

EXPERIMENTAL HYPERGAMMAGLOBULINEMIA IN MINK*

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(Received for publication, May 2, 1962)

Naturally occurring Aleutian disease (AD) of mink is characterized by hypergammaglobulinemia and lesions resembling many of those described in hypersensitivity or autoimmune diseases (1, 2, 7). There are segmental vasculitis; fibrinoid deposition in arterial walls and glomeruli; and marked perivascular and parenchymal lymphocyte and plasma cell infiltration. Henson *et al.* (1) reported absolute increases in serum gamma globulin and total protein and decreased albumin in mink with the natural disease. Studies of mink on an affected ranch have shown a familial occurrence of hypergammaglobulinemia (3).

Mink homozygous recessive for the Aleutian gene symbolized as (*aa*) are the usual genotype spontaneously affected, but the disease occasionally occurs in those which are heterozygous (*Aa*) or homozygous dominant (*AA*). When these genotypes are present on an affected ranch, the incidence is highest in *aa* mink.

Field observations suggest a marked increase of AD particularly in *aa* mink following the use of formalinized homologous tissue vaccines (2, 4).

Thus, it would appear that AD may be a hypersensitivity or autoimmune disease with an apparent genetic as well as familial occurrence. There is also a possibility that mink, especially *aa* mink, may be unusually susceptible to certain antigenic stimuli. The investigations reported here were conducted to: (*a*) produce experimental hypergammaglobulinemia in mink and determine whether there is a genetic predilection for its occurrence; (*b*) investigate the effect of formalin treatment of affected tissue suspensions on the experimental production of the disease; (*c*) determine the intensity of circulating gamma globulin following antigenic stimulation from various sources; (*d*) determine whether formalin treatment of normal tissues from different genotypes alters

*Aided in part by a grant from the Mink Farmers' Research Foundation, Washington Agricultural Experiment Station, Scientific Paper 2218 and by The National Institutes of Health Training Grant 2G-414, Division of General Medical Sciences and U. S. Public Health Service Grant A5400.

the tissue constituents sufficiently to evoke hypergammaglobulinemia when these tissues are injected into homologous and heterologous genotypes.

Materials and Methods

The 147 mink used in these trials were from our own herd in which spontaneous AD has not occurred during a 10-year observation period. These animals were selected at random as to sex and were approximately 2 months of age when these trials were initiated. The animals were paired or maintained individually within treatment groups. They were fed a commercial type mink ration and were not vaccinated. The genotypes were assessed by coat color and by pedigree (5).

Blood samples were taken from a lingual vessel (3) prior to inoculation, at monthly intervals during the investigations and at the termination of the trials. Only the preinjection samples

TABLE I
Total Number of Animals in Each Genotype and Treatment and the Number Developing Hypergammaglobulinemia

Group	Treatment	Recipient genotypes (affected/No. injected)			Total No. affected/total No. injected
		aa	Aa	AA	
A	Formalin-affected aa, 40 wks.	0/8	0/7	0/8	0/23
B	Formalin-normal aa, 2 wks.	0/8	0/7	0/8	0/23
C	Formalin-normal AA, 2 wks.	0/8	—*	0/8	0/16
D	Non-formalin affected aa	8/8	3/7	4/8	15/23
E	Bovine serum albumin, 5 per cent	0/8	—*	0/8	0/16
F	Non-injected controls	0/7	0/7	0/8	0/22
G	Feline tissue, 10 per cent	0/7	—*	0/4	0/11
H	Formalin-affected aa, 2 wks.	5/5	—*	3/8	8/13

* Insufficient numbers of Aa mink were available to include this genotype in these groups.

and the final serum samples were used in this paper. The gross and histologic changes will be reported at a later date.

Filter paper electrophoresis was carried out as previously described (1). Duplicate samples checked periodically revealed a very close reproducibility of the patterns.

The affected tissues used for injection were from aa mink with the natural disease obtained from a commercial ranch. Normal mink tissues were collected from animals in our own herd. In all instances, the spleens, kidneys, and livers were pooled, ground in a Waring blender and diluted to a 10^{-1} suspension with PSS. The non-formalinized-affected tissue suspension was maintained at -26°C until used. The formalinized tissue suspensions were prepared according to Hummon and Bushnell (9). Sufficient formalin was added to give a final concentration of 0.3 per cent. The formalinized suspensions were divided and held at 5°C for 2 weeks prior to injection. Part of the formalinized affected tissue suspension was maintained under the same conditions for 40 weeks. The remaining inocula consisted of bovine serum albumin (5 per cent, Armour) and 10^{-1} feline pooled tissue suspension prepared from spleen, liver, and intestine.

All mink were given 3 subcutaneous injections consisting of 2 ml each of inoculum at 2-week intervals. The experiments were terminated 4 months after the initial injections. The treatment groups, number injected, and genotypes are given in Table I.

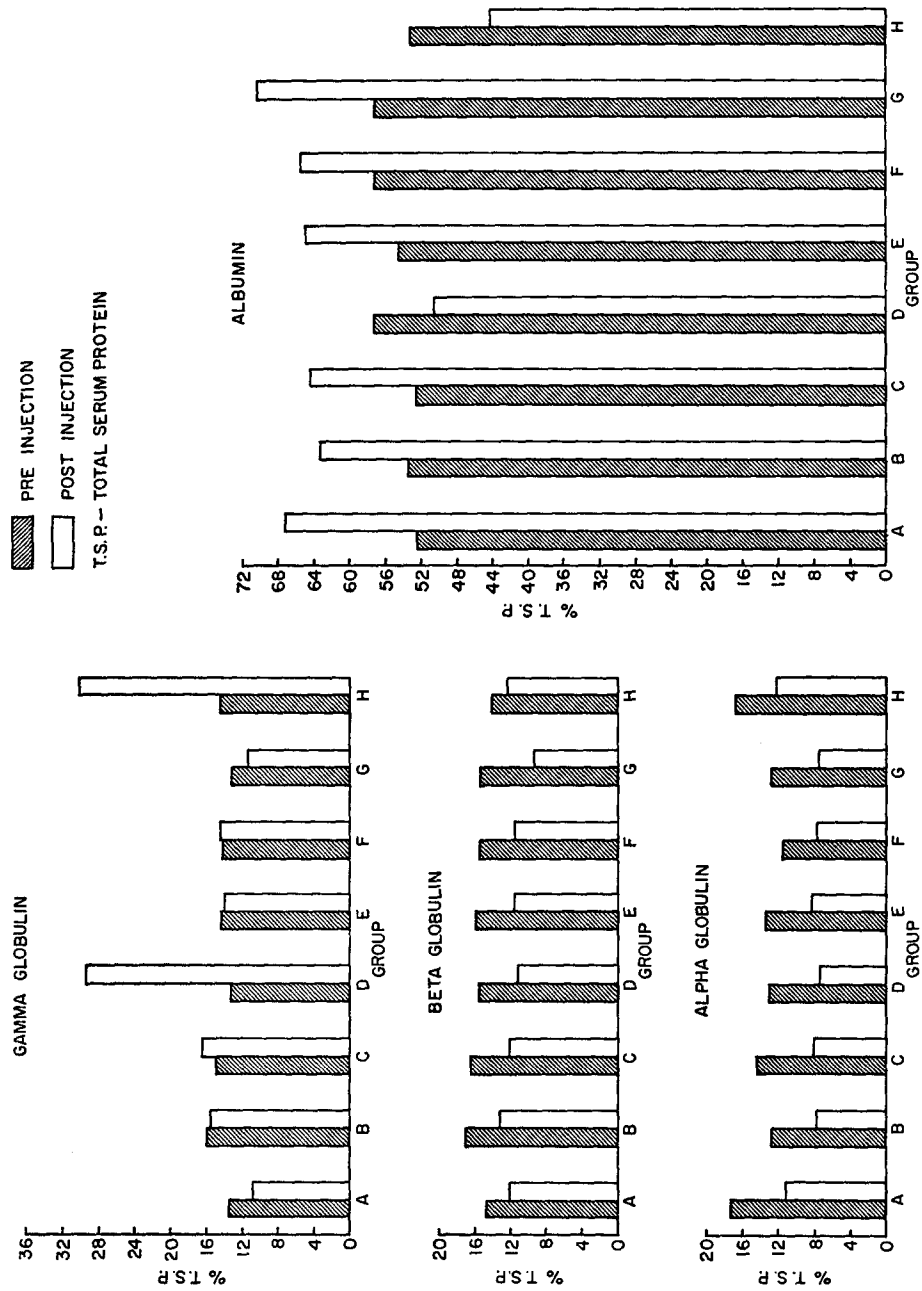


FIG. 1. The mean pre and post-injection serum protein values expressed as percentage of total serum proteins according to treatment groups in the 8 treatment-2 genotype (*aa AA*) analysis.

Statistical analyses were carried out on the data presented here. The percentage figures were converted to arcsin values so that they could be analyzed (10). Analysis of variance was used to determine the treatment and genotype effects as well as their interaction. The values for the albumin and alpha, beta and gamma globulin fractions were computed separately as an 8

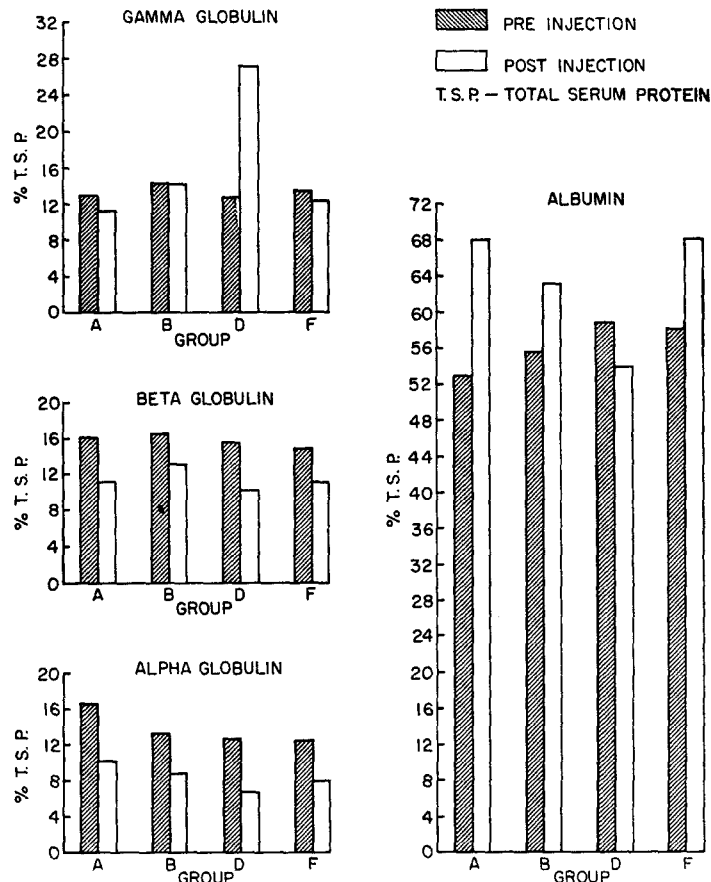


FIG. 2. The mean pre- and post-injection serum protein values expressed as percentage of total serum proteins according to treatment groups in the 4 treatment (A, B, D, F)-3 genotype (*aa*, *Aa*, *AA*) analysis.

treatment-2 genotype (*aa* and *AA*) analysis and as a 4 treatment (Groups A, B, D, and F)-3 genotype (*aa*, *Aa* and *AA*) analysis. The preinjection and the final serum protein values were compared on the basis of Least Significant Difference (LSD) (10).

RESULTS

The results are given as mean percentage of the total serum proteins in Figs. 1 and 2. The preinjection and final serum protein values for all the animals

within each treatment group are given separately for the four major serum protein fractions.

The results of the statistical analyses are given in Table II. Because of treatment effect, a statistically significant ($P < 0.01$) increase in gamma globulin and decrease in albumin were shown in groups D and H. A treatment-genotype effect was also demonstrated with a significant ($P < 0.01$) increase in gamma globulin in genotype *aa* as compared to the other genotype(s). The number of animals by genotypes in each treatment group that developed hypergamma-globulinemia is given in Table I.

TABLE II
Summary of Statistical Analyses

Analysis	Gamma globulin	Beta globulin	Alpha globulin	Albumin
8 Treatment-2 genotype (A B C D E F G H)(<i>aa</i> , <i>AA</i>)	$P < 0.01$ Sig. increase D H			$P < 0.01$ Sig. decrease D, H
4 Treatment-3 genotype (A B D F) (<i>aa</i> , <i>Aa</i> , <i>AA</i>)	$P < 0.01$ Sig. increase D		$P < 0.01$ Sig. decrease <i>AA</i> , <i>Aa</i> genotype effect	$P < 0.01$ Sig. decrease D
Treatment-genotype interaction	$P < 0.01$ Sig. increase <i>aa</i> -D, H		$P < 0.01$ Sig. decrease <i>AA</i> -A, D <i>Aa</i> -A, D	$P < 0.05$ Sig. decrease <i>aa</i> -H
Least significant difference (pre-injection vs. post-injection)	$P < 0.05$ Sig. increase <i>aa</i> -D, H <i>Aa</i> -D <i>AA</i> -D, H	$P < 0.05$ Sig. decrease 8 × 2 analysis <i>aa</i> -G 4 × 3 analysis <i>aa</i> -D, F <i>Aa</i> -A, D		$P < 0.05$ Sig. increase <i>aa</i> -A, G <i>Aa</i> -A, F <i>AA</i> -ABCF Sig. decrease <i>aa</i> -D, H

The finding of statistically increased albumin in the final serum samples in almost all groups except D and H may be associated with normal protein metabolism as a function of age. It was noted that in groups D and H the albumin was significantly decreased. The latter is consistent with the other changes reported here and in the natural disease. The alpha globulin values show a definite trend toward lower values in the final serum samples.

DISCUSSION

These trials reveal that hypergamma-globulinemia in mink can be evoked by the injection of affected tissue suspensions and a formalin-treated affected tissue suspension held for 2 weeks, but not when held for 40 weeks. The number of mink developing hypergamma-globulinemia varied between the different genotypes. The number of *aa* mink developing serum protein changes was significantly higher than the *AA* and *Aa* genotypes. These findings are similar to

epizootiologic observations on the predominance of the spontaneous disease in *aa* mink. Field observations indicate, however, that when AD becomes established in a herd of *AA* or *Aa* mink, the incidence will increase, thus overriding the genetic influence.

It seems possible that the gene for Aleutian coat color is not directly responsible for AD susceptibility. This gene may be a convenient marker for a group of linked genes in the residual inheritance that predispose to the susceptibility or resistance to AD.

The injection of foreign antigens did not induce hypergammaglobulinemia. These results indicate that mink of all genotypes used in these studies were not hyperresponsive to antigenic stimulation from the tissues used. Moreover, formalin treatment did not change normal mink tissue in such a manner as to initiate a response in the recipients regardless of genotype.

Spontaneous disease did not occur in the mink used in these trials as evidenced by the failure of the non-injected control group and all other groups except D and H to develop serum protein changes. The widespread occurrence of natural AD in mink herds makes it necessary to use caution in excluding the spontaneous disease in using mink of any color phase.

Several findings indicate the possible nature of the cause of AD. Injections of formalin-treated normal mink tissue did not cause altered serum proteins. Formalin-affected *aa* tissue was inactivated during 40 weeks' storage at 5°C. Furthermore, long term treatment with an anti-inflammatory drug (dexamethasone)¹ has doubled the incidence of spontaneous AD in a large group of mink on an affected ranch (6). In our laboratory, cell-free filtrates obtained by appropriate filtration and centrifugation of affected tissue suspensions have produced hypergammaglobulinemia. It appears that the cause is a filterable substance which can be almost completely removed from aqueous suspension by ultracentrifugation at 95,000 *g* for 1 hour. These trials exclude the possibility of the transmission of immunologically competent viable cells. This work is under study and will be the subject of a future report.

The initial serum protein changes can be detected 2 to 3 weeks following subcutaneous injection of affected tissue suspensions (6). The gamma globulin steadily rises until death ensues—often months following the initial globulin change. As anticipated, there is an increasing number of plasma cells and lymphocytes present in the parenchymatous organs, especially around arteries, and enlarged lymph nodes and spleen. Segmental arteritis is also present in some of the more advanced cases. It is noteworthy that the severity of the disease seems to parallel the increase in gamma globulin. It has also been found that the histochemical changes present in mink with AD resemble many of those found in the connective tissue diseases of man (7).

It is difficult to conceive that the gamma globulin is directed against the

¹ Azium, Schering corporation, Bloomfield, New Jersey.

"Aleutian agent" *per se*. The mink selected for transfer material were in advanced stages of the disease with an accompanying hypergammaglobulinemia. If the antibody is homotypic, the "Aleutian agent" may be inaccessible to its action. Another explanation would be that the antibody is directed against another antigen, conceivably a tissue antigen. Neither the mechanism of the response nor the relationship of the increased gamma globulin to the pathogenesis of the disease is clear.

Speculation as to the pathogenetic mechanisms involved in AD leads one to consider the possibility of an autoimmune disease. If the lesions observed are the result of the activity of "aggressive" or "forbidden" clones of cells in Burnet's sense (8), such cells might arise by somatic mutation with subsequent escape from the normal body homeostatic mechanisms. Regardless of the mechanisms involved, it appears that their pathological activity can be initiated by a filterable entity which appears to remain in the animal body in a transmissible form even though the circulating gamma globulin levels reach extraordinarily high values. This, along with the progression of changes in AD, suggests a "vicious cycle" involving "self" antigens.

It is interesting to note that the serum protein levels in the experimental disease can be held static or decreased by the oral administration of dexamethasone. Further, the expression of the disease as manifested by the serum protein changes can be suppressed by this drug for months after mink are injected with affected tissue suspensions. When the drug is removed, serum protein changes occur (6).

The transmission mechanism may be very intimate, perhaps in part vertical from dam to kittens. Family line studies on an affected ranch have shown over twice as many affected offspring from affected dams as compared to unaffected dams, but not all kits from an affected dam were diseased; nor were all kits from negative dams disease-free (3).

Aleutian disease of mink may be one of the first described naturally occurring, transmissible animal diseases that can be used as a laboratory model in studying the pathogenetic mechanisms involved in many immunologic and connective tissue diseases of man.

SUMMARY

Hypergammaglobulinemia in mink was produced by the injection of crude tissue suspensions from mink with spontaneous Aleutian disease. The initiating factor was found to be resistant to 0.3 per cent formalin for 2 weeks but not 40 weeks at 5°C. Foreign antigens as well as formalinized normal mink tissue from homologous and heterologous genotypes did not cause a detectable change in the serum protein values.

Mink homozygous recessive for the Aleutian gene were found to be significantly more susceptible to the experimental disease.

Possible pathogenetic mechanisms as well as similarities between the mink disease and certain immunologic and connective tissue diseases of man are discussed.

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