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IgA deficiency in wolves

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ABSTRACT

Low mean concentrations of serum immunoglobulin A (IgA) and an increased frequency of overt IgA deficiency (IgAD) in certain dog breeds raises the question whether it is a breeding-enriched phenomenon or a legacy from the dog's ancestor, the gray wolf (*Canis lupus*). The IgA concentration in 99 serum samples from 58 free-ranging and 13 captive Scandinavian wolves, was therefore measured by capture ELISA.

The concentrations were markedly lower in the wolf serum samples than in the dog controls. Potential differences in the IgA molecule between dogs and wolves were addressed by sequencing the wolf IgA heavy chain constant region encoding gene (*IGHA*). Complete amino acid sequence homology was found. Detection of wolf and dog IgA was ascertained by showing identity using double immunodiffusion. We suggest that the vast majority of wolves, the ancestor of the dog, are IgA deficient.

1. Introduction

Selective IgA deficiency (IgAD) is the most common primary immunodeficiency in humans with a prevalence of 1 in 600 in Caucasians (Hammarström and Smith, 2007). The disorder is defined as serum immunoglobulin A (IgA) concentrations equal to or less than 0.07 g/l with normal levels of serum IgG and IgM in children 4 years of age or older (Al-Herz et al., 2011). Even though most of the patients with IgAD are asymptomatic, 30% suffer from recurrent infections at mucosal sites (Al-Herz et al., 2011). The genetic background of IgAD is complex, but there is a strong association between IgAD and genes within the MHC region (Ferreira et al., 2012) and non-MHC genes, the latter including interferon induced with helicase C domain 1 (*IFIH1*), also known as MDA5 (melanoma differentiation-associated protein 5) C-type lectin domain family 16, *member A* (*CLEC16A*), and, albeit to a lesser extent, several other autoimmunity risk alleles (Ferreira et al., 2010).

Domestication of the dog is associated with two narrow genetic bottlenecks around 15,000 years, and, more recently, 200 years ago (Lindblad-Toh et al., 2005; Karlsson and Lindblad-Toh, 2008). At these occasions, only a limited number of wild ancestors contributed to generate a restricted gene pool for the domestic dog, leading to long haplotypes and long disequilibrium linkages, making the canine genome a suitable model for tracing the genetic background of complex human diseases (Lindblad-Toh et al., 2005; Karlsson and Lindblad-Toh, 2008).

Multiple studies on serum IgA concentrations in dogs have found "normal" IgA concentrations in most dog breeds tested (Felsburg et al., 1985; German et al., 1998; Griot-Wenk et al., 1999; Clemente et al., 2010). However, low concentrations, or even overt deficiency, have been found in selected breeds including the German shepherd dog (Whitbread et al., 1984; Griot-Wenk et al., 1999; Littler et al., 2006), shar pei dogs (Moroff et al., 1986; Rivas et al., 1995) and beagle dogs from selected kennels (Felsburg et al., 1985; Glickman et al., 1988).

Whereas low serum concentrations of IgA are not associated with clinical symptoms in a majority of animals (Griot-Wenk et al., 1999), infections, especially of the skin and the upper respiratory tract as well as intestinal bacterial overgrowth, are known to be associated with IgAD in dogs (Felsburg et al., 1985; Moroff et al., 1986; Batt et al., 1991).

With the exception of serum IgE concentrations, being approximately twice the concentration of those in dogs (Ledin et al., 2008), little is known about Ig concentrations in wolves. This is

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of particular interest, since the gray wolf is considered to be the ancestor of the dog (Vonholdt et al., 2010).

We therefore set out to determine whether the low concentrations of serum IgA in selected dog breeds was passed on from their wolf ancestors or whether it is a consequence of selective breeding during domestication.

2. Material and methods

2.1. Samples

Ninety-nine serum samples originating from 71 wolves, obtained between 1998 and 2004, were used. Fifty-eight animals were free-ranging and 13 individuals were captive. The former represent approximately 30% of the wolf population in Scandinavia (Ledin et al., 2008). Between one and four samples were taken per wolf at different occasions. For 53 of the animals (including the captive ones) only one sample was available. The time between repetitive sampling ranged from around one year to more than 4 years. All samples were kept at −18 °C until used.

2.2. Canine IgA ELISA

Antibodies previously successfully applied for measuring serum IgA concentrations in dogs (polyclonal goat anti-dog IgA antibodies (AbD Serotec), monoclonal mouse anti-dog IgA antibodies (AbD Serotec) and polyclonal, AP-conjugated goat anti-mouse antibodies (Jackson Immunoresearch)) were used as antibody set A. A second set of antibodies, antibody set B (polyclonal goat anti-dog IgA antibodies (AbD Serotec) and alkaline phosphatase (AP)-conjugated goat anti-dog IgA antibodies (Bethyl Laboratories)), was also used.

IgA levels in all serum samples were measured by capture ELISA. The protocols were previously successfully used to screen dog serum samples for IgA deficiency in our laboratory (Tengvall et al., 2013). In short, 100 μl of primary antibody, diluted 1:2,000 in carbonate/bicarbonate buffer, was coated onto polystyrene plates (Corning) and incubated at 4 °C overnight. The plates were then washed with phosphate-buffered saline with 0.05% Tween (PBST) and serum samples diluted 1:5,000, 1:10,000 and, 1:20,000 in PBST were added and incubated overnight at 4 °C. The plates were subsequently washed and the secondary antibody, diluted 1:2000 in Tris-buffered saline with 0.05% Tween (TBST) was added, followed by incubation for 2 h. The plates were then washed again and the substrate para-Nitrophenylphosphate (PNPP) was added (antibody set B). For antibody set A, the tertiary antibody was added in a 1:2000 dilution and incubated for 2 h before the plates were washed and substrate (PNPP) was added. Serum samples from dogs kindly provided by Professor M.J. Day, Bristol University (England) with previously determined IgA concentrations were used as controls for each individual measurement. All samples were measured twice using antibody set B and at least once using antibody set A.

2.3. Sequencing of the IGHA gene

Genomic DNA was prepared from blood samples. The IgA heavy chain constant region gene (*IGHA* gene), encoding the heavy chain constant region (C_H1 to C_H3) was amplified in two wolves using primers targeting the *IGHA* gene in dog (GenBank: L36871):

5′ CATGAAGACCTGTGCATTTCTCA 3′,

5′ AGGGACTCAATTGTGAGGAGGAA 3′.

The PCR conditions were: 94 °C for 5 min for 1 cycle; followed by 30 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 120 s and a final extension at 72 °C for 7 min. The resulting 1.7 kb PCR products were cloned into a pMD19-T vector and sequenced. For analyzing the sequence homology between dog and wolf, the obtained nucleotide sequence was aligned using MegAlign (DNASTAR). The used primer pair enables amplification of all four variants.

2.4. Immunodiffusion

Identity between wolf and dog IgA in serum samples was investigated by double immunodiffusion as previously described (Ouchterlony et al., 1950). Briefly, polyclonal goat anti-dog IgA antibodies (AbD Serotec) and monoclonal mouse anti-dog IgA antibodies (AbD serotec) were used to analyze serum samples from wolves and dogs. Antisera and serum samples were added to holes with a defined distance, punched in an 0.9% agarose matrix. The samples were allowed to diffuse in the matrix for 72 h at 4 °C.

3. Results

3.1. Immunoglobulin concentrations

IgA levels in all serum samples were measured by capture ELISA. A high correlation between the IgA concentrations obtained for 99 serum samples taken from the 71 wolves using two different sets of antibodies was observed ($r^2 = 0.91\%$, Fig. 1). The sample with the highest IgA concentration was found have 0.55 g/l (antibody set A) and 0.61 g/l (antibody set B) respectively. This sample may be considered an outlier, since its IgA concentration was three times higher than the second highest sample. Complete absence of IgA was observed for two of the samples using both antibody sets. Furthermore, five samples showed an IgA concentration between 0.23 and 0.11 g/l. All the remaining samples ($n = 93$) were found to have an IgA concentration of 0.1 g/l or less.

The IgA concentrations in the wolves were compared with those of 50 samples from various dog breeds (10 standard poodles, 10 miniature poodles, 10 Tibetan terriers, 10 Rottweilers, and 10 Boxers) and 154 serum samples from German shepherd dogs, a breed know to have low serum IgA concentrations (Whitbread et al.,

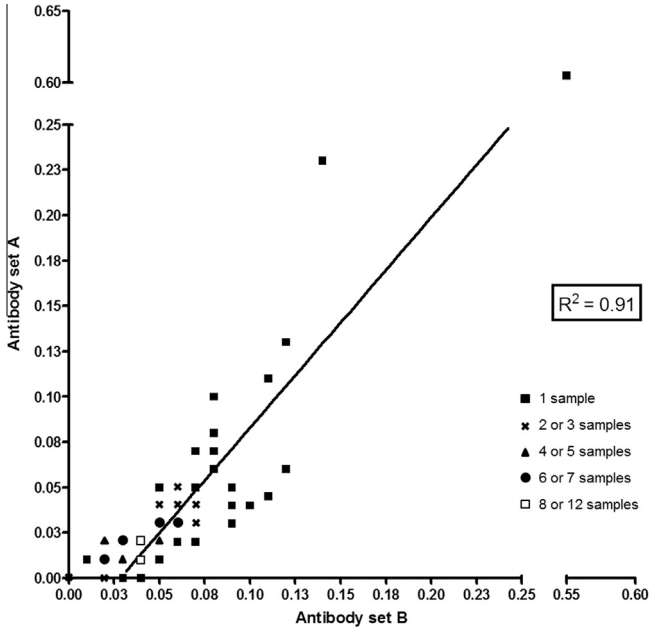

Fig. 1. IgA concentrations obtained for 99 serum samples from wolves using two different sets of antibodies. Samples with similar concentrations are displayed by one marker only.