A Case of Generalized Amyloidosis Associated With Cyclic Neutropenia

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A case of generalized amyloidosis associated with cyclic neutropenia is presented. A 24-yr-old female with cyclic neutropenia died from intestinal obstruction caused by necrosis and perforation of the small intestine. Post-mortem examination revealed generalized amyloidosis involving almost all organs. Amyloid deposits were prominent, especially in the alimentary tract, kidneys, spleen, and small blood vessels. As has been suggested in gray collie dogs with congenital cyclic neutropenia known to develop secondary amyloidosis in adulthood, an increase of antigenic stimulation during the intermittent bouts of acute infections may be one of the factors responsible for the development of secondary amyloidosis in this case. Although the association of amyloidosis and cyclic neutropenia in man has rarely been described, it is probable that amyloidosis is not a rare complication of human cyclic neutropenia, considering that patients with this hematologic disorder are chronically exposed to excessive antigenic stimulation.

HUMAN CYCLIC NEUTROPENIA is an unusual hematologic disorder characterized by episodes of neutropenia periodically occurring at approximately 21-day intervals. During the neutropenic periods, patients generally have symptoms such as fever, malaise and aphthous stomatitis, and occasionally suffer from headache, abdominal pain, and lymphadenitis.

In 1967, Lund et al. reported that cyclic neutropenia is present also in gray collie dogs and suggested that these dogs might serve as a model for studying cyclic neutropenia in man. Extensive studies on the mechanism of cyclic neutropenia in humans or in dogs have shown that cyclic fluctuations of neutrophil counts are the result of periodically occurring marrow failure in neutrophil production, and that canine cyclic neutropenia is caused by a stem cell defect and can be successfully treated by bone marrow transplantation.

In canine cyclic neutropenia, high incidence of secondary amyloidosis in adult dogs has been reported by Cheville et al. and Gregory et al. Although human cyclic neutropenia is similar to canine cyclic neutropenia in many respects, the report of Jennings et al., as far as we know, seems to be the only one describing the association of amyloidosis and cyclic neutropenia in man. However, neutropenia in their case probably did not fall neatly into the category of cyclic neutropenia in a strict sense, as discussed later in this article. The purpose of this report is to present a case of secondary amyloidosis associated with “typical” cyclic neutropenia in man.
CASE REPORT

The patient, T. M., was a 24-yr-old Japanese female who had been the subject of previous studies. She was first admitted to the Yamaguchi University Hospital on September 1, 1973, for the evaluation of periodic fever with aphthous stomatitis, gingival swelling, abdominal pain, and diarrhea.

At the age of 2 wk she had generalized impetigo and thereafter had recurrent fevers and infections. At the age of 6 yr, her parents became aware that her fever appeared periodically at intervals of about 3 wk and lasted for several days.

Physical examination at the time of admission revealed no remarkable findings except for moderate tenderness in the ileocecal region. Routine laboratory examinations revealed moderate iron-deficiency anemia, leukopenia, and normal platelet count. Serum protein level was 7.4 g/dl, and electrophoresis showed an albumin of 53.8%, alpha-1 globulin 2.3%, alpha-2 globulin 8.6%, beta globulin 8.1%, and gamma globulin 27.6%. A quantitative analysis of serum immunoglobulins revealed an IgG of 2150 mg/dl, IgA 375 mg/dl, and IgM 290 mg/dl (normal ranges 900-1700, 120-400, and 40-160, respectively). No abnormal serum immunoglobulins were detected by immunoelectrophoresis. Serologic tests revealed no autoantibodies. LE cells were negative. Urinalysis was normal, and other laboratory data were within normal limits.

Peripheral blood was examined every 2-3 days over a 6-wk period. Neutrophil counts regularly fluctuated with approximately 21-day periodicity (Fig. 1). During the neutropenic periods, neutrophils completely disappeared from the peripheral blood for 3-5 days. Serial bone marrow studies (five marrow samples during one cycle) revealed cyclic fluctuations in the number of neutrophil precursors, suggesting the periodic marrow failure in neutrophil production. These serial examinations of the peripheral blood and bone marrow led to the diagnosis of cyclic neutropenia. During the neutropenic periods, the patient developed aphthous stomatitis, gingivitis, lymphadenitis, arthritis, or enteritis. The level of alpha-2 globulin usually rose during the corresponding periods. On March 23, 1974, she was discharged on 45 mg/day of oxymetholone to be followed as an outpatient. Oxymetholone was discontinued 9 mo later without any improvement, and thereafter, folic acid and pyridoxine were orally given.

Over the next 2 yr, no improvement was noted in her symptoms and blood picture. Repeated...
laboratory examinations revealed mild hypoalbuminemia and moderate hypergammaglobulinemia. Urinalysis revealed only a small amount of protein occasionally.

She was admitted again on January 5, 1977, with a 4-mo history of epigastralgia, lumbago, and marked anorexia in addition to her usual periodic symptoms. Physical examination revealed no remarkable findings except for a markedly atrophied gingiva. Laboratory examinations showed moderate anemia and leukopenia. Serum protein was 7.4 g/dl, and electrophoresis disclosed an albumin of 43.8%, alpha-1 globulin 4.5%, alpha-2 globulin 8.9%, beta globulin 11.0%, and gamma globulin 31.7%. The concentrations of serum immunoglobulins were as follows: IgG 2100 mg/dl, IgA 290 mg/dl, and IgM 310 mg/dl. While blood urea nitrogen was normal (8.0 mg/dl) and urinalysis revealed only a small amount of protein (less than 30 mg/dl), decreased creatinine clearance (39.5 ml/min) suggested impaired renal function. Examinations of the upper gastrointestinal tract were not diagnostic, and orthopedic examinations failed to disclose any disorders of the bone responsible for lumbago.

Four weeks after admission, epigastralgia and lumbago began to occur even in a non-neutropenic period. Creatinine clearance at that time had decreased to 15–22 ml/min, though blood urea nitrogen remained within normal limits. Two weeks later, she was started on 60 mg/day of prednisolone that was intermittently administered orally for several days corresponding with the recovery phase from neutropenia, i.e., during the period when neutrophil production was considered to cease. After the initiation of treatment with prednisolone, the mode of fluctuation in neutrophil counts showed some alteration. Neutrophil counts stopped falling to zero, and after the third treatment, the duration of the cycle shortened slightly (Fig. 2). Concentrations of serum immunoglobulins gradually decreased. After the second treatment, IgG was 1200 mg/dl, IgA 120 mg/dl, and IgM 190 mg/dl. During the prednisolone treatment, the patient continued to have fever and gastrointestinal symptoms. At the beginning of March, severe vomiting and diarrhea developed, lasting through the neutropenic period. These symptoms reappeared in the next neutropenic period and continued thereafter. On April 18, 1977, symptoms of shock developed but soon subsided, responding to an adequate emergency care. The administration of prednisolone was discontinued thereafter. Regardless of careful supportive therapy, hypoalbuminemia progressed, and the elevation of blood urea nitrogen developed. Since the time of admission, there had been no change in the amount of urinary protein. On May 20, hematemesis and tarry stool appeared. Because she continued to have severe vomiting and bloody diarrhea, a laparotomy was performed on May 23, under the diagnosis of intestinal obstruction.
At surgery, a necrotic lesion measuring 50 cm in length was found in the ileum. A perforation of 3 cm in diameter, considered to be the cause of the previous shock, was located near the midpoint of the lesion, being completely covered with the omentum. The intestinal lumen was obstructed at the necrotic portion by a blood clot. Ninety centimeters of ileum was resected with an end-to-end anastomosis. The next morning, however, shock developed, and the patient died. The clinical course and daily neutrophil counts during the second hospitalization are shown in Fig. 2.

Post-mortem examination revealed multiple erosion and bleeding along the alimentary tract. The liver and spleen were enlarged, weighing 1900 g and 220 g, respectively. The weight of each kidney was about twice normal.

Histologic examination revealed marked deposits of eosinophilic, amorphous material in the spleen, lymph nodes, entire alimentary tract, liver, bone marrow, kidneys, heart, pancreas, thyroid, uterus, gallbladder, and blood vessels in all organs. Histochemical reactions of these deposits, including Congo red, periodic acid-Schiff, and thioflavin T, were consistent with amyloid. Amyloid deposition was marked, especially in the alimentary tract (Fig. 3), kidneys (Fig. 4), spleen and small peripheral blood vessels.

Tissue sections from the stomach, small intestine, spleen, and kidneys were subjected to the potassium permanganate reaction according to the method of Wright et al.15 The sections were then stained with alkaline Congo red and examined with conventional and polarizing microscopy. The amyloid deposits had lost Congo red affinity, and so it was suggested that the major component of amyloid was protein AA. These findings were consistent with the diagnosis of secondary amyloidosis.

DISCUSSION

In the present case, cyclic neutropenia had been present since childhood, and the patient had had recurrent episodes of infections during the neutropenic periods. Although she had serious infections and required antibiotic therapy several times in childhood, most infections in her adulthood were not life-threatening and were well tolerated. Post-mortem examination revealed that the cause of death was generalized secondary amyloidosis. Amyloidosis in the gastrointestinal tract resulted in
malabsorption and finally led to the necrosis and perforation of small intestine. Prominent deposition of amyloid in the kidneys resulted in impaired renal function, i.e., markedly decreased creatinine clearance and gradually elevated blood urea nitrogen in the terminal stage. Lesions of other organs, such as thyroid gland, pancreas, and heart, were also marked, though clinical manifestations were not yet apparent.

High incidence of amyloidosis in adult gray collie dogs with congenital cyclic neutropenia has been reported by Cheville et al.\textsuperscript{9,10} and Gregory et al.\textsuperscript{11} These dogs are periodically predisposed to infectious disease during the neutropenic period, i.e., enteritis, septicemia, arthrosis, and respiratory infection.\textsuperscript{3,9,10} Most affected dogs die within a few days of birth, but some survive and succumb in early adulthood to an infectious process.\textsuperscript{3,9,10} Cheville et al.\textsuperscript{10} and Gregory et al.\textsuperscript{11} carried out histopathologic studies on gray collie dogs and found that secondary amyloidosis developed in all dogs that survived to adulthood.

In gray collie dogs, a deficient development of lymphoid organs has been found in addition to other inherited abnormalities.\textsuperscript{11} Gregory et al.\textsuperscript{11} postulated that genetically defective lymphoid system and an increase in antigenic stimulation appear to be relevant to the etiology of amyloidosis in these dogs.

In the present case, moderate polyclonal hypergammaglobulinemia, as reported by some authors,\textsuperscript{2,3,9,10,16} was noted and considered to be the result of immunologic response to increased antigenic stimulation accompanying the recurrent infections. It is probable that excessive antigenic stimulation over a long period (more than 20 yr) caused amyloidosis, in combination with some masked immunologic abnormalities which, if present, could not be revealed by routine laboratory examinations.
Another factor that might have affected the course of amyloidosis in this case was the prednisolone administration. Various studies dealing with the effect of corticosteroid on amyloid formation in experimental animals have produced conflicting results. Some authors have reported that corticosteroid accelerates the amyloid deposition,\textsuperscript{17,19} while others have demonstrated that it has no effect\textsuperscript{20,22} or retards the progress of amyloidosis.\textsuperscript{20,23} It also has been reported that corticosteroid prevents amyloid formation when administered before amyloidogenesis\textsuperscript{23} or in large doses.\textsuperscript{24} There also have been conflicting reports concerning the effect of corticosteroid therapy on human amyloidosis.\textsuperscript{18,25} It has been assumed that the effect of corticosteroids in experimental amyloidosis may be related to dose, time of administration, species, and the inducing agents.\textsuperscript{20,21} In the present case, symptoms and abnormal laboratory data (gastrointestinal symptoms, renal dysfunction, etc.) that were retrospectively shown to be caused by amyloidosis had become more marked with time prior to the initiation of prednisolone therapy. Thus, it is reasonable to consider that amyloidosis had already been present and had progressed gradually prior to the start of steroid therapy, though the possibility that prednisolone might have influenced the course of amyloidosis cannot be excluded.

Although human cyclic neutropenia and canine cyclic neutropenia are similar in many respects,\textsuperscript{3} the association of amyloidosis and cyclic neutropenia in man has rarely been described. The spleen is one of the most frequently involved organs in secondary amyloidosis, but amyloid deposits have not been found in spleens removed from patients with cyclic neutropenia.\textsuperscript{2,26-29} Jennings et al.\textsuperscript{12} have described a patient with chronic granulocytopenia associated with vasculitis and amyloidosis. During the 22-yr follow-up, the patient showed various patterns of neutropenia, sometimes cyclical and sometimes almost agranulocytic. In addition to the hematologic disorder, collagen disease developed. These two disorders seemed to be closely related to each other and promptly and satisfactorily responded to steroid therapy with a following remission of 2-yr duration. Bone marrow examinations revealed the depletion of granulocyte series on one occasion, but were normal at other times even in neutropenic periods. The case of Jennings et al. is quite different from our present case in the following two respects: (1) there was an association of collagen disease, which by itself might cause secondary amyloidosis, and (2) neutropenia probably did not fall neatly into the category of cyclic neutropenia in a strict sense. Thus, the case presented in this article seems to be the first case of amyloidosis associated with "typical" cyclic neutropenia in man. It is, however, probable that amyloidosis is not a rare complication in human cyclic neutropenia, considering that patients with this condition are chronically exposed to excessive antigenic stimulation.

It is suggested that amyloidosis can be reversible, at least in some cases of the secondary type, if the preceding infection and the concomitant antigenic stimulation can be removed.\textsuperscript{30} Therefore, amyloidosis in cases with cyclic neutropenia may be prevented if cyclic neutropenia can be corrected. It has been shown that canine cyclic neutropenia can be corrected by bone marrow transplantation.\textsuperscript{7} Although it has not been clarified whether amyloidosis in gray collie dogs is reversible or not, amyloidosis in these dogs may be prevented by bone marrow transplantation in puppyhood. Various therapies have been tried to correct the hematologic abnormalities of human cyclic neutropenia without satisfactory effect.\textsuperscript{2,26-29, 31-33} In the
present case, intermittent administration of prednisolone seemed to be effective in correcting the blood picture, though a long-term observation was interrupted by death. During the preparation of this manuscript, Wright et al.34 reported that alternate-day prednisolone administration corrected human cyclic neutropenia in one case. If the treatment with prednisolone had been started early enough in this case, the development of amyloidosis might have been prevented. Further experimental studies in gray collie dogs concerning the effect of corticosteroid on both cyclic neutropenia and amyloidosis are necessary to verify the clinical usefulness of this drug in human cyclic neutropenia.

REFERENCES

1. Reimann HA, deBerardinis CT: Periodic (cyclic) neutropenia, an entity: A collection of sixteen cases. Blood 4:1109, 1949
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T Shiomura, Y Ishida, N Matsumoto, K Sasaki, T Ishihara and S Miwa