For five generations, pastel genotype mink (A/a) were selected and successfully bred for high and low antibody responsiveness to bovine serum albumin (BSA). They were then challenged with Aleutian mink disease virus (AMDV) and blood samples were taken regularly after infection. With a special focus on development of hypergammaglobulinaemia and lymphocytosis of CD8-positive T-lymphocytes, known to correlate with severity of Aleutian Disease (AD), no significant differences were observed between the high and low BSA-responder mink in the entire observation period for more than 6 months, leaving us to conclude that mink bred for high and low antibody response to BSA develop AD at equal rates.

Breeding of animals for high and low antibody response to various antigens has been documented in numerous cases (Biozzi et al. 1972, 1979). It is also well established that high responder animals to one particular antigen [including hapten groups] may be low/none responder to other antigens (McDevitt & Sela, 1965, Benacerraf & McDevitt, 1972). It is most clearly seen in studies in MHC defined inbred laboratory animals, but the phenomena of MHC restriction is in theory universal for all MHC-bearing animals. This would indicate that selection of animals on the basis of high and low antibody response to model antigens would have no (or very little) impact on immunity to infectious diseases in general, which requires many other immune functions than
just the magnitude of the humoral immune response to a particular antigen. Only if animals are bred for high and low antibody responsiveness to important antigens from the infectious agents (for instance neutralizable epitopes on cell-entry receptors of viruses or important parasite larvae antigens), may high responder animals show higher resistance to infection or milder disease outcome (Windon, 1996). In such cases the beneficial effect is restricted to that particular infection.

Aleutian Disease (AD), also known as mink plasmacytosis, is caused by persistent infection with Aleutian Mink Disease Parvovirus (Porter et al., 1980, Aasted, 1985, Alexandersen, 1990). Increased serum gammaglobulin production (hypergammaglobulinaemia, Porter et al., 1980) and elevated numbers of CD8-positive T-lymphocytes in peripheral blood (Aasted, 1989, Chen & Aasted, 1998) have together with mink death rates been used as relevant parameters for judgement of severity of AD (Aasted et al., 1998). Mink death is generally considered to be due to kidney failure (glomerulonephritis) induced by immune-complex deposition, by arteritis or by interstitial pneumonia in mink kits.

Materials and methods

Breeding of mink for high and low antibody production to BSA
Forty-five male and one-hundred-and-fifty-eight healthy, female pastel mink (Mustela vison, A/a genotype), housed in separate cages and fed a standard mink diet, were immunized with 1.0 mg of BSA (Hoechst-Boehring, Germany) in 1.2% aluminium hydroxide gel adjuvant (Superfos biosector, Vedbaek, Denmark) intramuscularly. Before experimentation, the mink were found negative for antibodies against AMDV (by the counter current method as described by Aasted et al. 1986). On day 22 after immunization, heparin-stabilized blood was drawn from vena cephalica and plasma samples analyzed for antibody quantity to BSA in a BSA ELISA (see below). The twenty-two males and 43 females with the highest antibody ELISA titres were bred (line 41) as was a similar amount of the lowest responding mink (line 42). This procedure was repeated for 5 generations. Figure 1 shows the development of average antibody titres against BSA with time of breeding of high and low responder mink.

Infection of high and low responder mink with AMDV
Sixteen healthy 7-8 month old, fifth generation high and low responder female mink were challenged with AMDV. Nine were of low responder genotype and seven of high responder genotype. Blood samples were taken by toenail-cuttings, except for terminal bleedings, which were taken by heart puncture. Heparinized capillary tubes were used for flowcytometric methods. For serum isolation, non-treated glass tubes were used. Macroscopic pathological examinations were performed on all animals sacrificed after 6.5 months of infection with a special focus at the mesenteric lymph node, spleen, kidney, liver, and lung (see the pathology section below).

Virus challenge
The AMDV used for mink challenge was a counter current antigen bought from United Vaccines (Madison, Wi, USA) and typed by Gottschalck et al.
(1994) to contain type 4 AMDV. The virus-containing material was titrated in mink by Aasted et al. (1998) and found to have a titer of $10^7 \text{ID}_{50}$ per ml of antigen solution. Mink were infected ip. with 10 µl of this material ($10^5 \text{ID}_{50}$ units) suspended in 1 ml of saline.

**Gammaglobulin quantitation**

Gammaglobulin quantitation was performed by serum electrophoresis in 1% agarose gel as described by Aasted (1989).

**BSA ELISA**

500 ng of BSA were added to each well in plastic microtiter plates (Nunc, Roskilde, Denmark) using 50 mM sodium carbonate buffer, pH 9.6 and incubated overnight. After a wash procedure, the plasma samples were diluted in a PBS buffer containing 0.05% Tween 20 and incubated (duplicated samples) for 2 h at room temperature. After three times wash, a peroxidase conjugated rabbit antibody to mouse immunoglobulins (code no P0161, DAKO, Glostrup, Denmark), known to be crossreactive with mink immunoglobulins, was added to each well in the recommended dilution given by the company. Finally (again after proper wash) the ELISA plates were incubated in the normal OPD staining solution and the color development in each well was measured by an ELISA reader. A pool of mink antibodies to BSA with a defined titer was titrated (duplicated samples) on each plate. The ELISA staining intensities of the different dilutions of the plasma samples were related to the staining intensities of the standard positive pool, giving relative titer estimates of the plasma samples.

**AMDV NS1 and 3b epitope DELFIA**

The NS1 and 3b epitope ELISA and DELFIA have recently been described (Christensen et al., 1995, Costello et al., 1999).

**Flow cytometry**

CD8-staining of mink leukocytes using a mink-crossreactive mouse monoclonal antibody to human CD8 (ATCC, cat.no CRL 8014, Aasted, 1989) was performed on fresh blood, followed by red cell lysing for 6 minutes in lysing buffer containing 155mM NH$_4$Cl, 10mM KHCO$_3$, 0.1mM EDTA, pH 8.3 followed by wash of the cell pellet. A FITC-conjugated F(ab')$_2$ fragment of a rabbit antibody to mouse immunoglobulin was used as secondary antibody (cat. no 313, DAKO. Glostrup, Denmark). Flow cytometry was done on a FACSCAN flow cytometer (Becton Dickinson, Calif., USA). The percentage of CD8-positive cells out of lymphocytes was calculated. These studies also gave relative estimates on the number of lymphocytes, monocytes and granulocytes using proper gates around the corresponding cell populations.

**Pathology**

Two of the high responder mink died of unknown reasons during the first months of the experiment. All other animals were necropsied. Gross pathology was done by opening the thoracic and abdominal cavities. For histopathological examination liver, mesenteric lymph node, spleen, kidney, and lung were fixed in 10% neutral buffered formalin. The tissue was embedded in Paraplast (R) according to routine procedures. Four µm sections were stained with haematoxylin and eosin. All sections were evaluated in a Leitz, Diaplan microscope at 25, 100 and 400 x magnification. The severity of the pathological changes were scored into the following 4 groups: 0 = no changes, + = slight changes, ++ = medium changes and +++ = severe changes. The pathological changes were typically perivascular accumulation of plasma cells and a few mononuclear cells. In the mesenteric lymph nodes the number of follicles were estimated, and few follicles were considered abnormal.

**Results**

Sixteen fifth generation female mink, 7 bred for high responsiveness to BSA (average relative ELISA titer 193) and 9 bred for low responsiveness (average relative ELISA titer of 4.8) were infected with $10^7 \text{ID}_{50}$ of AMDV. Blood samples were taken 0, 1, 2, 3.5 and 6.5 months after infection and analyzed for the following parameters: Serum gammaglobulin (Figure 2A), Serum antibodies to AMDV NS1 protein (Figure 2B) and the 3b-epitope (Figure 2C), percent CD8-positive lymphocytes (Figure 3A), lymphocytes (Figure 3B), monocytes (Figure 3C) and granulocytes (Figure 3D). No significant
differences were observed in any of these parameters when the high and low BSA-responder mink were compared. Similarly the pathological changes observed were of the same magnitude in both groups with medium, perivascular and interstitial accumulation of mainly plasma cells in the kidneys and liver of most animals, slight to medium, perivascular accumulation of plasma cells in the lungs, depletion of follicles in the mesenteric lymph nodes and slight depletion of the white pulp in the spleen.

**Discussion**

Seen in relation to the literature cited in the introduction, including the complex nature of the AMDV induced disease, we did not expect to observe any enhanced resistance to AMDV infection of mink bred for either high or low antibody response to bovine serum albumin. The experimental findings reported here verified this expectation. The reason for performing the experiment was primarily due to the fact that there was an interest among mink farmers for doing so, particularly because high and low responder mink were available for the first time. Already after 6.5 months of study this conclusion was obvious, so it was decided to stop the experiment. Consequently we were not able to record death statistics on the infected mink.

The number of animals was too small to perform regular statistical evaluations of the pathological recordings, but we noticed a slight tendency towards more severe changes in the kidneys and livers of the animals from the low responder group and more severe changes in the mesenterial lymph nodes and spleens of the high responder group, while the pathological changes in the lungs were uniform in the two groups. However it is usually difficult to differentiate chronic Aleutian Disease based on histopathology alone.

**Figure 2:** Development of serum gammaglobulin (A), antibody to AMDV NS1 antigen (B), and antibody to the linear dominant epitope 3b (C) with time after infection in high and low BSA-responder female 5th generation mink.
Development of relative percentage of CD8-positive lymphocytes (A), lymphocytes (B), monocytes (C) and granulocytes (D) with time after infection in high and low BSA-responder female 5th generation mink.

In conclusion we believe that our study clearly demonstrates that breeding of mink for high and low antibody responsiveness to a model antigen like BSA does not produce mink more or less resistant for development of a complex persistent viral disease like Aleutian Disease.

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