BRIEF REPORT

Amyloidosis Associated with Cyclic Neutropenia in the Dog

By N. F. Cheville

THE GRAY COLLIE SYNDROME is a lethal disease associated with hair coat abnormalities, cyclic neutropenia (1) and malabsorption (2). Episodes of fever, septicemia, enteritis, arthrosis and respiratory infection correlate with the cyclic recurrence of neutropenia. Most affected dogs die within a few days of birth, but some survive and succumb in early adulthood to an infectious process. The disease in dogs appears to be analogous in many respects to cyclic neutropenia in man (see reviews by Reimann, 1963, and Lund et al., 1967). Neutropenia in the human disease appears to be due to a defect in maturation of neutrophilic leukocytes at the promelocyte stage. Attempts to incriminate antileukocytic antibodies or hypersplenism have failed and evidence of correlation with estrogen or gonadotropin levels in serum have not been convincing. Although the disease in man is characterized by fever, oral disease, and lymphadenitis, amyloid has not heretofore been described.

Six cases of the gray collie syndrome which survived early puppyhood have been studied in this laboratory. Three, which were not treated clinically, died early in the course of the syndrome and did not have amyloidosis. Three were treated during neutropenic phases with penicillin, streptomycin, and hyperimmune serum. These dogs died after repeated episodes of fever and septicemia and had amyloidosis of the spleen, liver, and kidney. One remaining case is alive and, although in the late stages of the disease, has developed amyloidosis according to splenic biopsy.

The differential leukocyte count pattern in the affected dogs was characterized by neutropenia cycles which average 10.5 days. This is followed by a neutrophilic leukocytosis "rebound" phase and a phase of monocytosis. Preliminary ultrastructural examination of neutrophil leukocytes during the rebound phase has not revealed abnormalities in cell structure. Nuclear segmentation was complete and granules appear normal in size and number (Fig. 1).

The sera of all dogs were slightly hypergammaglobulinemic when examined early in the disease process by the ultraviolet spectrophotometric method for total protein determination and by electrophoretic separation of serum proteins. The sera of both amyloid cases examined at the terminus of their diseases had decreased levels of serum gammaglobulin.

Amyloid in the spleen was deposited around the arterioles in the periarterial
Fig. 1.—Electron micrograph of a neutrophilic leukocyte taken from a dog during an episode of fever and septicemia. Granules appear normal in number and size. Glutaraldehyde fixation and osmium and lead staining were used in preparations illustrated in Figs. 1, 3, and 4.

Fig. 2.—Histologic appearance of amyloid during its formation around the periarterial lymphoid sheath of the spleen. Note macrophages filled with pale hyaline substance (arrow) and dense area of amyloid in upper right. Hematoxylin-eosin.

Fig. 3.—Low power electron micrograph from the spleen near the termination of the arteriole in the marginal zone of the red pulp. Note: erythrocytes (A), endothelial cells (B), and reticular cell (C).

Fig. 4.—Electron micrograph from the spleen of an area of amyloid production. Cytoplasmic organelles from cells on the right have been displaced by amyloid fibrils.

lymphoid sheath (Fig. 2). Lymphoid tissue was displaced by large macrophages filled with a pale hyaline substance which was negative for amyloid. Canine amyloid however, like mouse amyloid, stains weakly in some congo red
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and methyl violet solutions. The histochemical reactions of dense accumulations of amyloid were: congo red, slight staining; alcian blue, negative; periodic acid-Schiff (PAS), positive; methyl violet, positive; thioflavin T, positive; polarization, anisotropic. Marked fluorescence occurred throughout areas of amyloid deposition when frozen sections of spleen were stained with fluorescein conjugated anti-canine globulin antiserum. Fluorescence was most severe around the central artery of the periaerioiolar lymphoid sheath.

Ultrastructural examination revealed amyloid fibrils at the cell surface of large reticular cells (Fig. 3). The cytoplasms contained scattered polyribosomes and small dense granules but only sparse endoplasmic reticulum. Similar cells in a more advanced state of amyloid production were adjacent to large masses of amyloid and were spindle shaped and surrounded by amyloid fibrils (Fig. 4). Endothelial cells although containing lipid droplets did not appear to be contributing directly to amyloid formation.

The "two-phase cellular theory of local amyloid secretion" of Teibum is based upon amyloid formation in situ by fixed RE cells due to secretion of insoluble polysaccharide-containing globulins. An initial pyroninophilic phase is characterized by proliferation of pyroninophilic RE cells and a rise in serum gammaglobulin. The second, or amyloid phase is characterized by development of PAS-positive cells and decrease in gammaglobulin levels. The transition from the pyroninophilic phase to the amyloid phase is dependent upon suppression of proliferating RE cells, either by exhaustion of the immune mechanism following protracted antigenic stimulation or by immunorepressive drugs. The breakdown of immunoglobulin production results in formation of amyloid in situ as glycoprotein products of PAS-positive plasmacytic cells. This theory, however, does not consider the presence of a circulating soluble precursor of amyloid as described by Vasquez and Dixon. Kennedy using autoradiographic techniques described a circulating sulphated glycoprotein formed by proliferating plasma cells which, he believed, formed the insoluble complex amyloid by combination with a second sulphated mucopolysaccharide produced by endothelial cells. Amyloid therefore would be primarily subendothelial in location, yet this did not appear to be true in the splenic amyloid of canine cyclic neutropenia. Circulating precursors of amyloid have not been sought in affected dogs. The morphological changes, however, are compatible with the concept of amyloid formation within proliferating pyroninophilic reticuloendothelial cells.

SUMMARY

Amyloid occurred in the spleen, liver, and kidney after recurrent episodes of fever and infection in dogs affected with cyclic neutropenia (gray collie syndrome). In areas of splenic amyloid formation, large reticular cells contained polyribosomal material and amyloid fibrils, but endothelial cells did not. Early in the disease, the dogs were slightly hypergammaglobulinemic, whereas those examined terminally with amyloidosis had lowered gammaglobulin levels Neutrophilic leukocytes appeared normal when examined by electron microscopy.
SUMMARIO IN INTERLINGUA

Amyloide occurreva in le splen, le hepate, e le renes post recurrente episodios de febre e infection in canes afficite de neutropenia cyclic (syndrome de collie gris). In areas de formation splenic de amyloide, grande cellulas reticular contineva material polyribosomal e fibrillas amyloide sed nulle tal esseva presente in cellulas endothelial. Precocemente in le curso de maladia le canes esseva levemente hypergammaglobulinemic sed le canes con amyloidosis que esseva examine terminalmente habeva reducite nivellos de globulina gamma. Leucocytes neutrophile examine per microscopia electronic pareva esser normal.

REFERENCES

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