The Development of a Myeloma-Like Condition in Mink with Aleutian Disease

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Multiple myeloma in man and the transplantable plasmacytomas in mice are the best studied malignant neoplasms of the plasma cell system. These tumors are generally associated with an increased production of one or more species of immunoglobulins. The increased globulin retains its specific qualitative characteristics throughout the course of the disease in man and through many generations of transplantation in the mouse.

Aleutian disease of mink is characterized by an extreme systemic proliferation of plasma cells without frank tumor formation. Secondary to this plasma cell proliferation, there is marked hyperglobulinemia, and renal, arterial and hepatic lesions are commonly found. The disease is readily transmissible to normal mink by cell free extracts of affected mink tissue, and the infectivity is sedimentable by ultracentrifugation, strongly suggesting a viral etiology.

The increased gamma globulin in Aleutian disease is usually quite heterogeneous in zone electrophoresis. The purpose of this paper is to report the transition from an electrophoretically heterogeneous hypergammaglobulinemia to a homogeneous myeloma-like hypergammaglobulinemia in mink affected with Aleutian disease.

Materials and Methods

Ranch raised mink were obtained from the Utah Mink Growers Cooperative.

Aleutian disease virus was prepared by homogenizing spleens from affected mink in isotonic phosphate buffered saline to make a 30 per cent suspension, centrifuging twice at 2400 g. for 20 minutes, retaining the supernatants. The virus stock was stored at −70 C. Mink with normal electrophoretic patterns were inoculated intraperitoneally with 2 ml. of the fluid.

Zone electrophoresis on paper and cellulose acetate was performed (Model R and R-100 systems, Beckman Instruments, Inc., Palo Alto, California) using barbital buffer pH 8.6, µ = 0.075. Strips were stained with bromphenol blue or Ponceau S and scanned (Analytrol, Beckman Instruments, Inc., Palo Alto, California). Immunoelectrophoresis employed 2 per cent agar (Ionagar #2, Consolidated Laboratories, Chicago, Illinois) in µ = 0.04 barbital buffer, pH 8.5, the slides were electrophoresed 2 to 2½ hours at 5 volts/cm. (Agafor-Gerat apparatus, Egaton AG, Berne, Switzerland) developed 48 to 72 hours with antiserum and photographed without staining.

Total serum proteins were determined with a refractometer.

Urine samples were tested for Bence Jones protein by the method of Putnam and co-workers. Urine was also concentrated by ultrafiltration and electrophoresis and immunoelectrophoresis performed as above. One concentrated urine was examined in the

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analytical ultracentrifuge (Model E, Beckman Instruments, Inc., Palo Alto, California) and the photographic plates measured with a microcomparator.

Normal mink gamma globulin was prepared by elution of serum protein insoluble in 50 per cent saturated ammonium sulfate from diethylaminoethyl cellulose using 0.0175 M sodium phosphate buffer, pH 7.0.

Antiserums to whole mink serum and gamma globulin were prepared in rabbits by 3 weekly injections of antigen in complete Freund’s adjuvant, followed by 12 subcutaneous injections of antigen in saline.

RESULTS

Of 25 mink naturally infected with the Aleutian disease agent on a ranch, and serially followed for a year or more with serum protein electrophoresis, 2 showed marked narrowing of the initially broadly elevated gamma globulin component, 5 and 9 months after the initial electrophoretic observation and an unknown time after the onset of Aleutian disease. Paper electrophoretic patterns of normal mink serum and mink before and after development of the narrow band is shown in figures 1 A-E.

Thirty adult mink were inoculated with a single preparation of the infectious agent and all started to show a heterogeneous elevation of their gamma globulin within 30 days. Three months after inoculation, 4 of the mink began showing narrowing gamma globulin patterns. The narrowing of the gamma globulin peak in zone electrophoresis is not dependent on concentration of the serum, since it persisted unchanged at 1:2 and 1:4 dilutions of the serum.

Sixteen further examples of myeloma-like hyperglobulinemia were found in a survey of 859 ranch mink with histlogic Aleutian disease, where the incidence of myeloma-like gamma globulin was 1 of 727 in mink 7 or 8 months old and 15 of 132 mink 19 or more months old. This indicates that the likelihood of myeloma-like globulin developing from multiclonal Aleutian disease is much greater in older mink, presumably those with a longer duration of the disease. Two examples of the paper electrophoretic patterns of this group of mink are shown in figures 1F and G.

Immunoelectrophoresis of the serum from mink with the myeloma-like hyperglobulinemia shows a narrow heavy bow of gamma globulin precipitation closer to the antibody trough than usual, and in most cases, a splitting of the gamma globulin precipitin arc, indicating that the paraprotein does not have all the antigenic determinants found in normal pooled mink gamma globulin. The position of the paraprotein in each of the myeloma-like mink serums is slightly different, but characteristic for that particular animal. Several examples of immunoelectrophoresis of the myeloma-like mink serums are shown in figure 2.

Five of the mink were tested for the presence of Bence Jones proteinuria, and all showed precipitation of a protein at 56 C with complete or partial solution of the precipitate at 100 C. Zone electrophoresis of the concentrated urine showed a protein with the mobility of gamma globulin in all cases, and the mobility was different from that of the myeloma-like globulin in the serum. The daily urinary protein loss ranged from 10 to 125 mg. per day in these animals, 75-95 per cent of which was Bence Jones protein. The 1 urine
Fig. 1.—(A) Paper zone electrophoresis of a normal mink serum for comparison with other patterns: 7.1 Gm./100 ml. total serum protein, 11 per cent γ-globulin. (B) Serum from a mink with Aleutian disease showing a heterogeneous elevation of the γ-globulin: 9.2 Gm./100 ml. total serum protein, 75 per cent γ-globulin. (C) Serum from the same mink as in figure 1B after the development of a homogeneous myeloma-like elevation of the γ-globulin: 11.1 Gm./100 ml. total serum protein, 72 per cent γ-globulin. (D) Serum from a mink with Aleutian disease showing a heterogeneous elevation of the γ-globulin: 8.8 Gm./100 ml. total serum protein, 49 per cent γ-globulin. (E) Serum from the same mink as in figure 1D after the development of a homogeneous myeloma-like elevation of the γ-globulin and the presence of Bence Jones proteinuria: 13.2 Gm./100 ml. total serum protein, 74 per cent γ-globulin. (F) Serum from a mink with homogeneous myeloma-like γ-globulin of beta mobility: 12.1 Gm./100 ml. total serum protein, 79 per cent γ-globulin. (G) Serum from a mink with homogeneous myeloma-like γ-globulin of slow γ mobility: 10.1 Gm./100 ml. total serum protein, 61 per cent γ-globulin.

examined in the analytical centrifuge showed 2 sedimentation peaks of $S_{20,w} = 1.6$ and 3.4, values in the range observed for human Bence Jones proteins.

Five of the mink were examined by x-ray for the presence of skeletal lesions, and none were noted. Three of these mink were sacrificed for gross and histologic examination, and they showed extensive characteristic lesions of Aleutian disease. No focal bone lesions were noted in multiple sections, although plasma cells were diffusely present in the bone marrow in large numbers. No difference between the tissue lesions of the 3 mink with the myeloma-like globulin and mink with a heterogeneous hypergammaglobulinemia were noted.
Fig. 2.—(A) Immunoelectrophoresis of same serum sample as figure 1D showing electrophoretically heterogeneous γ-globulin arc. Developed with rabbit antimink serum. (B) Same serum sample as figure 1E and same mink as figure 2A after the development of a myeloma-like γ-globulin showing a localized dip of the precipitin arc toward the antibody trough and doubling of the precipitin arc. Developed with rabbit antimink serum. (C) Same as figure 2A developed with rabbit antimink γ-globulin showing electrophoretically heterogeneous γ-globulin. (D) Same as figure 2B developed with rabbit antimink γ-globulin showing the dip and doubling of the precipitin arc in more detail. (E) Same serum as figure 1G showing the dip and doubling of the precipitin arc of a mink myeloma-like γ-globulin of slow γ mobility. Developed with rabbit antimink γ-globulin. (F) Same serum as figure 1F, showing the dip and doubling of the precipitin arc of a myeloma-like γ-globulin of beta mobility. Additional arc indicated with arrow may represent Bence Jones protein in the serum. Developed with rabbit antimink γ-globulin.
DISCUSSION

Multiple myeloma in man is a disease of unknown etiology. Diffuse plasmacytosis is known to occur with a variety of acute and chronic infectious diseases, but a transition from an infectious type of plasmacytosis to multiple myeloma has not been reported. Apparently no consistent antecedent history of infectious disease or other hypergammaglobulinemic condition can be elicited from patients with multiple myeloma.\(^3,4\)

Mouse plasmacytomas have been observed to develop spontaneously, or after a number of different experimental procedures, usually of a phlogogenic nature.\(^12,13\) The mouse tumors have been serially transplantable, but not transmissible by cell free materials. No isolation of a viral agent from these tumors has been achieved, although Howatson and McCulloch\(^14\) have observed virus-like particles in a line of a mouse plasmacytoma using the electron microscope.

Aleutian disease of mink is clearly infectious using cell-free materials from affected mink, and the infectious material is sedimentable by ultracentrifugation,\(^19\) strongly suggesting a viral etiology. This infectious disease initially results in a heterogeneous hypergammaglobulinemia. After a period of 3 months to a year, over 10 per cent of the affected mink showed a transition from the heterogeneous form of hypergammaglobulinemia to a myeloma-like condition differing from those in the human and mouse only by the lack of frank tumor formation.

Human multiple myeloma serum protein nearly always shows a narrow, apparently homogeneous globulin band upon paper zone electrophoresis of the serum protein of the affected patient. This narrow paraprotein band is in distinct contrast to the electrophoretic heterogeneity seen in non-neoplastic hypergammaglobulinemic conditions such as sarcoidosis, thyroiditis, lupus erythematosus and rheumatoid arthritis. Rundles and co-workers\(^2\) and Osserman\(^3,4\) have shown that an individual's myeloma protein retains its specific electrophoretic characteristic throughout the course of the disease. Immunochemical studies of a number of different serially transplantable mouse plasmacytomas done by Fahey\(^8\) and by Clausen and co-workers\(^5\) indicate that each tumor line is associated with a serum or urinary paraprotein which remains characteristic for that particular tumor throughout many generations of serial transplantations.

Although the individual human and mouse paraproteins appear to be homogeneous in paper zone electrophoresis and Tiselius free-cell electrophoresis, Fahey\(^15\) and Franglen\(^16\) have shown a limited degree of electrophoretic and polymer types of heterogeneity in human myeloma proteins, and Fahey\(^17\) has noted similar findings with the mouse paraproteins when examined by starch gel electrophoresis. The limited type of heterogeneity has been interpreted by Fahey as being compatible with the hypothesis that the myeloma proteins are derived from closely related clones of plasma cells, possibly derived from a single parent clone, or that the closely related proteins are derived from a single clone of malignant cells.

In contrast to the myeloma proteins in humans which are apparently
homogeneous from onset of the disease, the gamma globulins of mink affected
with Aleutian disease are initially heterogeneous, indicating that the viral
agent must be affecting a number of clones of plasma cells. This is similar to
the situation in the mouse described by Talal and co-workers\textsuperscript{13} where the
plasmacytoma inducing treatment causes either hyper- or hypogammaglobu-
linemia with increased heterogeneity of the gamma globulin before the
development of the plasmacytomas. Late in the course of Aleutian disease,
some mink show a transition from a heterogeneous hypergammaglobulinemia
to a homogeneous myeloma-like hypergammaglobulinemia together with ex-
cretion of Bence Jones protein in the urine, suggesting the ascendency of one,
or a few, related clones of plasma cells. Whether or not this observed transi-
tion from a multiclonal to a monoclonal plasma cell proliferation represents a
conversion of a hyperplastic to a neoplastic disease of viral etiology in these
mink is a matter of definition.

SUMMARY

Aleutian disease of mink, apparently of viral etiology, is characterized by
an extreme diffuse proliferation of plasma cells, and a marked hypergamma-
globulinemia, which is electrophoretically heterogeneous. In some mink with
this disease serially followed by serum protein electrophoresis, a transition to
a homogeneous myeloma-like hypergammaglobulinemia, together with the
appearance of Bence Jones proteinuria, was observed late in their course. Thus,
this viral disease shows plasma cell proliferation beginning as a multiclonal
hyperplasia which may terminate as a monoclonal, possibly neoplastic over-
growth of the plasma cell system.

SUMMARIO IN INTERLINGUA

Morbo aleutian de visones, apparentemente de etiologia virusal, es char-
acterisate per un extreme proliferation diffuse de plasmocytos e un marcate
hypergammaglobulinemia le qual es electrophoreticamente heterogenee. In
certe visones con iste morbo, le quales esseva studiate serialmente per electro-
phorese del proteinas seral, un transition esseva notate tardivemente in le
curso del morbo ad un homogenee hypergammaglobulinmia myeloma-simile
insimul con le apparition de proteinuria de Bence Jones. Assi iste morbo
virusal monstra un proliferation plasmocytic comenciante como hyperplasia
multiclonal que pote terminar se in un hypercrescentia monoclonal e possibile-
mente neoplastic del sistema plasmocytic.

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