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Molecular Analysis of the Third Component of Canine Complement (C3) and Identification of the Mutation Responsible for Hereditary Canine C3 Deficiency[1]

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Genetically determined deficiency of the third component of complement (C3) in the dog is characterized by a predisposition to recurrent bacterial infections and to type 1 membranoproliferative glomerulonephritis. The current studies were undertaken to characterize the cDNA for wild-type canine C3 and identify the molecular basis for hereditary canine C3 deficiency. Amplification, cloning, and sequence analysis indicated that canine C3 is highly conserved in comparison with human, mouse, and guinea pig C3. Southern blot analysis failed to show any gross deletions or rearrangements of DNA from C3-deficient animals. Northern blot analysis indicated that the livers of these animals contain markedly reduced quantities of a normal length C3 mRNA. The full-length 5.1-kb canine C3 cDNA was amplified in overlapping PCR fragments. Sequence analysis of these fragments has shown a deletion of a cytosine at position 2136 (codon 712), leading to a frameshift that generates a stop codon 11 amino acids downstream. The deletion has been confirmed in genomic DNA, and its inheritance has been demonstrated by allele-specific oligonucleotide hybridization. *The Journal of Immunology,* **1998, 160: 2824–2830.**

The third component of complement (C3) plays a critical role in the generation of the inflammatory and protective functions of the complement system (1, 2). Activation of C3 creates cleavage products that possess anaphylatoxic (C3a) and opsonic activities (C3b), as well as facilitate the clearance and processing of immune complexes (C3b). Since C3b also forms part of the enzymes that activate C5 to C9, it is also critical in the generation of the chemotactic and bactericidal activities of complement.

During the course of line breeding a colony of Brittany spaniels with hereditary canine spinal muscular atrophy (HCSMA) (3), a number of the dogs were noted to be unusually susceptible to recurrent bacterial infections. Further investigations revealed that the animals had a genetically determined deficiency of C3 (4). The disorder is inherited as an autosomal recessive trait and segregates independently of the HCSMA (4, 5).

Affected animals have less than 0.0003% of the normal amount of C3 in their serum and markedly reduced levels of serum opsonic and chemotactic activities (6). Like their human counterparts (2, 7, 8), C3-deficient dogs have an increased susceptibility to bacterial infections and develop type 1 membranoproliferative glomerulonephritis (9, 10).

Although previous studies have shown that C3 deficiency in the dog is the consequence of a null allele of the structural gene for C3 (5), the molecular basis of the defect has not been defined. The current studies were performed to characterize the cDNA for wild-type C3 in the dog and identify the mutation responsible for C3 deficiency. In this study, we demonstrate that canine C3 deficiency is the result of a deletion of a cytosine at position 2136, which results in a frameshift at codon 712 and the generation of a premature stop codon 11 amino acids downstream.

Materials and Methods

Animal tissues and serum

Animals for these studies were obtained from the colony of C3-deficient dogs housed at The Johns Hopkins Medical Institutions (Baltimore, MD). Animals were confirmed as C3 deficient by testing their serum for canine C3 by double immunodiffusion in gels using anti-canine C3 Ab (4). Tissues were obtained at the time of euthanasia and were cut into fragments weighing 10 to 50 g, snap frozen in liquid nitrogen, and stored at −70°C. Serum was obtained from clotted whole blood and was frozen and stored at −70°C.

Preparation of DNA and RNA

DNA was prepared from liver and peripheral blood leukocytes, as previously described (11). Total cellular RNA was obtained from liver, as previously described (11). Poly(A)$^+$ mRNA was extracted from total cellular RNA by oligo(dT) cellulose chromatography (12).

Northern blot analysis

Total cellular RNA or poly(A)$^+$ mRNA was electrophoresed in 1.5% agarose containing 2.2 M formaldehyde and transferred in 10× SSC to a Hybond nylon filter (Amersham, Arlington Heights, IL). The transferred RNA was hybridized to the appropriate cDNA probe in 50% formamide, 5× SSPE (1× = 0.18 M NaCl, 0.01 M Na phosphate, pH 6.8, and 0.001 M EDTA), 4× Denhart's solution (1× Ficoll, BSA, and polyvinyl pyrrolidone, each at 0.2 mg/ml) containing 2% SDS, and 100 μg/ml of sheared salmon sperm DNA at 42°C. The filters were then washed twice in 1× SSC containing 1% SDS for 30 min at room temperature and once in 0.1× SDS for 30 min at 50°C, and autoradiography was performed.

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Table I. *Primer positions and sequences used in these studies*[a]

Primer	Position	Sequence
PR11*	U(−60–30)	GGGACGCGTCTCCTCCCCATCCTCTCCCTCTGTCCCTCTGT
PR2*	L1098–1069	CACACGCGTGATCCCGCTGCGCTCTGCCTGCACCATGTC
PR3*	U935–967	GGGACGCGTACCTCCGAGCAGAAGACCTGGTGGGGAAGT
PR4*	L2484–2518	TTTACGCGTACAGAGTAGGGTAGCCGCAGGTCGATGAAG
PR12	U3598–3626	TGGACGCGTTAGACAAACTGGAGGGAGATAACCTCAG
PR15	L2508–2537	ACAGCCAAAGGTGATCATGTCATCAGTGAA
PR5*	U2448–2477	AGGACGCGTTGTGGCAGACCCCTTCGAGGTCACAGTAAT
PR9*	L3678–3649	TGTACGCGTTCTTGCCAGGGTCCACCCAGCGGTTAATGT
PR40	U2222–2252	CATGGACAACCTGGGACTGGCCAGGAGTGA
PR41	L3592–3562	CCAGGGCGTAGCCAGCAATGGCCACAGAAT
PR27	U2074–2100	GCTGCGAGGGCGGCATGCGGGACAAC
PR28	L2223–2193	TGCGGCTGTAGTTGAGCCGCAGCTGCGTGAT
PR33 ASO	U2129–2143	CCAGTTCGTCTCCC ASO
PR35 ASO	U2129–2144	CCAGTTGTCTCCCG ASO
11 + 12 (5′-1)	U295–312	TCAGAGAAGGGGAGCAAG
11 + 2 (5′-2)	U805–822	GCCTTCGTCATCTTTGGA
11 + 2 (5′-2.5)	U658–675	GCCGAGTTCGAGGTGAAG
11 + 12 (3′-1)	L687–669	CAGCACGTATTCCTTCAC
11 + 2 (3′-2)	L248–231	TACTGATTGGCGCTGGTC
3 + 4 (5′-1)	U1295–1313	GTCTCGACAGGCCACCAG
3 + 4 (5′-2)	U1446–1464	GAGGCTCAGATCCGCTAC
3 + 4 (5′-3)	U1975–1993	TTGAGTGCCCCAAACCAG
3 + 4 (3′-1)	L2241–2223	CAGTCCCAGGTTGTCCAT
3 + 4 (3′-2)	L1862–1844	GCCTCCACCACGTTCCAG
3 + 4 (3′-3)	L1396–1378	GCTTCAGTTCCATTCGAG
5 + 9 (5′-1)	U2807–2825	AATCCTAGTCAACCAAAC
5 + 9 (5′-2)	U3212–3230	GAACCGGCCATCCAGCAG
5 + 9 (5′-2a)	U3196–3214	CCTTCGCTGCCTTCCAGA
5 + 9 (3′-1)	L3350–3328	CCATCGGGCTTCTGCTTC
5 + 9 (3′-2)	L2880–2862	TCGCTGCACTCCCTCCTG
12 + 15 (5′-1)	U3952–3970	GCCTCCAACGGTCAGAAG
12 + 15 (5′-2)	U4481–4498	ACCACCCAGAGAAAGAAG
12 + 15 (3′-1)	L4608–4590	CTTGTCCAGCCGTTCATC
12 + 15 (3′-2)	L4025–4007	GTGCCTTGTCCTTTTCCT

[a] The positions are numbered from the start ATG (A = +1). L, lower (antisense); U, upper (sense) primer. *, Primers designed to human C3. The primer positions are numbered according to the coding region of human C3 cDNA. Some primers were designed with a Mlu 1 site engineered into the 5′ tail (ACGGCGT).

Southern blot analysis

Genomic DNA was digested with restriction endonucleases, electrophoresed in 1.5% agarose, and transferred in 10× SSC (1× SSC = 150 mM NaCl and 15 mM sodium citrate) to GeneScreen^{Plus} nylon membranes (DuPont NEN, Boston, MA) (12). The transferred DNA was hybridized to the appropriate cDNA probe in a solution of 50% formamide, 10% dextran sulfate, 1 mM NaCl, and 1% SDS. The filters were washed twice in 2× SSC with 1% SDS for 30 min at 65°C and twice in 0.1× SSC for 30 min at room temperature, and autoradiography was performed.

cDNA clones

Two cDNA clones for human C3 (pC3.11 and pC3.49) were obtained from American Type Culture Collection (Rockville, MD) (13). pC3.11 encompasses 4342 bp and represents approximately 90% of the 3′ coding region of human C3. pC3.49 encompasses 2.9 kb of the 5′ coding region of human C3 and overlaps the sequence of the pC3.11 clone.

A canine oligo(dT)-primed cDNA canine liver library in lamba gt11 (Clontech Laboratories) was screened with pC3.11. A 1.5-kb clone was isolated and subcloned into pGEM-4 before sequencing. The canine C3 cDNA clone (dC3.2) encompasses a region that corresponds to nucleotides 3540 to 5049 and includes 16 bp of the 3′ untranslated region and the poly(A)$^+$ tail.

PCR amplification of canine C3 cDNA

Alignment of mammalian C3 cDNA sequences (human (13), guinea pig (14), and murine (15)) indicated a high degree of sequence conservation, particularly for regions predicted to be important in its function (1). In addition, canine-specific sequence at the 5′ terminus was available (see Fig. 3). PCR primers were designed to the known canine sequence and to the mammalian consensus sequence. The full length of the wild-type and mutant canine C3 genes was amplified in overlapping fragments (see Fig. 3). All PCR amplifications were performed with a 1:1 combination of *Taq* polymerase (Boehringer Mannheim Corp., Indianapolis, IN; 2.5 U/tube) and *Pfu* polymerase (Stratagene, La Jolla, CA; 2.5 U/tube) to minimize *Taq* polymerase-induced errors. The PCR products were extracted from gels, purified (Qiagen, Valencia, CA), and cloned to the TA cloning vector (Invitrogen Corp., San Diego, CA). The amplification efficiencies varied for each primer pair. Amplification of the bands required between 35 and 40 cycles on a thermal cycler (Cetus, Emeryville, CA).

Transformation of bacteria and sequencing of PCR products

Transformation of competent *Escherichia coli* DH5 α cells was undertaken after a 42°C heat shock for 45 s. White colonies containing insert were identified in the presence of X-gal (Sigma Chemical Co., St. Louis, MO). The presence of insert was further confirmed by PCR analysis before automated sequencing (Applied Biosystems, Foster, CA). A series of internal primers were designed to sequence both strands of plasmids (Table I).

Confirmation of the mutation in genomic DNA and allele-specific oligonucleotides

To confirm the mutation found in the cDNA of C3-deficient dogs, the intron-exon boundaries of the human C3 were compared with the corresponding canine sequence. The region containing the canine C3 mutation corresponds to exon 17 in humans (16). Amplification of canine DNA was undertaken with primers 27 and 28, which were designed to prime within exon 17. Direct sequencing of PCR fragments extracted from gels was undertaken to confirm the presence of the mutation in canine DNA.

Analysis of the inheritance of the mutation was undertaken with allele-specific oligonucleotides ASO, as described (17). Fourteen-mer oligonucleotides corresponding to the wild-type (PR33ASO) and the mutant (PR35ASO) sequences were labeled with γ-^{32}P. PCR-amplified genomic products were slot blotted onto a filter and probed with ^{32}P-labeled oligonucleotides (Table I).

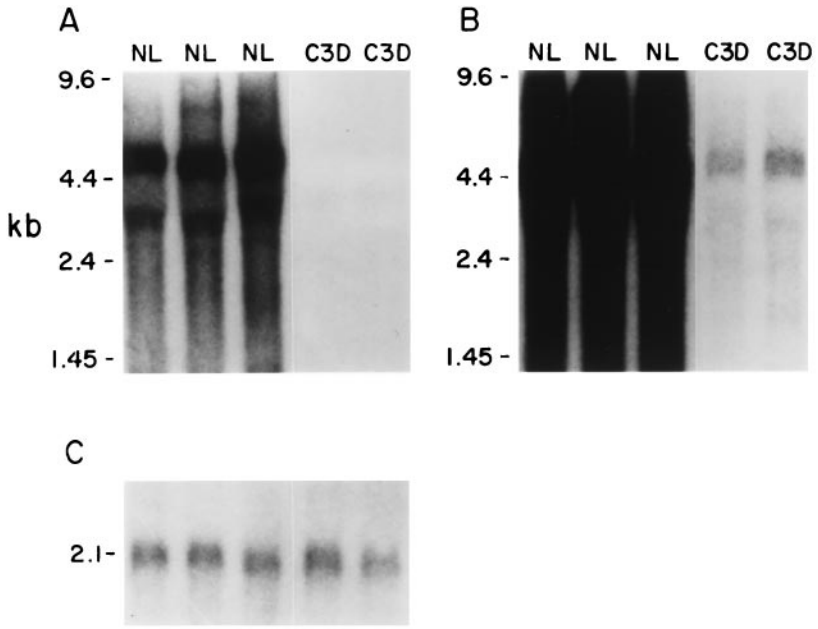

FIGURE 1. Northern blot analysis of total cellular RNA isolated from the livers of three normal (NL) and two C3-deficient (C3D) dogs. The livers of C3-deficient dogs had markedly reduced amounts of C3 mRNA, which appeared identical in size (~5.2 kb) to C3 mRNA from normal dogs. *A*, Each lane contains 10 μg of total RNA. The blot was probed with the canine C3 clone dC3.2 and was exposed for 18 h. In contrast to normal animals, no signal is seen in the C3-deficient dogs. *B*, The same filter exposed for 48 h. A faint band at 5.2 kb is clearly seen in the C3-deficient dogs. *C*, The same filter probed with a β-actin probe (PHA/629) indicating comparable loading of RNA from both wild-type and mutant animals.

Results

Southern blot analysis of DNA

Southern blot analysis was performed with DNA obtained from white blood cells from normal and C3-deficient dogs. DNA digested with a variety of enzymes (*Taq*I, *Hin*dIII, *Pst*I, *Bam*HI) showed the presence of common restriction fragments in both normal and C3-deficient dogs when probed with the cDNA for canine C3 (dC3.2, not shown). Since this probe covers only approximately 30% of the 3′ terminus of the full-length cDNA, we also repeated the Southern analysis using two human probes (pC3.11 and pC3.49) that together span the full coding region of human C3 (13). No differences were found between normal and deficient dogs (not shown), indicating that there was no evidence for a gross deletion or rearrangement of the C3 gene in the C3-deficient animals.

Northern blot analysis of liver RNA

Studies were also performed to determine whether C3-deficient dogs produced C3 mRNA. Liver was selected for study since it is the major site of C3 synthesis in man (18). When liver RNA was examined by Northern blot analysis using the probe for canine C3 (dC3.2), C3-deficient dogs had markedly reduced amounts of C3 mRNA (Fig. 1). In contrast, the content of β-actin mRNA was equivalent in RNA isolated from the livers of C3-deficient and normal dogs. The C3 mRNA from C3-deficient dogs appeared identical in size to C3 mRNA from normal dogs (~5.2 kb). Semiquantitative slot-blot analysis demonstrated that the livers of C3-deficient dogs had approximately 10% of the normal amount of C3 mRNA (Fig. 2). Northern blot analysis of poly(A)$^+$ mRNA confirmed the presence of reduced amounts of a normal sized mRNA in the livers of C3-deficient dogs (not shown).

PCR amplification and sequencing of canine cDNA

The full-length canine cDNA was amplified in overlapping fragments with primers designed to the consensus sequence of mammalian C3 cDNA and the canine-specific cDNA sequences (Fig. 3). Multiple products (>5 each) of independent reverse-transcriptase PCR amplifications of both wild-type and mutant canine C3 RNA were performed in both directions to confirm each sequence. The PCR fragments corresponded to the size of human C3 cDNA, and the full-length cDNA is consistent with the 5.2-kb C3 transcript seen on Northern blot analysis. No size differences were noted between the wild-type and mutant products.

The full-length canine C3 cDNA sequence was assembled with the assistance of the overlapping regions of the PCR products (Fig. 3). The composite sequence of both wild-type and mutant cDNAs was aligned, and any sequence discrepancies were further investigated by sequencing additional clones or by direct sequencing of PCR products. The nucleotide sequence was numbered with the A

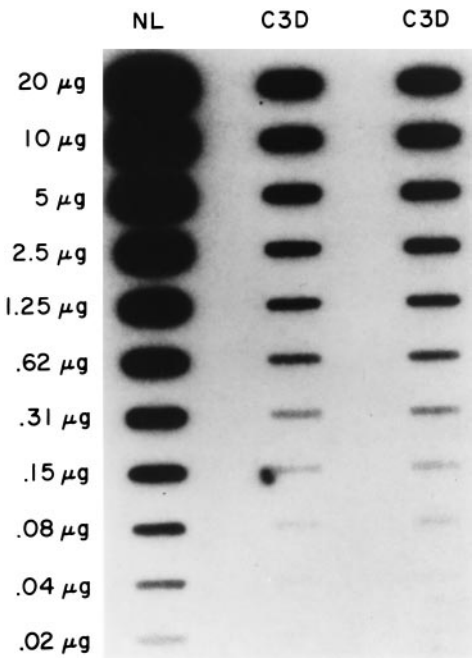

FIGURE 2. Slot-blot analysis of mRNA isolated from a normal (NL) and two C3-deficient (C3D) dogs, demonstrating that C3D dogs have approximately 10% of the normal amount of C3 mRNA in their liver.

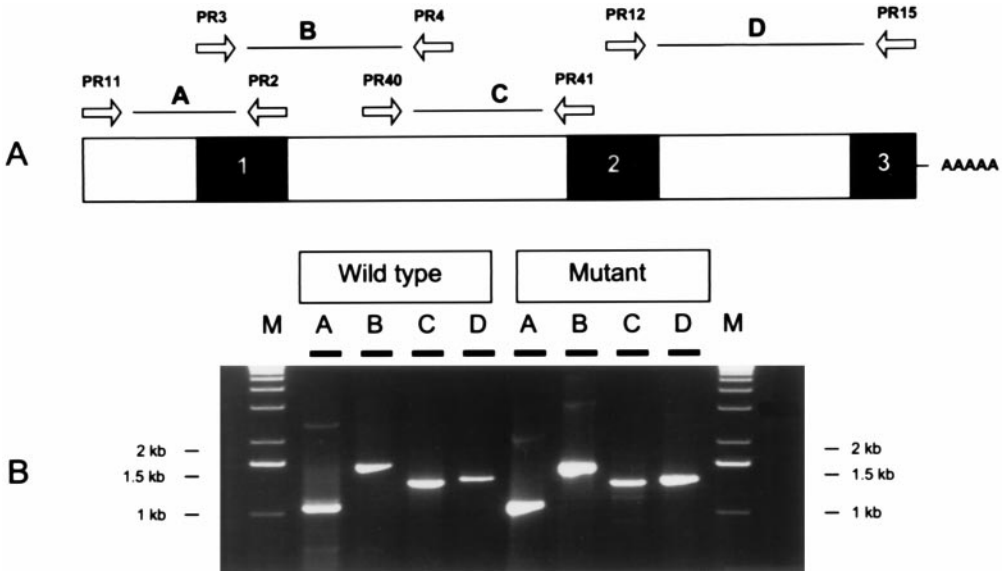

FIGURE 3. Amplification of the full-length canine C3 cDNA. *A*, The full length of the canine C3 cDNA was amplified in four overlapping fragments. The primers were designed to the mammalian consensus sequence or to known canine C3 sequence. Canine-specific sequence at 1 kb (shaded area 1) was identified by Dr. M. Binns, while the sequence at 3.5 and 5 kb (shaded areas 2 and 3) represents sequence obtained from canine clone dC3.2. *B*, PCR fragments obtained from wild-type and C3-deficient dogs were of identical size and are consistent with the 5.2-kb message seen on Northern analysis. The region between primers 41 and 12 was amplified by primers 5 and 9 (not shown).

of the initiation methionine codon as +1. Three synonymous mutations were found at positions 1590, 1727, and 2919. The wild-type sequence has been submitted to GenBank (accession bankit #27404).

Identification of conserved domains in the canine C3 gene

Alignment of canine C3 cDNA with known mammalian C3 cDNA sequences shows a high degree of conservation of regions thought to be critical for post-translational modification and for C3 function (Fig. 4). The canine C3 gene is predicted to produce a pre-pro-C3 protein of 1663 amino acids in length, which is identical to that of man. However, in contrast to the 22-amino-acid signal peptide in human C3, canine C3 has a 23-amino-acid signal peptide (Fig. 4). As seen in Figure 4, the amino acid sequence in the vicinity of the thioester bond is highly conserved in all of the mammalian species characterized to date. The tetra-arginine linker, which is cleaved to produce the β- and α-chains, is also highly conserved. The C3a cleavage site and the arginine required for mediating C3a activity are also seen in the canine C3 sequence (Fig. 4).

The regions thought to be important in ligand binding have also been highly conserved in canine C3, including binding sites for factor H, properdin, CR1, and CR2. Canine C3 also appears to be inactivated by similar mechanisms to that of its mammalian orthologs. The first two factor I cleavage sites at positions 1304 and 1321 are present in the canine sequence. However, the third factor I site at position 955 is not conserved with respect to the human sequence, but is conserved with respect to the guinea pig and mouse. The putative elastase, kallekrein, and trypsin cleavage sites have also been identified in the canine C3 protein.

Identification of the mutation responsible for canine C3 deficiency

The cytosine normally present at position 2136 is deleted in C3-deficient animals (Fig. 5). This deletion was present in four separate clones and also by direct sequencing of PCR products. The deletion results in a frameshift followed 10 codons downstream by a stop codon truncating the protein at amino acid 721. The mutant cDNA is expected to transcribe the β-chain and a small fragment of the α-chain. The mutation was seen in all six C3-deficient animals examined, but not in any of four normal animals. Direct PCR sequencing of cDNA from two heterozygotes showed the expected double sequence at the point of the single base deletion.

Genomic confirmation of the mutation and segregation analysis

To confirm the single base pair deletion, the intron-exon boundaries of the human C3 gene were aligned with the corresponding canine sequence. The region containing the mutation corresponds to exon 17 in humans. Amplification of this region with primers 27 and 28 produced a single band of equal size from either DNA or cDNA, indicating that the fragment was amplified from within a single exon (not shown). Direct sequencing of these bands in both directions showed the presence of the C2136 deletion in the C3-deficient animals.

Segregation analysis was undertaken with allele-specific oligonucleotides (Fig. 6) (17). Comparison of genotypic assignment based on C3 levels and ASO analysis of the C2136 deletion is shown in Figure 6. There is perfect agreement between genotype and C3 phenotype. They indicate that the wild-type probe (PR33ASO) binds only to genomic DNA of wild-type animals. Similarly, the mutant probe (PR35ASO) binds only to DNA of mutant animals. Heterozygous animals give bands of intermediate intensity with either the wild-type or mutant probes consistent with the presence of two alleles.

Discussion

The C3-deficient dog is unique in that it is the only naturally occurring experimental animal with a genetically determined complete deficiency of C3. Since their original description in 1981 (4), these animals have provided important information on the role of C3 in host defense against infection (9), in the generation of a normal humoral immune response (19), in protection against the development of membranoproliferative glomerulonephritis (9, 10)

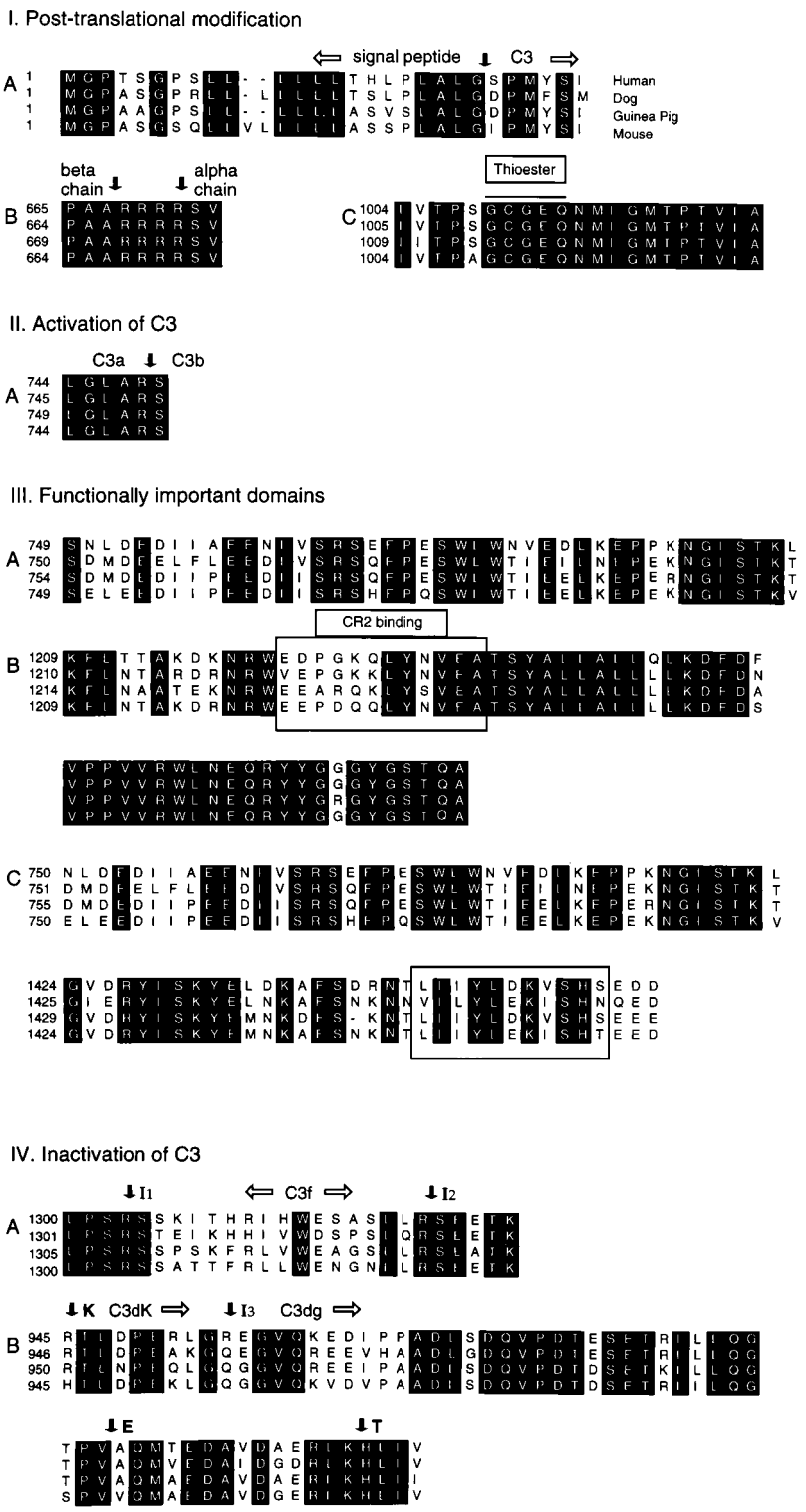

FIGURE 4. Comparison of canine C3 with other mammalian C3s. The predicted amino acid sequences of human, canine, guinea pig, and mouse are aligned to demonstrate their sequence identity. The sequences are numbered from the beginning of the coding region. *I*, Post-translational modification. *A*, Canine pre-pro-C3 contains a 23-amino-acid signal peptide that is cleaved during processing. The site of cleavage is shown by the vertical arrow. *B*, A tetra-arginine linker is removed similarly to generate the β- and α-chains of C3. *C*, The thioester domain shown under the bar is highly conserved in all mammalian species shown, attesting to its functional importance. *II*, Activation of C3. *A*, C3 is activated with cleavage of the C3a fragment from C3b. The classical and alternative pathway C3 convertases cleave the peptide bond between the arginine and the serine shown by the arrow. Activation of C3 results in the exposure of multiple ligand-binding domains. *III*, Functionally important domains. *A*, Region responsible for factors B, H, and CR1 binding (1, 30). Recent evidence suggests factors H and CR1 bind to separate sites within this region (31). *B*, The CR2 and the second factor H binding domains. The CR2 binding region is shown within the box. *C*, The properdin-binding domain. The amino acids thought to be critical for properdin binding are enclosed within the box (1). *IV*, Inactivation of C3. *A*, C3b is initially inactivated (iC3b) by the action of factor I in conjunction with factors H, CR1, CR2, or MCP. C3b is initially cleaved at positions 1304 and 1321 to release the C3f fragment. *B*, In contrast to human C3, canine C3 may not undergo further cleavage by factor I at position 955. The kallekrein (K), elastase (E), and trypsin (T) sites are, however, present in canine C3.

and in protection against endotoxin shock and multiple organ failure (20).

The current studies were performed to determine the molecular basis for the C3 deficiency. Initial Northern blot analysis demonstrated that the C3-deficient dogs have markedly reduced (10% of normal) levels of a normally sized C3 mRNA. Southern blot analysis did not reveal evidence of a major rearrangement of the C3 structural region.

Amplification of the full-length canine C3 cDNA was possible because of the highly conserved nature of the gene. Previous studies have established that mammalian complement proteins are frequently able to interact functionally with complement components from other species. This is characteristic of highly conserved systems that play a vital role in homeostasis. Analysis of the predicted amino acid sequence of the canine C3 gene has confirmed that domains responsible for critical functions show a high degree of identity with human, murine, and guinea pig C3. The canine C3 thioester domain, for example, has 100% sequence identity with that of related species. Minor interspecies differences are, however, present. For example, canine C3 would appear to lack a third

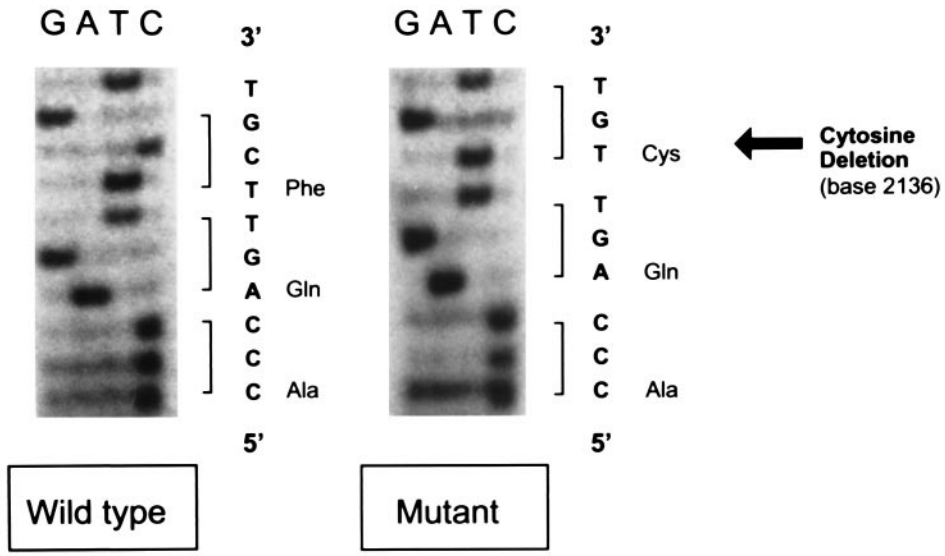

FIGURE 5. Sequence analysis showing deletion of the cytosine at position 2136 in C3-deficient animals. The deletion is predicted to cause a shift in the reading frame, resulting in premature truncation of the protein at amino acid 721.

factor I cleavage site at position 955 when compared with human C3, but not when compared with mouse or guinea pig C3.

Sequence analysis showed that the cytosine at position 2136 is deleted, resulting in a frameshift and premature termination 11 codons downstream. This deletion was not present in normal dogs. The presence of reduced amounts of a normal sized mRNA in the liver of the deficient dogs is consistent with the fact that mutations that result in premature termination of translation are associated with marked reductions in steady state levels of mRNA (21). The mutant allele would be expected to generate a protein containing the β-chain and a small part of α-chain. Analysis of the sequences in the vicinity of the deletion does not demonstrate any of the elements known to be associated with single base deletions (22).

C3 deficiency has been identified previously in one strain of guinea pigs and in one strain of rabbits. A strain of guinea pigs was identified in the 1980s with $<5\%$ serum C3 activity (23). Extensive molecular analysis of the gene has failed to identify a mutation within the coding region (14). It is currently thought that these animals may have a specific defect of the enzyme(s) responsible for generating the thioester bond. A strain of rabbits with 10% of normal serum C3 activity has also been identified (24). The nature of the defect in these animals has not been identified. Recently, C3-deficient mice have been generated by gene targeting. These mice lack any detectable C3 in their serum and are susceptible to recurrent bacterial infections (25).

C3 deficiency has been identified in 20 humans from 15 kindreds (2, 7, 8). The responsible mutations have been identified in four patients. Two patients have had splicing defects (26, 27). A G-A substitution of the 5′ splice donor site of intron 18 was found in the first patient, which resulted in 61-bp deletion of exon 18 (26). The second patient from Taiwan had G-T substitution of the splice donor site of intron 10. This resulted in transcripts that skipped exon 10 and were predicted to cause premature truncation of the C3 protein as a result of a frameshift (27). A third patient from South Africa was shown to have an 800-bp deletion leading to loss of exons 22 and 23 of the α-chain (28). Premature truncation of the protein is again predicted to occur as a result of a frameshift. The fourth patient characterized to date has a critical amino acid substitution resulting in a secretory defect (29). In this patient, an Asp549Asn substitution is thought to interfere with secretion of C3.

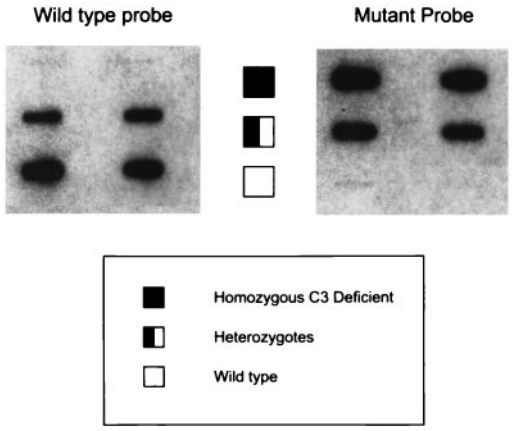

FIGURE 6. Allele-specific oligonucleotide blots with wild-type and mutant canine C3 probes. The mutant probe (PR33ASO) does not hybridize with the two wild-type sequences, and wild-type probe (PR35ASO) does not hybridize with the two mutant sequences. The two heterozygotes demonstrate intermediate binding with both wild-type and mutant probes consistent with the presence of both wild-type and mutant alleles.

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References

1. Lambris, J. D. 1993. The chemistry, biology and phylogeny of C3. In *Complement Profiles*. J. M. Cruse and F. R. Lewis. eds. Karger, Basel, p. 16.
2. Winkelstein, J. A., K. E. Sullivan, and H. R. Colten. 1995. Genetically determined disorders of the complement system. In *The Metabolic and Molecular Basis of Inherited Disease*. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle. eds. McGraw-Hill, New York, p. 3911.
3. Cork, L. C., J. W. Griffin, M. D. Mundell, R. J. Lorenz, R. J. Adams, and D. L. Price. 1979. Hereditary canine spinal muscular atrophy. *J. Neuropathol. Exp. Neurol. 38:209.*
4. Winkelstein, J. A., L. C. Cork, D. E. Griffin, J. W. Griffin, R. J. Adams, and D. L. Price. 1981. Genetically determined deficiency of the third component of complement in the dog. *Science 212:1169.*

5. Johnson, J. P., R. H. Mclean, L. C. Cork, and J. A. Winkelstein. 1986. Genetic analysis of an inherited deficiency of the third component of complement in the dog. *Am. J. Med. Genet. 25:557.*
6. Winkelstein, J. A., J. P. Johnson, A. J. Swift, F. Ferry, R. Yolken, and L. C. Cork. 1982. Genetically determined deficiency of the third component of complement in the dog: in vitro studies on the complement system and complement-mediated serum activities. *J. Immunol. 129:2598.*
7. Singer, L., H. R. Colten, and R. A. Wetsel. 1994. Complement C3 deficiency: human, animal and experimental models. *Pathobiology 62:14.*
8. Botto, M., and M. J. Walport. 1993. Hereditary deficiency of C3 in animals and humans. *Int. Rev. Immunol. 10:37.*
9. Blum, J. R., L. C. Cork, J. M. Morris, J. L. Olson, and J. A. Winkelstein. 1985. The clinical manifestations of a genetically determined deficiency of the third component of complement in the dog. *Clin. Immunol. Immunopathol. 34:304.*
10. Cork, L. C., J. M. Morris, J. L. Olson, S. Krackowa, A. J. Swift, and J. A. Winkelstein. 1991. Membranoproliferative glomerulonephritis in dogs with a genetically determined deficiency of the third component of complement. *Clin. Immunol. Immunopathol. 60:455.*
11. Ameratunga, R., J. McKee, J. French, R. Prestidge, W. Fanslow, and J. Marbrook. 1996. The molecular pathology of the hyper-immunoglobulin M syndrome: detection of wild type transcripts in a patient with a complex splicing defect of the CD40 ligand. *Clin. Diagn. Lab. Immunol. 3:722.*
12. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Vol. 1. Cold Spring Harbor Press, Plainview, NY, p. 7.26.
13. DeBruijn, M. H. L., and G. H. Fey. 1985. Human complement component 3: cDNA coding sequence and derived primary structure. *Proc. Natl. Acad. Sci. USA 82:708.*
14. Auerbach, H. S., R. Burger, A. Dodds, and H. R. Colten. 1990. Molecular basis of complement C3 deficiency in guinea pigs. *J. Clin. Invest. 86:96.*
15. Fey, G. H., A. Lundwall, R. A. Wetsel, B. F. Tack, M. H. L. de Bruijn, and H. Domdey. 1984. Nucelotide sequence of complementary DNA and derived amino acid sequence of murine complement protein C3. *Philos. Trans. R. Soc. Lond.-Biol. Sci. 306:333.*
16. Fong, K. K., M. Botto, M. J. Walport, and A. K. So. 1990. Genomic organization of human complement component C3. *Genomics 7:579.*
17. Marble, M., M. T. Geraghty, R. deFranchis, J. P. Kraus, and D. Valle. 1994. Characterization of a cystathionine B-synthase (CBS) allele with three mutations in *cis* in a patient with B6 non-responsive homocystinuria. *Hum. Mol. Genet. 3:1883.*
18. Alper, C. A., A. M. Johnson, A. G. Birtch, and F. D. Moore. 1969. Human C′3: evidence for the liver as the primary site of synthesis. *Science 163:286.*
19. O'Neil, K. M., H. D. Ochs, S. R. Heller, L. C. Cork, J. M. Morris, and J. A. Winkelstein. 1988. Role of C3 in humoral immunity: defective antibody production in C3-deficient dogs. *J. Immunol. 140:1939.*
20. Quezado, Z. M. N., W. D. Hoffman, J. A. Winkelstein, I. Yatsiv, C. A. Koev, L. C. Cork, R. J. Elin, P. Q. Eichacker, and C. Natanson. 1994. The third component of complement protects against *E. coli* endotoxin-induced shock and multiple organ failure. *J. Exp. Med. 179:569.*
21. Marquat, L. E. 1995. When cells stop making sense: effects of nonsense codons on RNA metabolism in vertebrate cells. *RNA 1:453.*
22. Cooper, D. N., M. Krawczak, and S. E. Antonorakis. 1996. The nature and mechanisms of human gene mutation. In *The Metabolic and Metabolic Basis of Inherited Diseases*. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, eds. McGraw-Hill, New York, p. 259.
23. Burger, R., J. Gordon, G. Stevenson, G. Ramadori, B. Zanker, U. Hadding, and D. Bitter-Suermann. 1986. An inherited deficiency of the third component of complement, C3, in guinea pigs. *Eur. J. Immunol. 16:7.*
24. Komatsu, M., K. Yamamoto, Y. Nakano, M. Nakazawa, A. Ozawa, and M. Mikami. 1988. Hereditary C3 hypocomplementemia in the rabbit. *Immunology 64:363.*
25. Wessels, M. R., P. Butko, M. Ma, H. B. Warren, A. L. Lager, and M. C. Carroll. 1995. Studies of group B streptococcal infection in mice deficient in complement component C3 or C4 demonstrate an essential role for complement in both innate and acquired immunity. *Proc. Natl. Acad. Sci. USA 92:11490.*
26. Botto, M., Y. Fong, A. So, A. Rudge, and M. Walport. 1990. Molecular basis of hereditary C3 deficiency. *J. Clin. Invest. 86:1158.*
27. Huang, J.-L., and C.-Y. Lin. 1994. A hereditary C3 deficiency due to aberrant splicing of exon 10. *Clin. Immunol. Immunopathol. 73:267.*
28. Botto, M., K. Y. Fong, A. K. So, R. Barlow, R. Routier, B. J. Morley, and M. Walport. 1992. Homozygous hereditary C3 deficiency due to partial gene deletion. *Proc. Natl. Acad. Sci. USA 89:4957.*
29. Singer, L., W. T. Whitehead, H. Akama, Y. Katz, Z. Fishelson, and R. Wetsel. 1994. Inherited human complement C3 deficiency. *J. Biol. Chem. 269:28494.*
30. Fishelson, Z. 1991. Complement C3: a molecular mosaic of binding sites. *Mol. Immunol. 28:545.*
31. Lambris, J. D., Z. Lao, T. J. Oglesby, J. P. Atkinson, C. E. Hack, and J. D. Becherer. 1996. Dissection of CR1, factor H, membrane cofactor protein, and factor B binding and functional sites in the third complement component. *J. Immunol. 156:4821.*