Genetically Determined Deficiency of the Third Component of Complement in the Dog

Abstract. *A genetically determined deficiency of the third component of complement (C3) has been identified in a colony of Brittany spaniels. Immunochemical methods show no detectable C3 in the serum of the affected dogs, and there is no evidence of an inhibitor of C3 in the serum. The C3 deficiency appears to be transmitted as an autosomal recessive trait.*

The third component of complement (C3) has a central role in generating the biologically significant effects of the complement system. Not only does the activation of C3 produce cleavage products capable of opsonic, anaphylatoxic, and chemotactic activities, but the larger cleavage product, C3b, also forms part of the enzyme that activates C5 to C9; thus, C3 is also important in the production of activities mediated by C5 to C9. The identification of animals and humans with genetically determined deficiencies of individual components of complement has been valuable in generating knowledge about the biology, biochemistry, and genetics of the complement system. Although a few humans with genetically determined C3 deficiency have been described (*1*), no animals with this deficiency have been identified. We now describe a colony of dogs with a genetically determined deficiency of C3.

A number of puppies from a large kindred of Brittany spaniels maintained for the study of hereditary canine spinal muscular atrophy (HCSMA) (*2*) were found to have recurrent bacterial sepsis and local bacterial infections. The serums of individual members of the kindred were tested for total hemolytic complement activity with sensitized sheep erythrocytes (*3*). Seven of the animals lacked hemolytic activity. Their serums had no C3 detectable by Ouchterlony or immunoelectrophoretic analysis with monospecific goat antiserum to canine C3 (Cappel Laboratories, Cochranville, Pennsylvania). A hemolytic assay (*4*) showed functional titers of 91 to 280 units of C3 per milliliter of serum compared to an average of 2514 units per milliliter in 18 normal mongrel dogs. The functional titers of C1, C2, C4, C5, C6, C7, C8, and C9 in the serums of the C3-deficient animals were tested with hemolytic assays (*5–9*), and all were within the range of normal established by the 18 healthy dogs.

To determine whether the markedly decreased levels of C3 were due to a serum inhibitor of C3, we added highly purified guinea pig C3 (*10*) to diluted C3-deficient serum in an attempt to restore total hemolytic complement activity (*11*). The purified C3 restored total hemolytic activity in a dose-dependent fashion. In addition, when undiluted C3-deficient serum was mixed with an equal volume of undiluted serum from a normal dog and incubated at 37°C for 30 minutes, immunoelectrophoresis showed no evidence of conversion of the C3 from its native to a cleaved form.

Prospective breeding studies and retrospective analyses of stored serums were performed to determine the manner in which the C3 deficiency was inherited. The levels of antigenic C3 in serum samples were determined by radial immunodiffusion with monospecific goat antiserum to canine C3 (*12*). This assay is capable of detecting as little as 0.8 percent of the amount of C3 found in normal dog serum. The C3 deficiency appears to be inherited as an autosomal recessive trait (Fig. 1). Heterozygous animals had C3 levels that were approximately 50 percent of the mean for normal dogs and that were equal to or less than the lower limit of the normal range. Two subsequent matings (not shown in this pedigree) also indicate an autosomal recessive mode of inheritance. A mating between a homozygous C3-deficient male and a normal unrelated female produced progeny all of whom had levels of C3 consistent with heterozygosity. When two putative heterozygotes were mated, one of their seven progeny was C3-deficient.

Each of the homozygous C3-deficient dogs has had an increased susceptibility to bacterial sepsis or local bacterial infections. There appears to be no direct relation between the expression of the HCSMA and the C3 deficiency. The HCSMA was expressed in both C3-deficient and non-C3-deficient members of the kindred. In addition, some of the C3-deficient dogs did not have HCSMA, although they may ultimately develop it, since this neurologic disease may not be expressed until after the third year of life (*2*).

These dogs, therefore, appear to have a genetically determined deficiency of C3. No C3 is found in their serums by immunochemical techniques that detect as little as 0.8 percent of the normal amount of serum C3, but they have C3-like hemolytic activity in their serums that is between 3 and 10 percent of the amount found in normal canine serum. The nature of this functional C3-like activity is unknown. A form of C3 may be present in the C3-deficient serums that does not cross-react immunologically with normal C3, or there may be a pathway to activation of C5 to C9 that is not dependent on C3. Two human subjects with genetically determined C3 deficiency have also had small amounts of C3 hemolytic activity in their serums despite immunochemically undetectable C3 (*13*).

A number of genetically determined

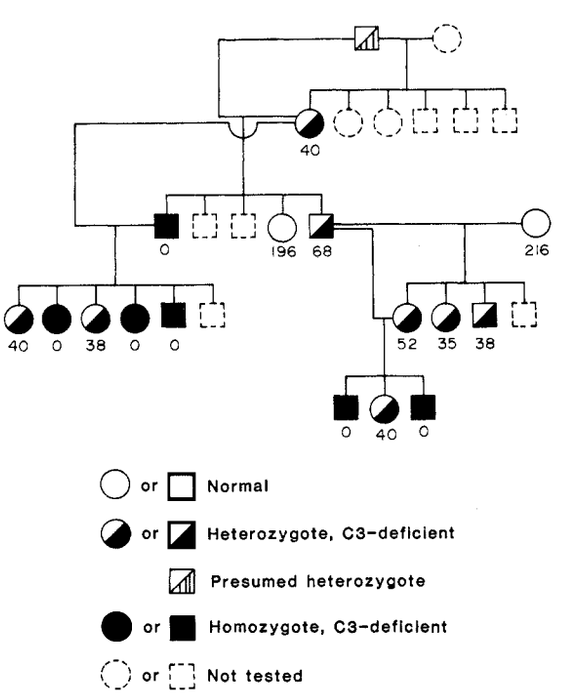

Fig. 1. Pedigree analysis of C3-deficient kindred. The number below each symbol refers to the level of immunochemically detectable serum C3, expressed as a percentage of a standard reference serum. Analyses of 18 mongrel normal dogs gave a mean of 106 with a range of 70 to 192. Analysis of 10 unrelated Brittany spaniels gave a mean of 123 with a range of 92 to 140.

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deficiencies of individual complement components have been described in animals. These have included C4 deficiency in the guinea pig (*14*) and rat (*15*), C5 deficiency in the mouse (*16*), and C6 deficiency in the rabbit (*17*). Although pharmacologic agents, such as cobra venom factor (*18*), have been used as an experimental tool for the study of C3 in vivo, the resulting C3 depletion is neither complete nor sustained. The availability of animals with C3 deficiency will permit the study of the immunobiology of C3 in vivo.

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11. Sheep erythrocytes were sensitized with rabbit antiserum to sheep erythrocytes. One hundred microliters of sensitized erythrocytes at a concentration of 1.5×10^8 per milliliter were added to 500 μl of C3-deficient dog serum diluted 1:50 and 150 μl of C3 at a concentration of 30 μg/ml. The mixtures were incubated at 37°C for 60 minutes and the percentage of lysis was determined.
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Gallamine Triethiodide (Flaxedil): Tetraethylammonium- and Pancuronium-Like Effects in Myelinated Nerve Fibers

Abstract. *Gallamine triethiodide (Flaxedil) is commonly used as a neuromuscular blocking agent. Voltage-clamp studies show that gallamine also directly affects amphibian and mammalian myelinated nerve fibers. Externally, gallamine is about five times more potent than tetraethylammonium in blocking potassium conductance, where this is present, but has no effect on the sodium channel. Internal application slows sodium inactivation, which in addition is often incomplete. At positive potentials, gallamine can occlude sodium channels, thereby almost eliminating outward sodium currents.*

Since its introduction in 1947 (*1*), gallamine triethiodide (Flaxedil) has become one of the most commonly used neuromuscular blocking agents. The widespread use of gallamine, especially in neurophysiological studies, has depended in part on the belief that gallamine has no direct effect on nerve. However, the voltage-clamp studies described here reveal that externally applied gallamine has a potent blocking effect on potassium channels, where these are present, in both mammalian and amphibian nerve fibers. The blocking effect is sufficiently great that gallamine may have a direct effect on neural activity in gallamine-paralyzed preparations. Internal application of gallamine also has significant effects on the sodium channel; these effects resemble those produced by pancuronium bromide in squid giant axons (*2*).

Frog and rat myelinated nerve fibers were dissected and voltage-clamped by conventional methods (*3–5*). The nodes were held at potentials of −80 to −85 mV by adjusting for a steady-state inactivation of 0.8, and a 50-mV hyperpolarizing prepulse was applied to remove resting inactivation. Leakage and capacity currents were subtracted by an analog circuit, and membrane currents were re-

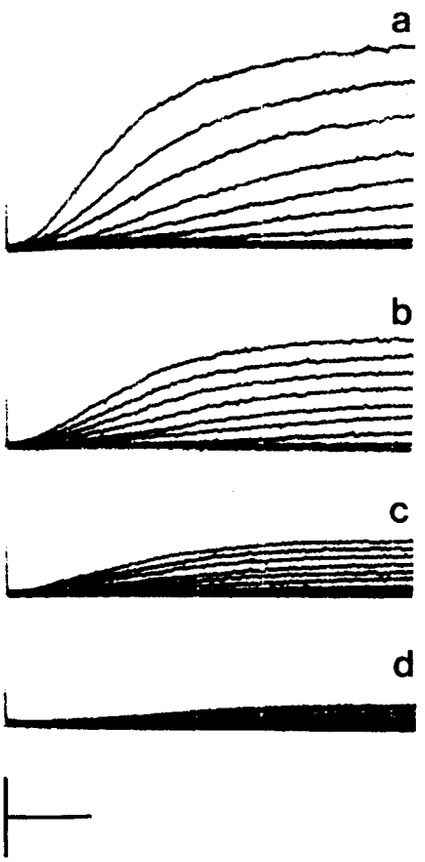

Fig. 1. Inhibition of potassium currents by gallamine in a frog fiber initially treated with $10^{-6}M$ tetrodotoxin to block the sodium conductance. (a) Control records in Ringer solution (115 m*M* Na^+; 2.5 m*M* K^+; 1.8 m*M* Ca^{2+}; 5 m*M* tris) for step depolarizations of 30 to 110 mV in 10-mV increments. (b to d) Records for the same depolarizations as in (a) but recorded 10 minutes after the addition of 0.1 m*M* (b), 1.0 m*M* (c), and 10.0 m*M* (d) gallamine triethiodide to the node in the A pool of the voltage-clamp chamber [see (*3*)]. Leakage current has been eliminated by the use of an analog circuit adjusted to null out those currents flowing during a 100-mV hyperpolarizing step. Calibrations are 8 nA and 2 msec. Holding potential, −85 mV; temperature, 15°C.

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