Isolation, Characterization, and Cloning of Porcine Complement Component C7

Azin Agah, Michael C. Montalto, Cheri L. Kiesecker, Margaret Morrissey, Meera Grover, Kara L. Whoolery, Russell P. Rother and Gregory L. Stahl

http://www.jimmunol.org/content/165/2/1059

References
This article cites 35 articles, 22 of which you can access for free at:
http://www.jimmunol.org/content/165/2/1059.full#ref-list-1

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Isolation, Characterization, and Cloning of Porcine Complement Component C7

Azin Agah,* Michael C. Montalto,* Cheri L. Kiesecker, † Margaret Morrissey,* Meera Grover,* Kara L. Whoolery,* Russell P. Rother, † and Gregory L. Stahl2 *

Activation of the complement system through the classical, alternative, or lectin pathway results in the formation of the terminal complement complex. C7 plays an integral role in the assembly of this complex with target cell membranes. To date, only human C7 has been cloned and characterized; thus, in this study, we characterized the porcine complement component C7. Porcine C7 was isolated by affinity chromatography as a single glycoprotein with an approximate molecular mass of 90 kDa and 100 kDa under reducing and nonreducing conditions, respectively. The full-length porcine C7 cDNA was isolated, and the predicted amino acid sequence exhibited 80% identity with human C7 with conservation of the cysteine backbone and two putative N-linked glycosylation sites. Porcine C7 mRNA expression was detected in all tissues investigated, except polymorphonuclear and mononuclear leukocytes. Addition of purified porcine C7 restored the hemolytic activity of C7-depleted human sera in a dose-dependent manner. A functionally inhibitory mAb against porcine C7 attenuated the hemolytic activity of human, rabbit, or rat sera, suggesting an important conserved C7 epitope among species. These data demonstrate that porcine and human C7 are highly conserved, sharing structural and functional characteristics. The Journal of Immunology, 2000, 165: 1059–1065.

H uman complement is a component of the innate immune system that plays a key role in the elimination of pathogens and initiation of inflammatory responses. Based on its mode of activation, the complement system has been subdivided into the classical, alternative, and lectin pathways. Complement activation ultimately results in formation of the terminal complement complex (C5b-9). Formation of the C5b-9 is initiated when C5 is cleaved into C5a and C5b. C5a, a potent anaphylatoxin, mediates various inflammatory events, while C6 binds to C5b to form a stable complex C5b-6. Upon binding of C7 to C5b-6, the trimeric C5b-9 complex undergoes a conformational transition and allows insertion of this complex into lipid membranes (1–4). The incorporation of C8 into C5b-7 brings about the addition of C9 molecules, resulting in formation of a transmembrane pore. The C5b-9 can contain as many as 18 C9 molecules that polymerize into barrel-like structures and can vary dramatically in size (5, 6). As a result, formation of C5b-9 can induce changes in lipid bilayer permeability and ultimately result in cellular activation or death (7).

Sublytic amounts of C5b-9 can activate neutrophils, endothelial, and epithelial cells, leading to a proinflammatory state (7). In addition, C5b-9 induces endothelial expression of IL-8 and P-selectin (8, 9), augments TNF-induced ICAM-1 and E-selectin expression (9, 10), and directly attenuates endothelium-dependent relaxation of vascular smooth muscle (11–13). C5b-9 also induces a loss of acetylcholine-induced increases in intracellular cGMP in human endothelial cells, leading to translocation of NF-κB and up-regulation of VCAM-1 (14). Inhibition of C5a and C5b-9 attenuates neutrophil infiltration and limits infarct size following myocardial ischemia and reperfusion (15). C5b-9 also plays a major role in tissue injury following renal ischemia/reperfusion (16). Collectively, these data demonstrate a significant role of C5b-9 in the inflammatory process. Thus, understanding and regulating C5b-9 formation may lead to potential therapeutics that inhibit C5b-9-mediated tissue injury.

Similar to C5b-9, hemolytically inactive C5b-7 complex induces cellular activation (17, 18). Since C7 plays a pivotal role in formation of biologically active terminal complement complexes, C7 may be a potential therapeutic target for inhibition of C5b-9 formation and inflammation. At present, only human complement C7 has been cloned and characterized (3, 4, 19, 20). To gain further insight into the structural/functional architecture of this complement component, we have characterized and generated mAbs to porcine C7. The data demonstrate a striking homology between human and porcine C7 at structural and functional levels.

Materials and Methods

Purification of porcine C7

A commercially available mAb to human C7 (CL326; Quidel, San Diego, CA) was found in preliminary studies to recognize porcine C7 and was conjugated to cyanogen bromide-activated Sepharose 4B (Pharmacia Biotech, Piscataway, NJ). Fresh porcine plasma was collected in acid-citrated dextrose and then diluted with PBS (pH 7.2; 1:3 v/v). Dilute porcine plasma containing PMSF (2 mmol/L) was loaded onto an anti-human C7 mAb affinity column (30 ml bed volume). The column was washed extensively (10 mmol/L EDTA; 750 mmol/L NaCl in PBS, pH 7.2) and bound proteins were eluted (100 mmol/L glycine, pH 3). The porcine C7-containing peak was immediately neutralized (1 mol/L Tris, pH 10; 1:10 v/v) and dialyzed against PBS (pH 7.2, 4°C).

SDS-PAGE and Western blot analysis

Rabbits were immunized s.c. (four sites/rabbit) with 50 μg porcine C7 in PBS and CFA. Rabbits were boosted at 4 wk intervals with 25 μg porcine
C7 in incomplete Freund’s adjuvant. Rabbit sera were collected via a marginal ear vein 10 days after each immunization. The IgG-containing fraction of the rabbit sera was purified by protein G affinity chromatography, Rabbit polyclonal Abs (PAb) against porcine C7 were obtained with HRP (Pierce, Rockford, IL). The fractions containing HRP-conjugated PAb were isolated by a G50 column and identified by addition of 2.2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS), pooled, stabilized with BSA (10 mg/ml), and dialyzed against PBS (pH 7.4).

Purified porcine C7 (2 μg) and human C7 (2 μg) (Advanced Research Technologies, San Diego, CA) were fractionated by SDS-PAGE on a 9% gel under reducing (2% 2-ME) and nonreducing conditions. The protein bands were visualized with Coomassie, and a broad range protein standard (Bio-Rad, Hercules, CA) was used to establish the relative m.w. of porcine and human C7. Porcine and human C7 were analyzed under nonreducing conditions on additional gels and transferred onto nitrocellulose, and the membranes were blocked with PBS-TB (PBS containing 0.1% Tween 20 and 0.1% BSA) containing 10% nonfat dry milk overnight. The membrane was then incubated with HRP-conjugated anti-porcine C7 PAb (1:100) in PBS-TB containing 3% nonfat dry milk for 1 h at 4°C. The membrane was then washed with PBS-TB and developed with the ECL system (Amer sham International, Little Chalfont, U.K.).

Peptide sequencing and mass spectroscopy
Porcine C7 (10 μg) was resolved on a 9% polyacrylamide gel under nonreducing conditions. The protein was transferred onto a polyvinylidene difluoride membrane (Bio-Rad) and sent to the Harvard Microchemistry Facility for tryptic digestion and internal sequencing by Edman degradation.

Porcine C7 cDNA isolation
Reverse transcription and PCR amplification (RT-PCR) were utilized to determine the presence of a C7 transcript in porcine lung tissue. Briefly, total RNA was purified from freshly isolated porcine lung tissue (Stratagene, La Jolla, CA). Purified RNA was used as template for oligo(dT)-primed reverse transcription using the Access RT-PCR System (Promega, Madison, WI). A short porcine C7 internal peptide corresponding to the sequence LQSHPQKPFYTV was obtained by Edman degradation and was utilized to degenerate oligonucleotide (5’-CACCTCCCTCTGTAGAAAGG-3’). An additional oligonucleotide (5’-CTTCTCAGACCTGTAGAAAGG-3’), based on the published human C7 cDNA sequence (GenBank accession number NM000587), was designed. These primers were subsequently used in RT-PCR reactions. The reverse-transcribed cocktail (2 μl) was used as template for PCR amplification using 10 μl of each primer, 1 μl of DNA Taq polymerase, and 10 μmol/L of each dNTP, and 5 μl of 10× PCR buffer containing MgCl₂ in a total volume of 50 μl was added to the reaction mixture. PCR amplification was performed using the following conditions: a single cycle of 94°C for 1 min before adding 20 μl of each primer, Pig C7 primers (forward, 5’-ACCCCTCTCTGATGTAGAAAGG-3’ and reverse, 5’-CTTCTCCACCACCGTCTGAGAAAGG-3’) amplified a 1.1-kb fragment (bases 845-1962; GenBank accession number AF16224) using the following conditions; 94°C for 5 min, 28 cycles of 94°C for 1 min, 60°C for 45 s, and 72°C for 1 min, followed by a final 10-min extension at 72°C. Identical C7 cDNA reactions that contained RNA as template were performed on all tissues. No amplified product(s) was observed, indicating that the RNA samples did not contain genomic DNA (data not shown). Porcine GAPDH was amplified using commercially available primers (Stratagene), according to the manufacturer’s instructions. Amplification products were resolved on a 1.8% agarose gel containing 0.06 μg/ml of ethidium bromide. Bands were digitized using the Kodak 1D electrophoresis documentation and analysis system.

Production of anti-porcine C7 mAbs
mAb against porcine C7 were produced by immunization of female BALB/c mice with an i.p. injection of porcine C7 (50 μg in Titermax, Sigma, St. Louis, MO), followed by three weekly i.p. injections of porcine C7 (25 μg in PBS). Fusions were performed with a myeloma cell line (P301), as previously described (24). Hybridomas were screened initially by a solid-phase Ab-capture porcine C7 ELISA. A secondary hemolytic screening assay (see below) using sensitized chicken RBCs was used to identify parent hybridomas that eliminated the hemolytic activity of porcine serum. In the secondary screening assay, porcine serum was initially diluted in gelatin veronal buffer saline (0.1% gelatin, 141 mM NaCl, 0.5 mM MgCl₂, 0.15 mM LiCl, 0.18 mM sodium barbital) to establish the serum concentration that yielded 100% hemolysis of sensitized chicken RBCs. The porcine serum was then doubled and incubated 1:2 (v:v) with tissue culture supernatant from porcine C7-positive ELISA wells. Control experiments consisted of sera receiving tissue culture media. Monoclonal hybridoma cell lines were established by limiting dilution. mAbs were raised in tissue culture, purified by protein G affinity chromatography, dialyzed against PBS, concentrated, and sterilized filtered.

Fab fragments were made by adding 2 mg of mAb PC7.1 to 1 ml of protein G-agarose (10 mg/ml) or PC7.1 Fab fragments (18 μg of papain in 5 ml of PBS containing 20 mM EDTA and 1-cysteine) and incubated for 16 h at 37°C. The reaction was stopped by addition of iodoacetamide (Sigma; 11.1 mg). The mixture was passed over a protein A affinity column. Digestion and Fab production were confirmed by SDS-PAGE. Fab fragment protein concentration was determined by a commercially available kit (Bio-Rad).

Hemolytic assay
Hemolytic assays were conducted as previously described (15). Briefly, porcine, rat, rabbit, human sera, or C7-depleted human sera (Advanced Research Technologies, San Diego, CA) were diluted serially (1/2) in gelatin veronal buffer saline and added in triplicate (40 μl/weil) to a 96-well plate. In some experiments, porcine C7 (0, 0.01, 0.1, 1, 10, and 40 μg/ml) was added to the C7-depleted human sera before serial dilution. In additional experiments, PC7.1 (40 or 80 μg/ml) or PC7.1 Fab fragments (40 μg/ml) were added to porcine, human, or rabbit sera and incubated on ice for 30 min before serial dilution. The sera were then added to sensitized chicken RBC (1 × 10⁵ cells; InterCell Technologies, Hopewell, NJ), mixed, and incubated at 37°C for 30 min. The plate was then centrifuged (1000 × g for 20 min), and supernatant (85 μl) from each well was transferred to a new microtiter plate. The plate was read at 415 nm using a microplate reader (Molecular Devices, Sunnyvale, CA), and the percent serum complement hemolytic activity was determined (15).

Immunoprecipitation of C7 from sera
PC7.1 Abs were conjugated to cyanogen bromide-activated Sepharose 4B (5 mg mAb/ml of beads). Porcine, human, rat, and rabbit sera (3.5 ml each) were treated with PMSF (2 mM/L). C7 was immunoprecipitated (100 μl of PC7.1-coupled resin) for 16 h at 4°C. GS1 (mAb against porcine C5a (24)) was also used for immunoprecipitation to control for nonspecific protein binding with each sera sample. Following immunoprecipitation, the beads were collected by centrifugation and washed with a low ionic, followed by high ionic and isotonic strength wash buffers. The beads were
then boiled in reduced (2% 2-ME) sample buffer. The samples and purified, reduced human C7 (6 µg; Advanced Research Technologies) were fractionated by SDS-PAGE on a 9% gel under reducing conditions and visualized with Coomassie blue.

**Results**

**Purification of porcine C7**

We isolated a single prominent porcine protein peak by affinity chromatography using the mAb anti-human C7 affinity column. Polyclonal Abs raised against this protein recognized nonreduced human C7 by Western analysis (Fig. 1, lane 1). The isolated porcine protein was of similar m.w. as human C7 and was thus determined to be porcine C7 (Fig. 1, lane 2). Purified fragment C7 was fractionated by 9% SDS-PAGE under reducing (Fig. 2, lane 2) and nonreducing (Fig. 2, lane 3) conditions and was compared with human C7 protein under the same conditions (Fig. 2, lanes 1 and 4, respectively). Affinity-purified porcine C7 contained a single band with approximate molecular mass of 90 kDa and 100 kDa under reducing and nonreducing conditions, respectively. These data indicate that porcine C7 is a single polypeptide chain with similar electrophoretic mobility as human C7. In addition, cross-reactivity of the anti-porcine C7 PAb indicates that porcine and human C7 share antigenic epitopes.

**Cloning of porcine C7 cDNA**

An initial RT-PCR reaction was conducted on porcine lung mRNA using an oligonucleotide deduced from the internal amino acid sequence of a small porcine C7 peptide and an oligonucleotide was generated from the human C7 cDNA sequence. A 1.1-kb PCR band was obtained that, following nucleotide sequencing, was positively identified as a human C7 homologue. This fragment corresponded to position 900-1900 bp of human C7 cDNA. The 5′ and 3′ ends of the porcine C7 cDNA were obtained by RACE using porcine lung mRNA. The full-length porcine C7 cDNA of 2532 bp and an open reading frame of 843 aa was obtained (GenBank accession number AF162274). Alignment of the deduced amino acid sequence of porcine C7 and human C7 sequence (GenBank accession number NM 000587) demonstrated ~80% identity at the protein level (Fig. 3). The deduced amino acid sequence revealed that all 56 cysteine residues (see asterisks, Fig. 3) are conserved between species, including the cysteine-rich carboxyl terminus domain. Two potential N-linked glycosylation sites (Asn-X-Ser/Thr) at positions 202 and 754 were also conserved in porcine C7 (see plusines, Fig. 3). The sequenced internal peptide obtained by tryptic digestion and Edman degradation is present at positions 640–651 (see underlined sequence, Fig. 3).

**Tissue distribution of porcine C7**

We examined the tissue expression profile of C7 mRNA by RT-PCR. As shown in Fig. 4, we observed C7 expression in the bone marrow (lane 2), heart (lane 4), intestine (lane 5), lung (lane 6), spleen (lane 7), kidney (lane 8), liver (lane 9), and thymus (lane 10). We did not observe C7 expression in circulating neutrophils (lane 3) or mononuclear leukocytes (lane 1). Thus, C7 expression is widely distributed in the pig, but not present in circulating leukocytes.

**Hemolytic assay**

A high degree of biochemical and structural similarity between porcine and human C7 protein was observed. Hemolytic assays were performed to test whether porcine C7 could functionally replace human C7 in C7-deficient human sera. Human sera depleted of the complement component C7 failed to induce significant hemolysis of sensitized chicken RBC (Fig. 5). Addition of porcine C7 to C7-depleted human sera restored hemolytic activity in a dose-dependent manner. The hemolytic activity of C7-depleted human sera was restored to that of normal human sera at a concentration of 40 µg/ml porcine C7. This concentration of C7 is similar to that present in normal human plasma.

Screening of several hundred positive anti-porcine C7 mAbs yielded one non-IgM mAb that recognized porcine C7 by Ab capture ELISA and attenuated the hemolytic activity of porcine serum. mAb PC7.1 was identified as an IgG2a isotype. As shown in Fig. 6A, the hemolytic activity of porcine serum was attenuated by PC7.1 (40 µg/ml) in vitro. Increasing the concentration to 80 µg/ml did not further attenuate the hemolytic activity of porcine sera. Furthermore, addition of PC7.1 to porcine sera did not increase the chemotactic activity of porcine neutrophils (under agarose assay) compared with untreated porcine sera (data not shown). These data suggested that the attenuation of hemolytic activity was a direct action of the mAb to C7 and not through complement activation (immune complex formation) and complement depletion. Furthermore, as demonstrated in Fig. 6B, Fab fragments of PC7.1 (40 µg/ml) significantly attenuated the hemolytic
activity of porcine sera. Administration of PC7.1 (50 mg/kg, i.v.) in vivo to anesthetized pigs failed to attenuate completely the hemolytic activity of porcine plasma ex vivo. Thus, while this mAb attenuates porcine serum hemolytic activity, it does not completely inhibit C5b-9 formation.

Since we observed functional and structural similarities between human and porcine C7, we investigated whether PC7.1 would attenuate the hemolytic activity of human, rat, and rabbit sera. As shown in Fig. 6C, PC7.1 (80 μg/ml) attenuated the hemolytic activity of human, rat, or rabbit sera. Another mAb against human C7 (clone CL326; Quidel, San Diego, CA) did not attenuate the hemolytic activity of these sera (data not shown).

Immunoprecipitation of C7

To demonstrate that PC7.1 recognizes a conserved epitope in rat, porcine, rabbit, and human C7, we immunoprecipitated C7 from sera. As shown in Fig. 7, PC7.1 immunoprecipitated a protein with a m.w. that is consistent with that of human and pig C7 from rat, porcine, human, and rabbit sera. Of interest, the rat C7 immunoprecipitate band was of slightly lower m.w. than human, porcine, or rabbit C7 immunoprecipitate bands. This difference in m.w. may be a result of glycosylation or protein size. These data demonstrate that PC7.1 recognizes a conserved epitope in several species. Furthermore, this C7 epitope appears to play an important role in the formation of the terminal complement complex. Because the hemolytic activity of these sera could not be completely inhibited, additional epitopes may be involved in the formation of an active C5b-9 complex.

Discussion

To provide a better understanding of the molecular basis of cell-mediated injury by C5b-9, it is crucial to study the molecular architecture of the five individual complement proteins that act in
concert to form the transmembrane pore, C5b-9. C7 plays an undeniable crucial role in the assembly of this complex because its attachment to C5b-6 leads to its initial insertion into cell membranes. Furthermore, the hemolytically inactive C5b-7 complex induces cellular activation in vitro and inflammation in vivo (17, 18). The primary sequence of human C7 has been elucidated, and the five domains of the protein have been characterized based on its homology with other complement proteins. However, the specific contributions of these domains to its overall structure/function still remain unknown (1–4). Because only human C7 has been cloned and sequenced, characterization of C7 in other species and their relationships to human C7 may provide structural and functional information about important human C7 domains.

The cDNA cloning of porcine lung complement C7 unveiled the striking overall structural homology to human C7. Similar to human C7, the predicted amino acid sequence of precursor porcine C7 also consists of 843 aa residues. Human and porcine C7 share an overall identity of ~80% at the protein level. The two potential N-linked glycosylation sites in human C7 are also conserved in porcine C7. Both human and porcine C7 have 56 cysteines, the majority of which are located either at the amino or carboxyl terminus of the protein, giving rise to cysteine-rich domains. Although the function of these cysteine-rich motifs remains unclear, one speculation is that they play a role in the stabilization of β-turns that are presumably involved in protein-protein interactions (3). In addition, the carboxyl termini of C6, factors H and I, also have cysteine-rich motifs (termed factor I domains), and this domain has been implicated in the capacity of these proteins to specifically interact with C5 (25–27).

Although liver has been demonstrated to be the major site for the synthesis of C6 and C8, the major site of C7 synthesis has not yet been elucidated. Although human cDNA was obtained from liver, C7 is not detected in the human hepatoma-derived cell line HepG2, thus suggesting that human hepatocytes do not primarily synthesize C7 (3, 28). Interestingly, circulating C7 initially switches to 50% of the transplanted allotype following allogeneic liver transplantation, but completely reverts to the original allotype by 6 wk (28). These data suggest that mature mononuclear white blood cells (i.e., Kupffer cells in the liver) may be a major source

![FIGURE 4](http://www.jimmunol.org/)

**FIGURE 4.** Expression of C7 in porcine tissues. mRNA from various tissues was isolated and subjected to RT-PCR using porcine C7-specific primers. We observed C7 expression in the bone marrow (lane 2), heart (lane 4), intestine (lane 5), lung (lane 6), spleen (lane 7), kidney (lane 8), liver (lane 9), and thymus (lane 10). We did not observe C7 expression in circulating polymorphonuclear (lane 3) or mononuclear leukocytes (lane 1). Thus, C7 expression is widely distributed in the pig, but not present in circulating leukocytes. In contrast, GAPDH was amplified/present in these C7-negative cells.

![FIGURE 5](http://www.jimmunol.org/)

**FIGURE 5.** Functional analysis of porcine C7. Various concentrations of purified porcine C7 were added to C7-depleted human serum, and the hemolytic activity of the serum was measured on sensitized chicken RBC (n = 3). Values are mean ± SEM of triplicate determinations. NHS, normal human sera.

![FIGURE 6](http://www.jimmunol.org/)

**FIGURE 6.** Hemolytic activity of sera. *A*, Porcine serum (PS) was treated with or without PC7.1 (40 or 80 μg/ml) and tested for hemolytic activity in serial dilutions. Values shown are mean ± SEM of triplicate determinations. B, Porcine serum (PS) was treated with or without Fab fragments of PC7.1 (40 μg/ml) and tested for hemolytic activity in serial dilutions. Values shown are mean ± SEM of triplicate determinations. C, Human, rabbit, and rat serum were treated with or without PC7.1 (80 μg/ml) and tested for hemolytic activity in serial dilutions. HS, human serum; RS, rat serum; RAB, rabbit serum. Plus means addition of PC7.1 at 80 μg/ml. Values shown are mean ± SEM of triplicate determinations.
porcine C7 mRNA expression in all tissues examined, with the exception of circulating leukocytes. Thus, in the normal pig, C7 expression is widely distributed. The absence of C7 mRNA in circulating mononuclear leukocytes suggests that tissue resident mature macrophages may be a major site of C7 production. Similarly, mononuclear phagocytes exhibit developmental potential for plasmigen inhibitor type 1 expression (i.e., not present in circulating normal monocytes, but observed in mature tissue resident macrophages) (29).

Inhibition of complement activation has been demonstrated to provide tissue protection in a variety of models (30–33). However, the role of the early (iC3b, C3a, and C3b) vs late (C5a and C5b-9) complement components is only now beginning to be investigated. Inhibition of the terminal complement complex through C5 attenuates myocardial ischemia/reperfusion injury (15). Protection to the ischemia/reperfused myocardium by inhibition of C5a and C5b-9 provided more protection than inhibition of C5a alone (15, 34). Furthermore, C5b-9 and not C5a appears to be the major mediator of renal ischemia/reperfusion injury (16). These are not unexpected observations, considering the multitude of proinflamma-
tory actions induced by the terminal complement components (9, 13, 14, 18, 35). Thus, identification and formation of specific inhibitors of C5b-9 may have therapeutic value. Since one of the rate-limiting steps in formation of membrane-bound C5b-9 is C7 assembly to C5b-6, an attractive therapeutic approach would be inhibition of C7. Along these lines, we attempted multiple fusions to generate a functionally inhibitory Ab to porcine C7 with limited success. mAb PC7.1 marginally inhibited the hemolytic activity of porcine sera. Importantly, Fab fragments of PC7.1 also attenuated the hemolytic activity of porcine sera, further suggesting that this mAb recognizes an important epitope and that inhibition of more than one epitope on C7 may be needed to inhibit completely formation of a stable C5b-7 complex. Thus, inhibition of C7 may require multiple inhibitory interactions. Epitope mapping of PC7.1 should aid in identification of one of these important domains.

In summary, we demonstrate the highly conserved nature of porcine and human C7 at the molecular and functional level. Porcine C7 mRNA expression is widely distributed. There appears to be a conserved epitope that is important in the formation of C5b-9 that is recognized by mAb PC7.1. Epitope mapping of PC7.1 may aid in the elucidation of an important C7 epitope in the formation of C5b-9.

References


12. Friedman, M., S. Y. Wang, G. L. Stahl, R. G. Johnson, and F. W. Sellke. 1995. Inhibition of the terminal complement components and inhibition by anti-C5 therapy or GMP an-


23. Frohman, M. A. 1993. Rapid amplification of complementary DNA ends for generation of full-length complementary DNAs: thermal RACE. Methods Enzy-


30. Frohman, M. A. 1993. Rapid amplification of complementary DNA ends for generation of full-length complementary DNAs: thermal RACE. Methods Enzy-


32. Frohman, M. A. 1993. Rapid amplification of complementary DNA ends for generation of full-length complementary DNAs: thermal RACE. Methods Enzy-


34. Frohman, M. A. 1993. Rapid amplification of complementary DNA ends for generation of full-length complementary DNAs: thermal RACE. Methods Enzy-