MYXOVIRUS-LIKE STRUCTURES IN THE GLOMERULAR ENDOTHELIAL CELL CYTOPLASM IN CANINE NEPHRITIS

KAI KROHN and MARKUS SANDHOLM

Institute of Biomedical Sciences, University of Tampere, Tampere and from the Department of Medicine, College of Veterinary Medicine, Helsinki, Finland


Electron microscopic examinations revealed the presence of myxovirus-like tubular structures, 200Å in diameter, in the endothelial cytoplasm of the glomeruli in four dogs with chronic interstitial nephritis and in one healthy control dog. The structures were found inside the endoplasmic reticulum and in some longitudinal sections, the tubuli seemed to have a helical or spiral substructure. The morphology and cellular localization of the structures resembled those seen in systemic lupus erythematosus in man, and in cultures of cells from tissue infected with canine distemper. Precipitating canine distemper virus antibodies were not in evidence in dogs in which such structures were demonstrable by electron microscopy while these antibodies were found in other dogs.

Key words: Canine interstitial nephritis; myxoviruses.

Kai Krohn, Institute of Biomedical Sciences, University of Tampere, Teiskontie 37, SF 33520 Tampere 52, Finland.

Received 7.1.74 Accepted 20.xii.74

Tubular or spherical particles, morphologically resembling paramyxoviruses, have been found in human glomeruli in some pathological conditions, especially in connection with systemic lupus erythematosus (SLE) (1, 2, 4, 5, 10). Similar particles have been found in some animal diseases, such as Aleutian mink disease (11). The character of these particles is unknown, but it has been suggested that they either might be viruses (1, 4, 5) or represent an unspecific cellular response to a variety of external stimuli (9, 12).

We have studied the electron microscopic morphology of the glomeruli in chronic canine interstitial nephritis (CIN). In this disease, the glomerular lesions resemble those seen in SLE (6). In some of the electron micrographs, virus-like structures were observed which were subjected to a closer study.

MATERIAL AND METHODS

Eighteen dogs were investigated. In six of these, aged from 6 to 13 years, a clinical diagnosis of chronic renal failure was established and chronic interstitial nephritis was confirmed by renal histology (6, 7). A control group was composed of four young dogs (aged ½-1 years) and of eight old dogs (aged 6-17 years) without clinical and histological signs of renal disease. All dogs had been vaccinated against canine distemper and canine infectious hepatitis (Candur SH, Boehringer-werke, Germany).

The dogs were anaesthetized intravenously with
thiopentone sodium (Intraval®) and samples were taken for electron microscopy and histology. Several pieces of about 1 mm³ were removed from the cortical region of each kidney and fixed for 60 minutes in 2.5 per cent glutaraldehyde, pH 7.4, with 0.1 M phosphate buffer. After washing with the same buffer, the samples were postfixed for 1 hour with 1 per cent osmium tetroxide and embedded in Epon 812. The sections were stained with uranyl acetate and lead citrate and studied using a Siemens A1 Elmiskope.

Precipitating antibodies in undiluted sera were assayed by micro-immunodiffusion in 1 per cent agarose in phosphate buffered saline, pH 7.2. A homogenate of culture of the Rockhorn strain, distemper virus (kidney cell culture) was used as antigen. After 72 hours diffusion at room temperature, the plates were washed in PBS for 24 hours at +4°C and stained with amidoblack. By this method, about 90 per cent of all adult dogs admitted to the clinic for small animals at the College of Veterinary Medicine, Helsinki, were found to harbour precipitating antibodies.

**Fig. 2.** Electron micrograph of glomerular endothelial cell (EN) showing tubular structures inside the cisternae of the smooth endoplasmic reticulum (ER). The tubuli show a helical substructure. 100,000 x.

**Fig. 1.** Electron micrograph of glomerular endothelial cell showing aggregates of tubular structures in the cytoplasm. 28,000 x.

**RESULTS**

Paramyxovirus-like structures were seen in the endothelial cytoplasm of the glomeruli in one of the eight older control dogs and in four of the six nephritic dogs but in none of the four young controls.

Occasional longitudinal sections of tubuli of uniform size were seen, arranged in a fairly regular pattern (Fig. 1 and 3). The diameter of the tubuli was about 200Å, and the distance between the tubuli in aggregates was about 100Å. Hexagonal formations were sometimes seen in which six tubuli surrounded one central tubular structure (Fig. 3). In longitudinal sections, helical form, resembling the nucleocapsid structures of paramyxoviruses, were observed (Fig. 2). The structures were located in the endothelial cytoplasm in the cisternae of the smooth endoplasmatic reticulum.

Structures of similar appearance, but ar-
Fig. 3. Higher magnification of the area shown in Fig. 1. In some oblique sections, hexagonal arrangement of six tubuli around a single tubulus is seen. A smooth membrane continuous with the endoplasmic membrane lower most in the figure surrounds the aggregates 100,000 X.

ranged in strictly regular crystalline patterns, were observed in the mesangial cytoplasm of the two nephritic dogs (Fig. 4). These structures were also surrounded by the membranes of the endoplasmic reticulum.

The distribution of precipitating antibodies against canine distemper virus in the study group is shown in Table 1.

DISCUSSION

Chronic canine interstitial nephritis is a disease which characteristically affects old animals of both sexes. A prominent histological alteration in the affected kidneys is the interstitial infiltration of lymphocytes and plasma cells. As a rule, however, the glomeruli are also affected and the lesion in the glomeruli resembles the membranoproliferative nephritis seen in SLE (6). In previ-

| TABLE 1. Occurrence of Precipitating Antibodies against Canine Distemper Virus in Nephritic and Control Dogs and the Presence of Myxovirus-like Structures in the Glomerular Endothelial Cells |
|-------------------------------------------------|-----------------|-----------------|
| Kidneys containing myxovirus-like structures   | 1/4             | 0/1             |
| Kidneys without myxovirus-like structures     | 2/2             | 4/4             |
ous studies, the occurrence of electron dense deposits in the thickened glomerular basement membranes were seen, probably having a connection with the interstitial-glomerular nephritis in the dog (6). IgG type antibodies and the complement component C3 were deposited in the glomerular capillary wall (7). Elution and recombination studies showed that these antibodies probably originated from antigen-antibody complexes and that the antigen part of the complex was not endogenous and could thus be of viral origin.

The results of the present study indicate that there may be a connection between the renal disease and the presence of the paramyxovirus-like structures located in the glomerular endothelial cells. As regards the morphology, these structures are similar to those occurring in SLE described earlier in various other collagen diseases in man and in some animal diseases, such as the Aleutian mink disease. It has been suggested that nuclear crystalloid structures in canine glomeruli may have some connection with infectious canine hepatitis (ICH). No doubt endothelial and mesangial cytoplasmic crystalloid structures can be found in apparently healthy dogs (3). Although it has been suggested that these structures could be due to various exogenous stimuli (9, 12), it seems reasonable to assume that they represent viruses which could be of aetiological importance. Among the known canine viruses the most likely would be the distemper virus. In tissue culture, this virus resembles morphologically the structures observed in the present study (8).

An inverse correlation between the finding of the virus-like structures in the endothelial cell and the occurrence of precipitating antibodies against distemper virus in the serum was observed.

It is possible that the immune response
against the virus normally occurring in vaccinated dogs fades in the course of ageing, thus leading to a continuous viral replication and excessive production of viral antigen. This process in turn would result in the formation of antigen-antibody complexes and might explain the presence of basement membrane-bound deposits in the glomeruli as observed in canine interstitial nephritis (7) and the disappearance of free (precipitating) antibody from the serum.

REFERENCES