

# Alterations of the gut associated lymphoid tissue (galt) in harbor seals (*phoca vitulina vitulina*) during the epidemic in 1988

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identified in rabbits (Takeuchi et al., 1978) and humans (Rothbaum et al., 1983). Similar *E. coli* were identified in the small intestine of naturally infected calves (Pospischil et al., 1987; Pearson et al., 1988) and experimentally inoculated calves (Wray et al., 1989). In addition some of these strains produce a toxin which is toxic to vero cells (Verocytotoxin (VT)) (Konowalchuk et al., 1977) and are now known as VTEC; some produce haemorrhage in the large bowel (Riley et al., 1983) and are termed enterohaemorrhagic *E. coli* (EHEC), and may also produce VT.

With this proliferation of names the description *E. coli* diseases has become extremely complex. Thus it is suggested that *E. coli* associated with enteric disease should be termed either enterotoxigenic (ETEC), with the understanding that some of these strains may be associated with pathological changes, and «attaching and effacing» *E. coli* (AEEC). Unfortunately both types require ultrastructural examination for a definitive pathological diagnosis.

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### ALTERATIONS OF THE GUT ASSOCIATED LYMPHOID TISSUE (GALT) IN HARBOR SEALS (PHOCA VITULINA VITULINA) DURING THE EPIDEMIC IN 1988

*G. Pohlmeier, J. Pohlenz, E. Liebler*

In 1988 the seal population of the northern European coasts was severely decimated by an epidemic associated with distemper-like clinical symptoms. At necropsy bronchopneumonia, depletion of lymphoid organs and degenerative or inflammatory changes of the liver were found (Breuer et al., 1988; Kennedy et al., 1989; Friedhoff & Pohlenz, 1989). As cause infection with a Morbillivirus (PDV = Phocine Distemper Virus) and with several secondary infectious agents is considered (Osterhaus & Vedder, 1988; Liess et al., 1989; Kirchhoff et al., 1989). Since GALT has been recognized as a major pathway for entry of infectious agents into the host (Owen, 1983), it was the objective of this work to examine involvement of the intestine in the epidemic by morphological investigation of gut mucosa, especially of GALT.

#### Material and methods

Intraluminally fixed intestines or intestinal specimens from 33 diseased seals and from one healthy control animal were examined macroscopically and/or by light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Distribution and amount of aggregated and solitary lymphoid follicles were determined. LM, SEM and TEM were performed on specimens from jejunal and ileal Peyer's patches and from solitary and aggregated lymphoid follicles of colorectum.

#### Results

In phocine small intestine about 20 Peyer's patches and an individually varying number of solitary lymphoid follicles are present. In large intestine solitary follicles are distributed irregularly, patch-like aggregations occur in the middle of colorectum.

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Paramyxovirus and Reovirus each were detected in the intestinal epithelium of three animals. Trematodes, Cryptosporidium and bacteria were present as single or combined infections in several animals. Infiltration of lamina propria with granulocytes was found in the intestines of most animals. In several seals villus atrophy and/or crypt abscesses were observed.

Compared to the control animal lymphoid follicles in small intestines of infected seals were characterized by varying degrees of depletion and reduced numbers of intraepithelial cells associated with M cells in the FAE. In four animals, three of which had Paramyxovirus inclusions in gut epithelial cells and intraepithelial cells, dome epithelium consisted mainly of immature epithelial cells. Findings in the large intestine were similar. Depleted lymphoid follicles were demarcated indistinctly and epithelium was relapsed in varying degrees. FAE was frequently composed of cuboidal immature epithelial cells.

#### Discussion

Whereas symptoms of gastrointestinal disease were not reported to be clinically prevalent, our examinations revealed alterations of intestinal mucosa and of gut associated immune system and the presence of several microorganisms, some of which known to be pathogenic. Main finding was a depletion of GALT of varying degree. An infection with PDV is most likely to be the cause. In the course of Canine Distemper inflammation of the intestinal tract (Potel, 1951; Cornwell et al., 1965; Appel, 1970) as well as depletion of lymphatic tissue and necrosis of lymphatic cells are well known to occur (Stevens & Osburn, 1976; Krakowka et al., 1980). A preexisting immunosuppression of any other origin might have promoted an infection with PDV as well as with other pathogens. The presence of predominantly immature dome epithelial cells in

intestines of PDV-positive seals suggests an increased turnover of FAE in these animals. We believe that PDV and/or other pathogenic organisms may have detrimental effects on FAE.

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### A CASE OF GLOMERULONEPHRITIS IN A DOG RESEMBLING IGA NEPHROPATHY IN MAN

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IgA nephropathy first described by Berger et al. in 1968 is being recognized increasingly as a common form of glomerulonephritis (GN) in humans (D'Amico, 1987). There is little documentation up to now about IgA nephropathy in dogs; high serum IgA values were found in dogs affected with several diseases such as pyoderma, demodicosis, aspergillosis, neoplasia, systemic lupus erythematosus, diabetes mellitus, rheumatoid arthritis, autoimmune haemolytic anaemia, idiopathic thrombocytopenia and Evan's syndrome.

In these subjects large numbers of circulating IgA bearing lymphocytes and a generalized IgA response within the tissues was found (Day and Phenale, 1988). Some of these changes were similar to those reported in Berger's disease of man. Salient clinical and pathological features of the disease in human patients are microhematuria, mesangial proliferative GN with IgA as the predominant Ig deposited, C<sub>3</sub> deposits, smaller amount of IgM and sometimes IgG deposits. The purpose of this study is to describe, in a German Shepherd dog, a case of nephropathy resembling Berger's disease in man.

### Material and methods

Kidneys from a 3-year-old, male, German Shepherd dog affected by *Leishmania infantum* were examined by light and electron microscopy. Immunohistochemistry and biochemical assays on urine and blood samples were also performed.

**Light microscopy:** For light microscopy renal tissue was fixed in 10% formalin buffered solution, embedded in paraffin and sectioned at 2 µm. The sections were stained with Hematoxylin-Eosin, Periodic Acid-Schiff and Periodic Acid Silver Methenamine (PASM). Five µm thick sections were stained with Congo-Red stain.

**Electron microscopy:** For electron microscopy specimens were fixed in 3% glutaraldehyde, postfixed in Osmium Tetroxide and embedded in Araldite. Thin sections were stained with uranyl and lead acetate and examined with an Elmiskope electron microscope.

**Immunohistochemistry:** Immunohistochemistry was performed on paraffin embedded sections using the immunoperoxidase technique of Sternberger. Antibodies used included rabbit anti-dog IgA, IgG, IgM and C<sub>3</sub>. Specificity of labeling was demonstrated by omitting primary antiserum.

**Biochemical assays:** Serum chemistry analysis was performed to detect BUN, creatinine and total serum protein levels. Serum concentrations of IgA, IgG and immune complex were also determined. Urine samples were investigated for sediment analysis and quantitative and qualitative proteinuria was assessed by BioRad protein assay and SDS-PAGE electrophoresis respectively.

### Results

**Clinical findings:** Circulating immuno complex (77%I) and gamma globulin (6.5 g/dl) concentrations, particularly IgA fraction (0.6 g/dl), were significantly increased. Serum albumin level (2.55 g/dl) was decreased while BUN (103 mg/dl) and serum creatinine (1.9 mg/dl) concentrations were increased. Marked glomerular non selective and tubular proteinuria (3.02 g/l), and microhematuria were present.

**Morphological findings:** Kidney lesion was predominantly a mild diffuse mesangial proliferative GN. Mesangium was widened by increased number of mesangial cells and mesangial matrix. A large number of infiltrating cells were found in the interstitium. Both lymphocytes and plasmacells were distributed in a diffuse infiltrate pattern with focal areas of dense cell collections mainly in periglomerular areas. Scattered foci of microscopic tubular hematuria were present. By means of immunohistochemistry IgA deposits were detected together with C<sub>3</sub>, and occasional IgM and IgG deposits, and their distribution was mostly mesangial with the greatest intensity for IgA and C<sub>3</sub> and lesser for the other tested. Light microscopic changes were confirmed by electron microscopy that showed the presence of electron-dense mesangial and paramesangial deposits.

### Discussion

Our results are compatible with the previously reported on IgA nephropathy in humans particularly for the morphological and immunochemical aspect. IgA nephropathy is thought to be an immuno complex disease resulting from a poorly controlled mucosal immune response to environmental antigens to which the host is chronically subjected. Several exogenous antigens such as viruses or diet antigens have been implicated in IgA disease (Emancipator and Lamm, 1989). The case here reported was a dog affected by *Leishmania infantum* infection and a possible relationship between the presence of the parasite and an altered immunological response in the parasi-