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Familial Nephropathy in Bernese Mountain Dogs

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Abstract. Between January 1988 and March 1992 nephropathies were frequently diagnosed in Bernese Mountain Dogs. During this period, 20 animals (16 females, four males), ages 2–5 years (average age at time of diagnosis = 3.3 years) presented with clinically renal insufficiency. Morphologic diagnosis of the renal lesions was identical in all cases, i.e., membranoproliferative glomerulonephritis (MPGN) with concomitant interstitial nephritis. Deposits of immunoglobulin-M (IgM) and of the third complement component were regularly demonstrated immunohistochemically in the glomeruli; deposits of immunoglobulin-A (IgA) and immunoglobulin-G (IgG) were found only in isolated cases. Reduplication of glomerular basement membranes, mesangial interposition, and subendothelial deposits of the immunocomplex type were also detected by electron microscopy. A pedigree analysis indicated that the MPGN in these 20 Bernese Mountain Dogs of approximately the same age was of hereditary genesis. Thus, MPGN should be allocated to the group of familial nephropathies. Serologically, high IgG titers against *Borrelia burgdorferi* were found in 17 dogs. These findings are discussed in relation to familial nephropathies in humans.

Key words: Bernese Mountain Dogs; familial nephropathy; membranoproliferative glomerulonephritis.

Familial nephropathy in dogs cannot be classed as a single morphological entity but comprises a group of nephropathies that includes renal dysplasia, juvenile renal disease, glomerulopathy, and tubular transport dysfunction.[46] Cases of familial nephropathy have been reported in different species, including humans,[18] sheep,[4] and dogs,[46] and is suspected whenever renal disease occurs in young related individuals and a specific cause such as toxins or infective agents is not identified. The majority of reports concern single animals or littermates. Several breeds of dogs are involved, e.g., the Norwegian Elkhound,[12] Basenji,[11] Cocker Spaniel,[30] Wheaton Terrier,[41] Llaso Apso,[42] Shih Tzu,[27,42] Doberman Pinscher,[47] Samoyed,[5,29] and Bull Terrier.[26] The age of clinical onset of disease is variable, ranging from a few weeks to several years.[40] In some breeds, there is good evidence that the disease is familial, but the mode of inheritance has so far only been elucidated in four breeds (Cocker Spaniel, Samoyed, Shih Tzu, and Bull Terrier).

We identified a nephropathy in a number of related Bernese Mountain Dogs. In this report, we describe the gross, light, and electron microscopic and immunohistologic features of a membranoproliferative glomerulonephritis (MPGN) occurring in dogs of that breed and report the results of pedigree analysis and serologic studies. Further clinical aspects of the disease will be published elsewhere.

Material and Methods

Eighteen Bernese Mountain Dogs were submitted to our institute for necropsy. Samples obtained from the kidneys, heart, skin, lung, stomach, small intestine, liver, spleen, pancreas, urinary bladder, joints (carpal, tarsal, hip, knee, shoulder), lymph nodes (ln. cervicalis superficialis, ln. mesenterialis), bone marrow, thyroid, parathyroid, adrenal gland, brain, spinal cord, and eyes were collected in 7% buffered formalin and processed in paraffin. Sections were cut at 5 μm and stained with hematoxylin and eosin (HE) for light microscopic examination.

In addition, specimens were fixed in 4% buffered paraformaldehyde and embedded in plastic as described elsewhere.[22] For two dogs, only biopsy specimens were available, which was completely used up in plastic histology. The following staining procedures were performed on sections of 2 μm thickness: HE, Giemsa, periodic acid–Schiff's reaction, and silver impregnation.

Immunohistochemical staining was performed on paraffin material utilizing the avidin–biotin complex (ABC) method.[28] Antibodies employed were rabbit anti-human IgA (Dako, Glostrup, Denmark; diluted 1 : 2,000), rabbit anti-human IgG (Dako; diluted 1 : 2,000), rabbit anti-human IgM (Dako, diluted 1 :400), rabbit anti-human von Willebrand factor (Dako; diluted 1 : 1,500), rabbit anti-dog C3 (ICN Flow, Meckenheim, Germany; diluted 1 : 100), and rabbit whole serum anti-*Borrelia burgdorferi* (BAG Diagnostika, Lich, Germany; diluted 1 : 4,000, paraffin-embedded skin biopsy with subcutaneously injected borreliae served as a positive control). Goat anti-rabbit IgG (Dako) was used as the second-layer

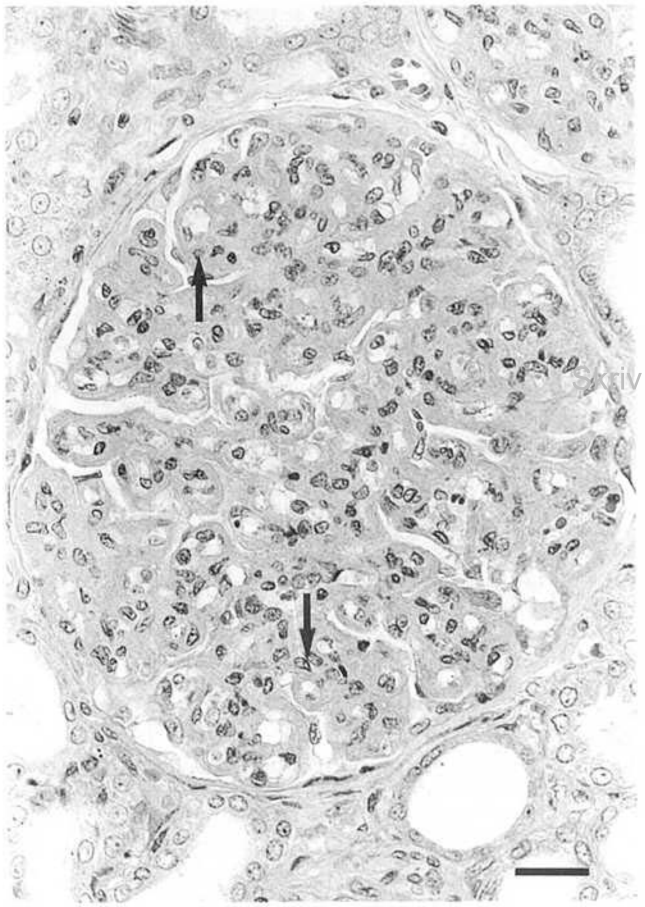

Fig. 1. Light photomicrograph. Glomerulus; Bernese Mountain Dog. Note expansion of the glomerular tuft, accentuation of lobular pattern, increase of cells in mesangial position, considerable thickening of capillary walls (arrows), and proliferation of endothelial cells. HE. Bar = 25 μm.

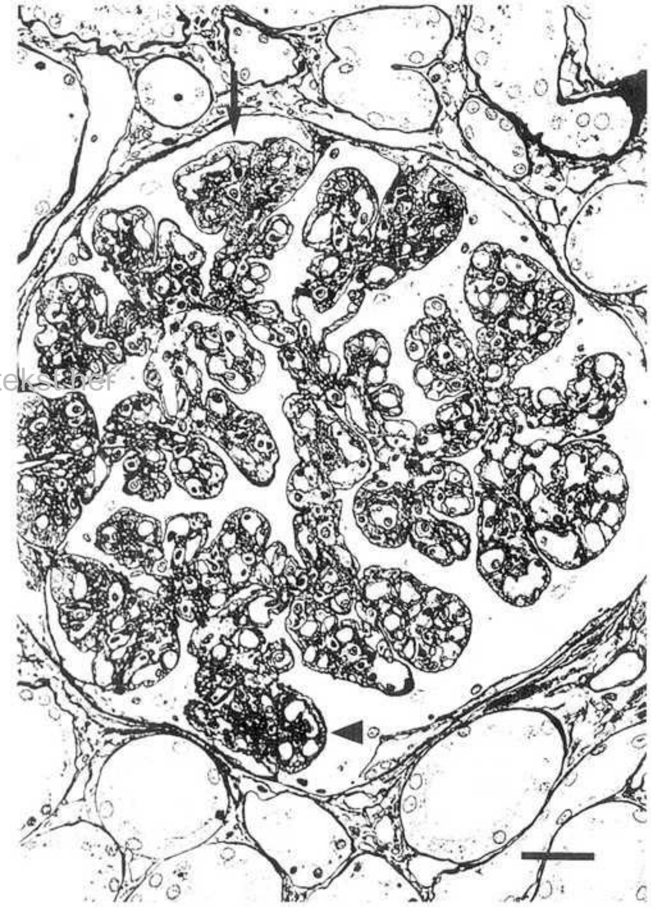

Fig. 2. Light photomicrograph. Glomerulus; Bernese Mountain Dog. Note split double-contoured basement membranes (arrow) and focal mesangial sclerosis (arrowhead). Silver impregnation. Bar = 25 μm.

antibody (dilutions ranging from 1:50 to 1:2,000). Diaminobenzidine tetrahydrochloride (Sigma, Deisenhofen, Germany) served as the chromogen, and the sections were finally counterstained with hematoxylin. Negative controls omitting the first antibodies were treated in the same way as described above. For electron microscopic examination, kidney specimens were cut into fragments 1 mm in diameter, fixed in cold 1% glutaraldehyde/4% formalin,[45] and processed in the conventional manner for embedment in epoxy resins (epon). Ultrathin sections were stained with lead citrate/uranyl acetate and examined with an electron microscope (Zeiss EM 10, Toberkochen, Germany). Blood samples were taken for routine hematologic examination (complete blood count, blood urea nitrogen, serum creatinine, serum total protein, and urine analysis was performed (urinary creatinine, urinary total protein, microbiological examination, sodium dodecyl sulfate (SDS) gel electrophoresis). An indirect immunofluorescent assay (IFA[52]) was used to evaluate IgG anti-*Borrelia burgdorferi* antibodies. In accordance with other reports, IFA titers > 1 : 64 are considered positive because of negative heterologous bacterial cross-reactivities.[14,17,36] Kidney, heart, synovial membrane, and blood specimens have so far only been taken for cultural isolation of *B. burgdorferi* on one occasion, immediately after euthanasia. Genetic analysis of the ancestral background was also performed, using the available pedigrees of the 20 Bernese Mountain Dogs. In most cases, data for only three generations of ancestors were available.

Results

Clinical examination, serologic, and microbiological findings

All dogs had been suffering for several weeks from weight loss, vomiting, polydipsia, and polyuria. All but one dog displayed azotemia (blood urea nitrogen: 40–245 mg/dl, serum creatinine: 1.6–16.5 mg/dl); all dogs had nonregenerative anemia with packed cell values between 13% and 38%; leucocytes were between 6,600/μl and 25,900/μl (14 dogs exhibited white blood cell counts above the reference range). Nine dogs were also suffering from hypoproteinemia (total protein: 3.3–6.7 mg/dl). The animals had pronounced proteinuria (381–2,377 mg/dl) and an elevated urine protein : creatinine

Fig. 3. Electron photomicrograph. Glomerulus; Bernese Mountain Dog. Note thickening of the peripheral capillary loop due to mesangial interposition (N = mesangial cell nucleus) and reduplication of basement membrane-like material (arrow) and native capillary basement membrane (arrowhead). Bar = 1 μm.

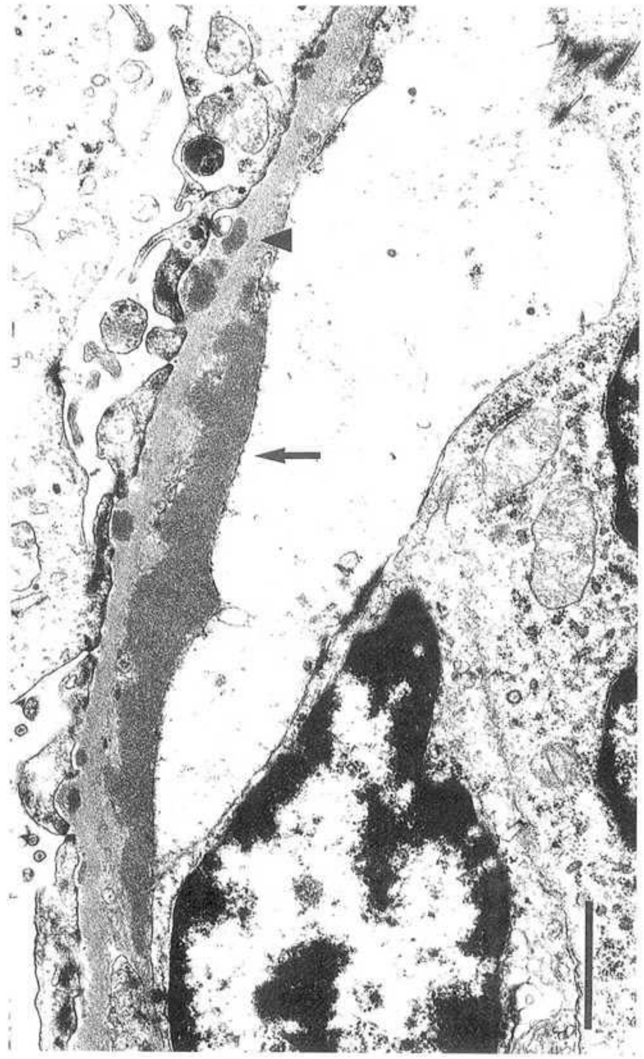

Fig. 4. Electron photomicrograph. Glomerulus; Bernese Mountain Dog. Peripheral capillary loop has large subendothelial (arrow) and small subepithelial (arrowhead) electron-dense deposits. Bar = 1 μm.

ratio (4.1–30.1); macroproteinuria was identified using SDS gel electrophoresis. Nine dogs with ascites and generalized subdermal edema developed a nephrotic syndrome. Serologically, high IgG IFA titers against *B. burgdorferi* were found in 17 of the dogs examined (1 : 512–1 : 32,768). The cultural isolation of *B. burgdorferi* was negative in the one case examined.

Necropsy findings

Anemia was a common feature; generalized subdermal edema and hydroperitoneum of various grades of severity were diagnosed in nine dogs. In all animals, both kidneys were equally involved. They were pale and firm, and the surface of the cortex zone, which had become smaller in most cases, was finely granulated. The glomeruli were prominent. Uremic gastritis with bleeding erosions and ulcers were common. In six dogs, necrotic foci were detected in the myocardium of the left heart ventricle. Atrophy of skeletal muscles, enlarged parathyroids, and bone demineralization had been diagnosed in all animals and were due to renal protein loss and renal osteodystrophy.

Microscopic findings

All glomeruli were involved, and they were enlarged and often showed accentuation of the lobular pattern. There was an increase in the number of cells in the mesangial position and often considerable thickening of the capillary walls. The capillar lumina were often reduced due to proliferation of the endothelial cells

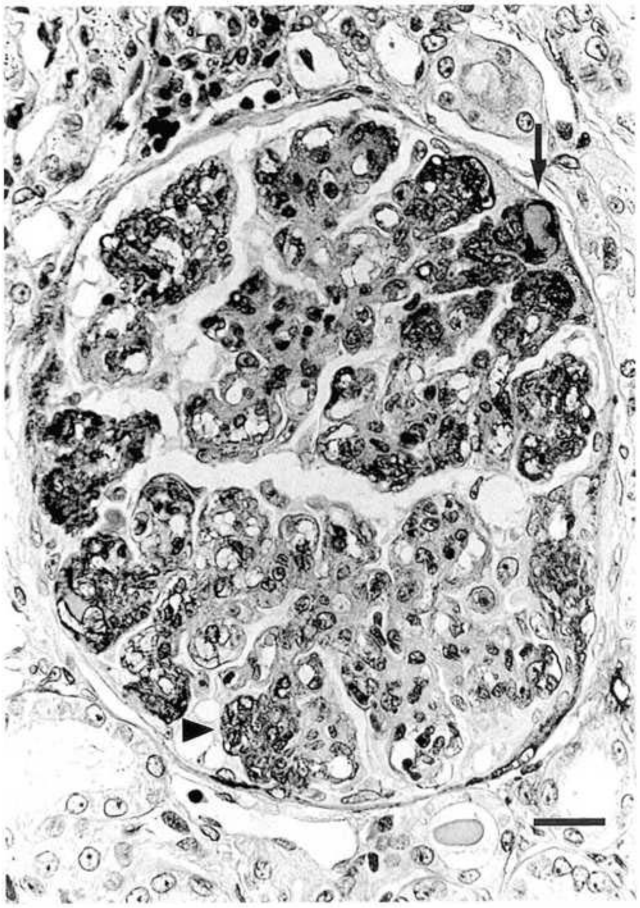

Fig. 5. Light photomicrograph. Kidney; Bernese Mountain Dog. Section shows granular deposition of C3 along the capillary walls (arrow) and within the mesangium (arrowhead). Immunoperoxidase stain. Bar = 25 μm.

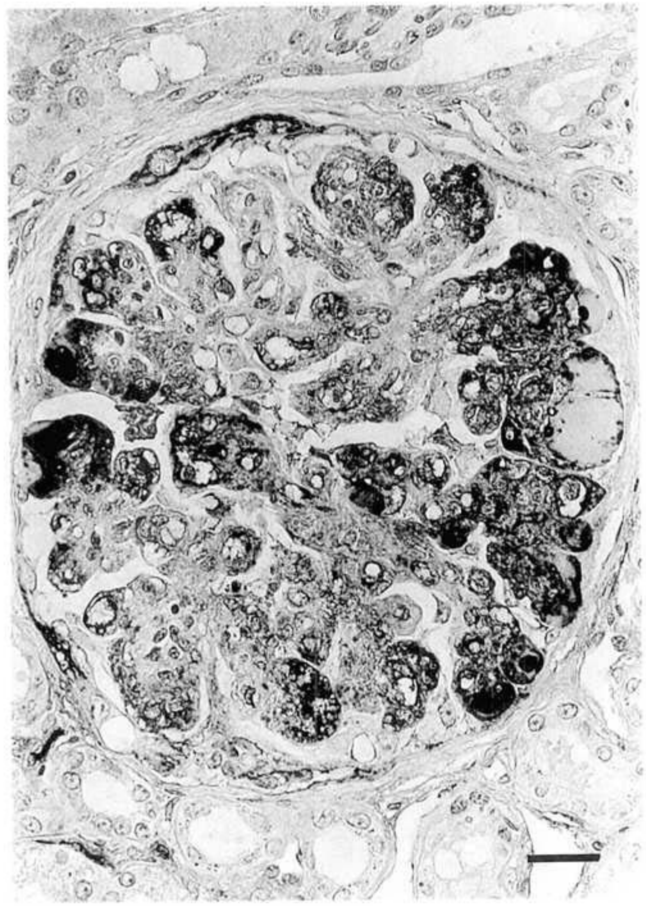

Fig. 6. Light photomicrograph. Kidney; Bernese Mountain Dog. Section shows deposition of IgM within the mesangium and along the capillary walls. Immunoperoxidase stain. Bar = 25 μm.

(Fig. 1). Silver staining of the thickened capillary walls revealed the characteristic feature of split, double-contoured basement membranes (Fig. 2). In some cases, singular capillary loops showed subepithelial spikes, as recorded in cases of MPGN. Hyalinoses of capillary loops, synechias of glomerular tufts with Bowman's capsules, and fibrous crescents, as well as an increase in the mesangial matrix, were found in the advanced stage of the disease. Mesangial sclerosis was the main lesion in the advanced stage. The cells of the tubules contained numerous hyaline droplets. In the course of the disease and with the development of concomitant chronic interstitial inflammation, several tubules became dystrophic. Parenchymatous degeneration of the liver was diagnosed in 16 (89%) of the dogs. Necrotizing myoarteritis and foci of necrotic myocardial cells associated with inflammatory infiltrates (neutrophil granulocytes, lymphocytes, and plasma cells) were other major extrarenal lesions. No causative agents were detected with special stains (Giemsa, silver impregnation). Other organs, specifically the joints, skin, brain, spinal cord, and eyes, did not show any pathologic changes.

Electron microscopic findings

Examination with the electron microscope confirmed the renal changes observed with light microscopy, but certain additional features were also revealed. The thickened capillary walls consisted of two layers: the native capillary basement membrane without changes in its ultrastructure (e.g., no intramembranous dense deposits or multilaminar splitting) and a layer of newly formed basement membrane-like material, a change that gave rise to the reduplication observed with silver staining. Between these layers, there were cytoplasmic processes of mesangial cells (Fig. 3). Electron-dense deposits of the immunocomplex type were present on the endothelial side of the capillary basement membrane and to a lesser extent within the mesangium and on the subepithelial side of the cap-

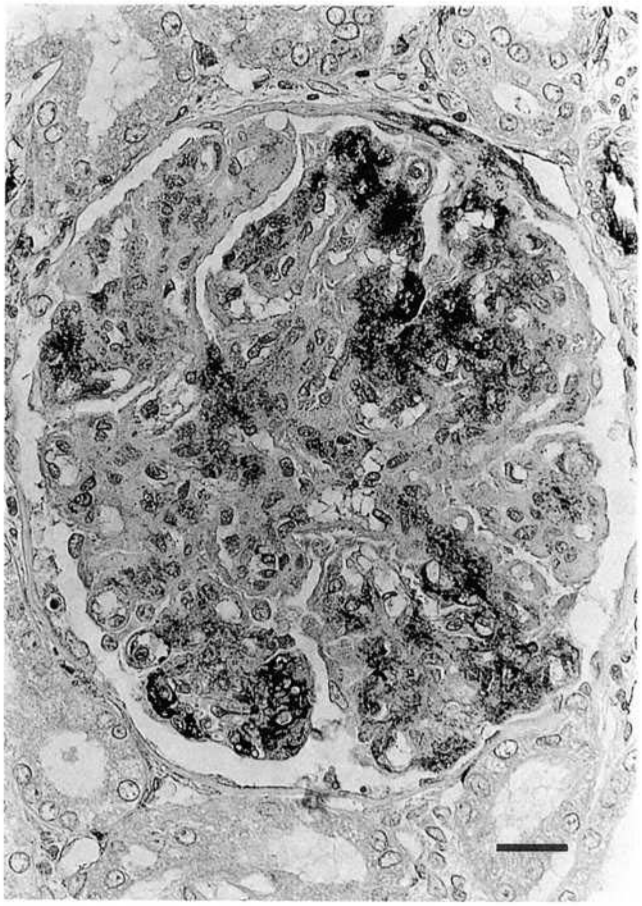

Fig. 7. Light photomicrograph. Kidney; Bernese Mountain Dog. Section shows granular deposition of von Willebrand factor within the mesangium and along the capillary walls. Immunoperoxidase stain. Bar = 25 μm.

illary loops (Fig. 4). Some of the deposits were small and discrete, others were large and elongated. Foot-process effacement was also a general feature. The tubular basement membranes were thickened whenever interstitial inflammation was present but did not show changes in their original lamilar structure.

Immunohistochemical findings

Positive staining for C3 (Fig. 5) and IgM (Fig. 6) was always observed (18/18), with a granular pattern along the capillary loops and less frequently within the mesangium. IgA deposits were demonstrated in 72% of the dogs (13/18), and IgG deposits were seen in 11% of the dogs (2/18) with a comparable distribution as described for IgM and C3. Von Willebrand factor could again be demonstrated (Fig. 7) in all dogs (18/18) within the mesangium and along the capillary loops. Immunohistochemical examination for *B. burgdorferi* on sections of myocardium and kidney was negative in all dogs.

Pedigree analysis

The pedigrees of the 20 Bernese Mountain Dogs were available for analysis. The sex ratio of the affected dogs was 1:4 (four males, 16 females). In three litters, two female full sibs were affected, and the father of one of these litters also showed renal lesions 2 years after the parturition of his offspring. One full sister of two affected female dogs was the mother of another affected female. The grandfather of one full sib pair was also grandparent of an affected male. Two affected females had the same father, and four other male dogs were found at least three times among the ancestors of various affected animals. Altogether, pedigree analysis showed that all affected animals were related to each other, sharing at least one ancestor with one or more other affected animals. In more than 50% of these Bernese Mountain Dogs, both the paternal and the maternal pathways showed links with ancestors of one or more affected animals.

Discussion

The primary renal lesions of MPGN were comparable in all the Bernese Mountain Dogs. Secondary glomerular alterations (e.g., mesangial sclerosis[8]) and interstitial alterations of various degrees occurred, depending on the duration of the disease. Morphologically, two major types of MPGN in humans have been defined in the literature.[20] The main morphologic feature of type 1 MPGN is mesangial expansion, which is secondary to an increase in cells and matrix, capillary wall thickening due to mesangial interposition, presence of two layers of regularly formed basement membranes (the newly formed track can alternately consist of a compacted layer of mesangial matrix), and deposits of immunocomplexes, mostly subendothelial and occasionally subepithelial, along the capillary wall and often within the mesangium.[31,53] Type 2 MPGN differs from type 1 because of the presence of intramembranous deposits within the lamina densa.[19] Similar features such as endocapillary cell proliferation are observed, whereas proliferation of the mesangial cells is often only slight. Humans suffering from hereditary nephritis/Alport's syndrome sometimes develop MPGN characterized by multilaminar splitting of the basement membranes. Further distinct morphologic variants have been described.[3,6] Despite the morphologic differences (subepithelial deposits, disruption of lamina densa), a new variant, type 3 MPGN, has been described[6] and includes lesions associated with type 1 MPGN. We therefore regard this new classification as redundant.

The electron microscopic features (presence of two layers of basement membranes or basement membrane-like material but no changes in the native glomerular basement membrane, the absence of intramembranous dense deposits and multilaminar splitting of original basement membranes) observed in our study indicate that MPGN in this group of Bernese Mountain Dogs is similar to type 1 MPGN.

In human nephrology, the different types of MPGN have different etiopathologies. Various bacterial organisms (e.g., *Staphylococcus albus, S. epidermidis, Corynebacterium bovis,* and *Mycobacterium leprae*) have been identified and are presumably responsible for type 1 MPGN.[24,53] Disorders such as systemic immune complex diseases, neoplasms (e.g., lymphomas), chronic liver diseases, and a number of other diseases (e.g., polyarteritis or sarcoidosis) are also associated with MPGN.[24,53]

Further reasons for spontaneous MPGN in dogs (review by Slauson and Lewis[49]), such as pyometra, systemic lupus erthematodes, infectious canine hepatitis, and dirofilariosis, can be excluded because of the absence of characteristic lesions. There are few reports concerning familial MPGN in animals. Finnish Landrace lambs have been diagnosed with MPGN (similar to type 1 MPGN) on the basis of a severe deficiency of C3,[4] with ultrastructural alterations resembling those of the Bernese Mountain Dogs. C3 deficiency is possibly also the cause of MPGN in dogs (Brittany Spaniels,[9] ultrastructure comparable to the lambs), and there are reports of familial MPGN in other dog breeds: Samoyeds,[5] Doberman Pinschers,[47] and Bull Terriers.[25] As described in Alport's syndrome in humans,[23] the characteristic ultrastructural lesion in these three dog breeds is splitting of the capillary basement membranes into multiple layers, and therefore the findings are different from those observed in the Bernese Mountain Dogs.

The deposition patterns of C3 and the various immunoglobulins in our study are similar to those of MPGN in humans; however, no characteristic pattern has been identified for any of the MPGN types. As reported by other investigators,[44] mesangial location of von Willebrand factor is probably due to translocation from plasma or from glomerular endothelial cells and is secondary to and part of the inflammatory process.

The pedigree analysis has shown with a high degree of probability that the susceptibility of MPGN observed in these Bernese Mountain Dogs was inherited, although the exact mode cannot be explained definitively by analysis of the available information. A sex-influenced mode of inheritance seems very probable because of the significant deviation in the 50:50 sex ratio in affected animals (sign test of Dixon and Mood). This second locus should act epistatically with the glomerulonephritis locus. The pedigree analysis indicates an autosomal recessive mode of genetic transmission. In most litters, only one member was affected, and the three litters with two affected sibs can be explained by the high number of offspring per litter (seven on average). A second autosomal locus appears to be responsible for the sex-influenced genetic determination (penetrance) of the trait, showing so-called sex-dependent dominance exchange in the heterozygote. In this mode of inheritance, the allele is dominant in one sex and recessive in the other. Using this hypothesis, the frequency of the alleles can be estimated from the inequality of the distribution. A ratio of 1 : 4 results in frequencies of 0.6 and 0.4 for the two corresponding alleles. We therefore conclude that susceptibility to MPGN in Bernese Mountain Dogs is inherited as an autosomal recessive trait and assume that its expression is influenced by the epistatic action of a second gene locus with a sex-influenced dominance exchange.

At present, it is difficult to reconcile the various findings—occurrence of the same type of nephropathy in animals of one breed with a preference for a certain age group, presumed autosomal recessive heredity of the disease on the basis of the pedigree analysis, and positive serology for *B. burgdorferi.*

Development of MPGN as the result of circulating immune complexes becoming trapped in the glomeruli has already been described in Lyme disease in humans.[21] *Borrelia burgdorferi* can also induce MPGN in dogs, as has been shown in one case by immunohistochemical demonstration of this pathogen in the kidneys.[16] Special staining methods, such as Giemsa, silver staining, or immunohistochemical methods,[2] which can be used to demonstrate borreliae, did not reveal borreliae in any organ of the affected Bernese Mountain Dogs (i.e., kidneys, myocardium, joints, skin). Borreliosis was not confirmed by cultural methods, although only one dog has been investigated so far and positive results are difficult to obtain even in experimental studies.[17] In addition, borreliae are rarely isolated by culture, suggesting that spirochaetemia is mild or intermittent, and therefore the involvement of organs as well as the presence of *B. burgdorferi* in tissue is obviously stage related.[17]

Myocardial alterations comparable to those in our study have also been described in canine borreliosis[34] and in uremic animals on the basis of arteriolar lesions causing ischemia and dystrophy of myocardial cells.[37] Parenchymatous degeneration of the liver is a common lesion in uremic patients due to acidosis and uremic toxins. This lesion is distinct from those described in borreliosis in humans[7,15] and in an experimental study of mice.[48] Furthermore, contrary to clinical reports of arthritis and the meningitic form of borreliosis in seropositive dogs,[10,32,33,35] no inflammation could be seen in the joints and brains of these Bernese Mountain Dogs.

The only established cause and effect of familial

MPGN in dogs is the multilaminar splitting of glomerular basement membranes in Samoyeds associated with a defect of the NC1 domain of type VI collagen.[51] The cause of MPGN in Bernese Mountain Dogs has not yet been elucidated. It might be an immunogenetic disposition as in humans[13] and/or autoimmunity (possibly triggered by *B. burgdorferi*) against renal, presumably glomerular, antigen. Autoimmunity has been assumed to cause or to be associated with Lyme disease in humans on the basis of common antigenic determinants shared between *B. burgdorferi* and human tissue (nerve cells, synovial fluid, myosin).[1,39,50] This phenomenon of molecular mimicry is the result of similar structures being shared by molecules from dissimilar genes or shared by their protein products, for example a virus and a normal host determinant.[43] Further investigations using other methods (e.g., polymerase chain reaction,[38] complement assay for C3,[9] and immunoblotting studies[54]) are necessary to find the underlying cause of this disease.

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