VK1)	96	2
B22	6.0×10^{-5} M	6-IG1 (VH6)	94	d5r, 21/10r	4	A20 (VK1)	98	3
B27	1.5×10^{-6} M	6-IG1 (VH6)	94	d5r, 21/10r	4	L15 (VK1)	96	4
P11	1.6×10^{-6} M[a]	VH4.33 (VH4)	93	23/07, 21/10r,				
	3.2×10^{-6} M[b]			22/12, Dxp'1	1	A30 (VK1)	95	3
P13	1.4×10^{-6} M	6-IG1 (VH6)	94	d5r, 21/10r				
				dn4	4	L5 (VK1)	93	1

[a] K_d of anti-β_2 GP-1 reactivity of mAb P11.
[b] K_d for anti-II response of mAb P11.

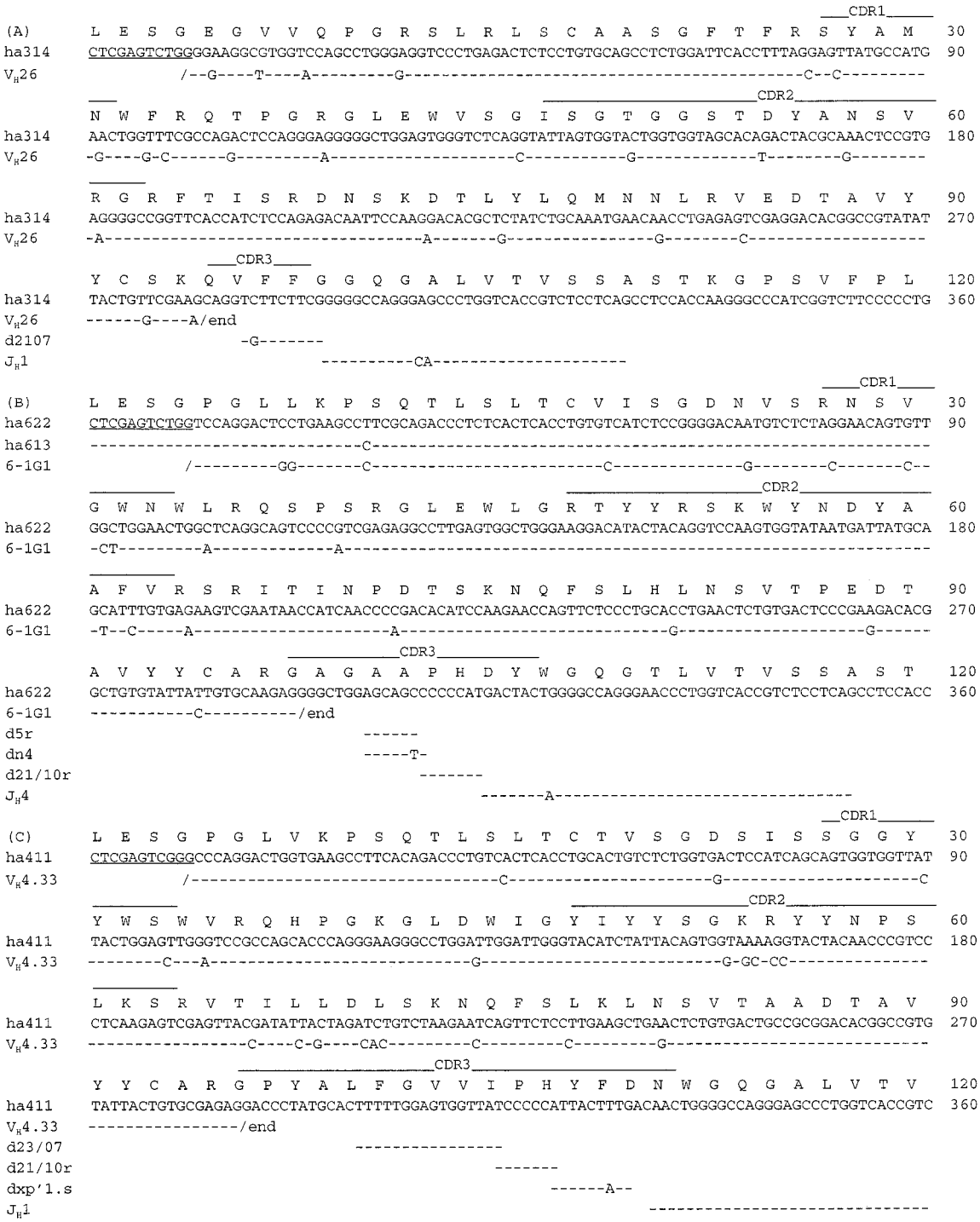

FIGURE 6. The nucleotide and deduced amino acid sequences of Ab H chain V regions B14 (*A*), B22 and P13 (*B*), and P11 (*C*), which are designated Humha314, Humha622, Humha613, and Humha411 and are abbreviated ha314, ha622, ha613, and ha411, respectively. The H chain of B27 is identical with that of B22 and thus is represented by ha622; ha613 differs from ha622 by a single silent base and is given for this region only. The putative corresponding germline gene sequences are included for comparison (29–31). In each panel, the complete nucleotide and amino acid sequences of a H chain are given, while the corresponding germline sequences (and other related H chain sequence) are given only at the positions where they differ from V_H cDNA in the overlapping regions. Dashes denote the identities, the PCR primers are underlined. The CDRs are indicated, and D region and JH genes of all clones are included.

Competitive inhibition ELISA

Binding specificity was confirmed by demonstration that soluble β_2GP-1 and/or prothrombin inhibited the mAb interactions with immobilized β_2GP-1/prothrombin as previously described (26). As shown in Figs. 4 and 5, four Fab clones were specific for β_2GP-1 while two clones were reactive with prothrombin. The reactivity of mAb P11 to β_2GP-1 and prothrombin was inhibited by soluble Ags, suggesting that mAb P11 may recognize an

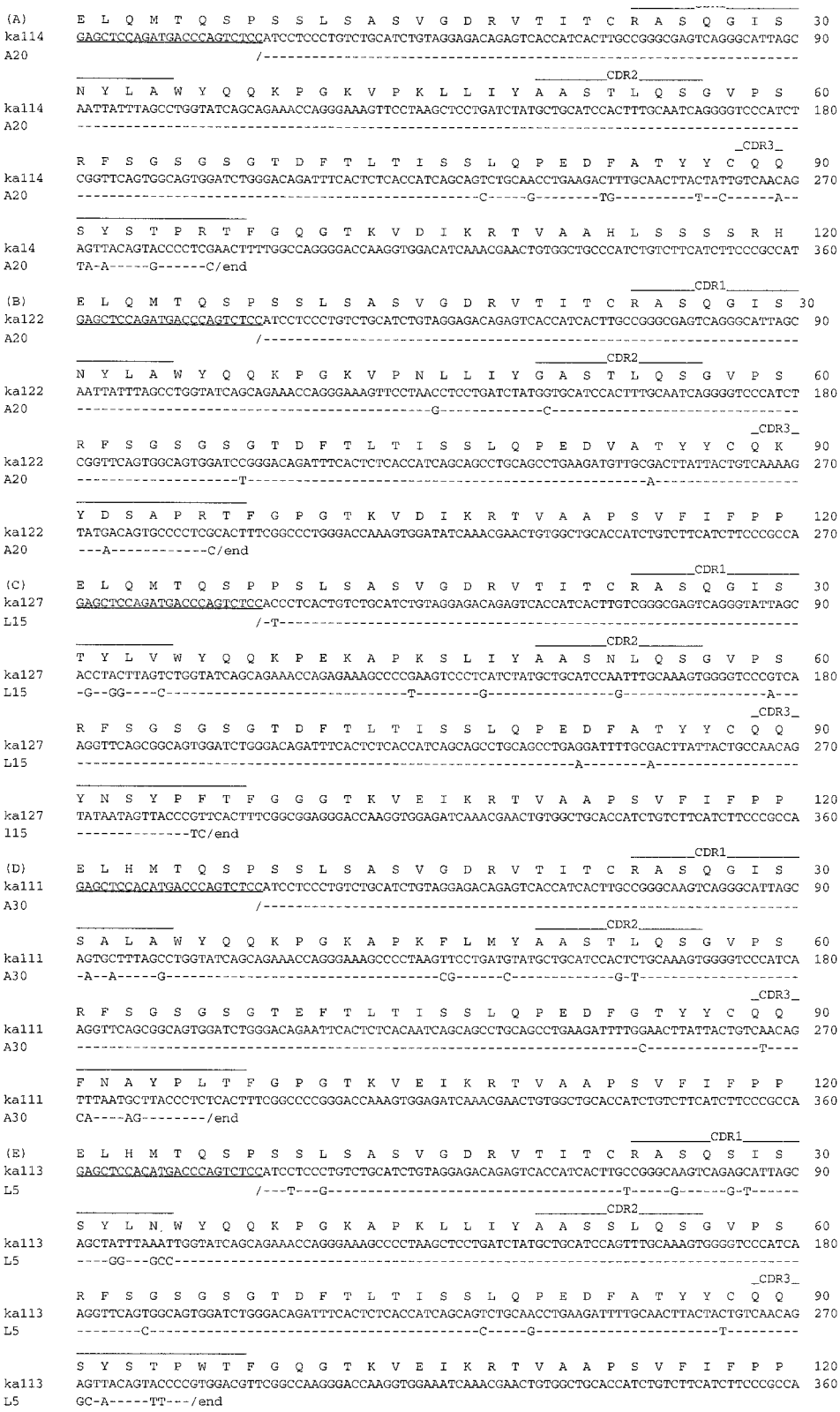

FIGURE 7. The nucleotide and deduced amino acid sequences of Ab L chain V regions: (A) B14, (B) B22, (C) B27, (D) P11 and (E) P13 which are designated Humka114, Humka122, Humka127, Humka111 and Humka113 and are abbreviated ka114, ka122, ka127, ka111 and ka113. The putative corresponding germline L chain V gene sequences are included for comparison (32–35). In each panel, the complete nucleotide and amino acid sequences of a L chain are given, while the germline sequence is given only at the positions where it differs from the L chain in the overlapping regions. Bars denote the identities, the PCR primers are underlined, and the CDRs are indicated.

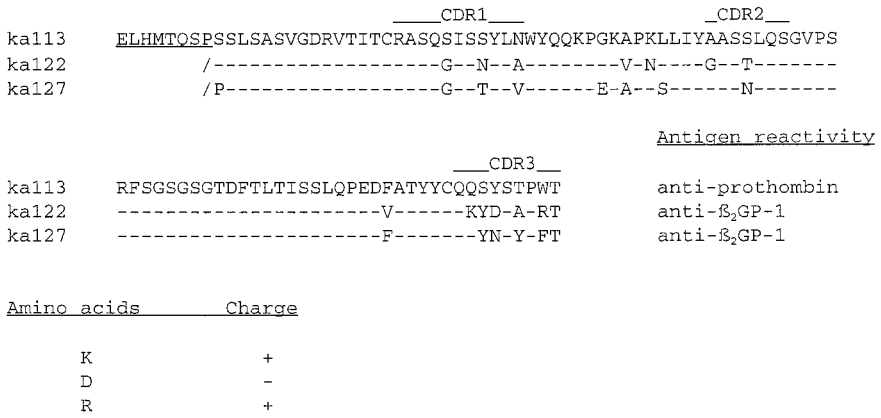

FIGURE 8. The deduced amino acid sequence of clone P13, B22, and B27 L chain cDNA V regions, designated Humka113, abbreviated ka113; Humka122, abbreviated ka122; and Humka127, abbreviated ka127, respectively. The complete amino acid sequence of ka113 are given, while all others are given only at the positions where they differ from the sequence of ka113. Dashes denote identities, while the CDRs are indicated. The underlined amino acid sequence is coded by the PCR primer.

epitope shared by prothrombin and β_2GP-1. The inhibitions range from 23 to 61% and are consistent with the low affinity of anti-β_2GP-1 Abs, which remains free in the presence of 200 μg/ml of β_2GP-1 in the plasma (28). The binding affinities (K_d) were estimated from the inhibition ELISAs and are shown in Table I.

Sequence analysis of the aPL Fab clones

Sequence analysis of the H and L chain V regions of all Fab clones (Figs. 6 and 7) revealed that B22, B27, and P13 shared an almost identical rearranged VH6-D-J gene segment termed Humha622. It consists of VH6, D5, and D21/10 in the reverse orientation, and JH4 (Fig. 6*B*). The putative D5-encoded segment may derive from DN4. The V_H sequences for B22 and B27 are identical and are represented by ha622 (Fig. 6*B*); they differed from that of P13, denoted as ha613 in Fig. 6*B*, by one silent change in the framework (FR) 1. However, these three clones used different L chains termed Humka122, Humka127, and Humka113, respectively (denoted as ka122 [B], ka127 [C], and ka113 [E] in Fig. 7); each employed different members of the Vk1 family.

The mAb B14 employed the V_H-26 VH3 gene termed Humha314 and the A20 Vk1 gene termed Humka114 (Figs. 6*A* and 7*A*). Of note, the invariant tryptophan residue that represents the beginning of the fourth FR in ha314 is absent (Fig. 6A). To the best of our knowledge, this is the first V_H sequence of a functional Ab that has no invariant tryptophan. Finally, clone P11 used the VH4.33 gene, termed Humha411, and the A30 Vk1 gene, termed Humka111 (Figs. 6*C* and 7*D*).

It was difficult to discern the closest germline D gene used in all five clones because of extensive modification, but there appears to be certain germline genes that could have been employed by these clones as shown in Fig. 6 and Table I. As for the JH gene usage, the mAbs P11 and B14 employed JH1 while others used JH4 gene segments (Fig. 6).

There was no restriction in Jk usage in the clones analyzed (Table I). ka113 used Jk1, ka114 used Jk2, ka122 and ka111 employed Jk3, and ka127 used Jk4. The Jk2 employed by ka114 and the Jk3 in ka111 each contained two mutations (Fig. 7, *A* and *D*).

A comparison of the V gene-encoded regions in all five clones with both GenBank and EMBL databases as well as all published sequences revealed that the expressed V_H showed a range of 92 to 94% homology with their nearest germline genes for an average of 93.4% (Table I) (29–35). When compared with these putative germline counterparts, the replacement to silent changes (R/S) in the complementarity-determining regions (CDRs) was 5.0 for mAbs B14, B22, B27, and P13 and $<$1.0 for mAb P11. In contrast, the R/S ratios in the FRs was 4.0 for mAb B14 and $<$1.4 for mAbs B22, B27, P11 and P13.

Sequence analysis of the L chains of all five clones revealed significant mutations that range from 6 to 20 nt per L chain V region, which results in a mutation frequencies of 2.1–7.0%. In Fig. 7, the germline nucleotide sequences are included for comparison. When compared with their germline counterparts, the R/S ratios in the CDRs was 2.0 for mAb B22, 4.0 for mAbs B14 and B27. This ratio was 8.0 and 6.0, respectively, for mAbs P11 and P13. In contrast, the R/S ratios in the FRs was 3.0 for mAb P11 and $<$0.5 for mAbs B14, B22, B27, and P13.

Discussion

In an attempt to define the structural basis of anti-β_2GP-1 and anti-prothrombin activities obtained from a coronary artery bypass patient, we employed the phage display method to isolate and analyze the structural features of five monoclonal IgG aPL Fab. The results show that all three β_2GP-1-enriched clones, mAbs B14, B22, and B27, specifically recognized the plasma cofactor, β_2GP-1 (Fig. 1). The affinities of these three clones range from 7×10^{-5}

Table II. *aPL share Ig V genes with other anti-DNA autoantibodies in SLE*

Germline V Gene[a]	aPL		Patient-Derived Autoantibody[b]	
	Name	Homology (%)	Name	Homology (%)
VH26 VH3	B14	92	18/2 IgM anti-DNA	100
4.33 VH4	P11	93	None	n/a
VH6	B22	94	A10 IgM anti-DNA	99
A20 VK1	B14, B22	96,98	III-2R IgM anti-DNA	100
A30 VK1	P11	95	SC17 IgG anti-DNA	98
L5 VK1	P13	93	NE-13 IgM anti-DNA	100
L15 VK1	B27	96	None	n/a

[a] The references for germline Ig V genes are VH26 (29), 4.33 (31), VH6 (30), A20 (34), A30 (35), L5 (33), and L15 (32).
[b] The references for patient-derived autoantibodies are 18/2 (41), A10 (42), III-2R (43), SC17 (40), and NE13 (44).

to 1.5×10^{-6} M and are comparable to the reported K_d values of serum anti-β_2GP-1 Abs ranging from 10^{-5} to $3.4\ 10^{-6}$ M (14, 36). The mAb B27 had the highest affinity of the three β_2GP-1 binding clones and was the most specific Ab.

Both prothrombin-enriched clones (mAbs P11 and P13) reacted with prothrombin (Fig. 2). Intriguingly, mAb P11 also reacted strongly with β_2GP-1, suggesting that P11 may recognize an epitope shared by prothrombin and β_2GP-1. It is possible that similar dual-reactive autoantibodies may be present in APS patients. In the future, it would be important to study the presence of mAb P11-like aPL in APS patients and the role of such aPL in APS pathogenesis. To this end, it will be required to first define the epitope recognized by mAb P11. The conversion of clone P11 into intact IgG secretor would allow the screening of β_2GP-1 and prothrombin peptide libraries to identify the shared epitope. Subsequently, the peptide representing this shared epitope can then be used to screen patients serum samples for the putative P11-like aPL.

The mAbs B22, B27, and P13 shared identical VH6-D-J H chain but different L chains. The first two mAbs recognized β_2GP-1, while P13 bound to prothrombin, suggesting that while VH6-D-J gene may have an intrinsic binding affinity for β_2GP-1, certain L chain pairings apparently influence that binding. To decipher the role of L chains in binding to β_2GP-1, we compared their amino acid sequences. Fig. 8 shows that the two β_2GP-1-reactive L chains, ka122 and ka127, are not more similar to each other than to the prothrombin-reactive ka13.

The H chains of mAbs B22, B27, P13, and B14 derive from VH6/V6–1 and VH26/V3–23 genes segments, respectively. These V_H gene segments belong to a set of V_H genes that have been shown to be preferentially expressed in ontogeny (37, 38). Previously, analysis of several natural autoantibodies derived from normal individuals revealed usage of the same restricted set of V genes. In this context, the natural IgM autoantibody Kim 13.1, which is encoded by the 51P1 gene in germline configuration, displays anti-CL and rheumatoid factor activity (39). Taken together, these data suggest that some aPL autoantibodies may arise from natural autoantibodies.

The mAb P11 used the A30 Vk1 L chain, which is rarely employed in the functional Ig Vk gene repertoire. In contrast, it was reported recently that in systemic lupus erythematosus (SLE) patients A30 is rearranged to the Jk2 gene and encodes the nephritogenic anti-DNA Ab L chain (35, 40). In addition, several of the presently characterized aPL-associated V genes have been shown to encode diagnostic anti-DNA autoantibodies in SLE (Table II) (40–44). In particular, VH26 and VH6 encode, respectively, the H chains of the 18/2 and the A10 anti-DNA Ab; A20, A30, and L5 encode the L chain of the III-2R, SC17, and NE-3 anti-DNA autoantibodies, respectively. The meaning of these findings are not clear. However, the extensive overlap of the V gene usage in the aPL with that of characteristic anti-DNA autoantibodies in SLE suggests that some aPL in SLE patients may arise as the byproducts of receptor editing of autoreactive B cells, in which one of the original H and L chain V regions is replaced with a newly rearranged V gene (45, 46). In support of this, sequence analysis of three monoclonal LAC Abs revealed extensive overlap in the Ig V genes with anti-DNA Abs found in lupus patients (47).

In conclusion, we isolated five mAbs aPL representing two of the major groups of autoantibodies found in APS patients. The structural features of these Abs revealed that certain H and L chain combinations may be important in the development of aPL reactivity.

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