Antibody-Forming Cells and Serum Hemolysin Responses of Pastel and Sapphire Mink Inoculated with Aleutian Disease Virus

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The effect of Aleutian disease virus (ADV) on serum hemolysin titers and antibody-forming cells in lymph nodes and spleens of sapphire and pastel mink inoculated with goat erythrocytes (G-RBC) was investigated. ADV injected 1 day after primary antigenic stimulation with G-RBC did not depress the immune responses of either color phase for a period of 26 days. However, when G-RBC were injected 47 days after ADV, both the number of antibody-forming cells and hemolysin titers were more markedly depressed in sapphire than in pastel mink. The results are discussed in relation to the greater susceptibility of sapphire mink and the variable susceptibility of pastel mink to the Pullman isolate of ADV.

Aleutian disease (AD) is a viral infection of mink in which the primary lesion is systemic plasmacytosis with hypergammaglobulinemia resulting from overproduction of immunoglobulin G (15, 16). Sapphire mink, a color phase homozygous for the Aleutian gene, are more uniformly susceptible to experimental infection with Aleutian disease virus (ADV) than are royal pastel mink, a color phase not homozygous for this autosomal recessive gene (5). The disease, leading to death from immune complex glomerulopathy, usually progresses much more slowly in pastel than in sapphire mink. Although AD is always fatal in sapphire mink, occasionally an affected pastel mink recovers. Whether these differences in response to ADV injection may be explained by differences in immunologic responsiveness of the two color phases is as yet unanswered.

A previous study (10) demonstrated that the responses of normal sapphire and pastel mink to goat erythrocytes (G-RBC) were similar. Yet, differences were observed, especially in the anamnestic response. In another study (11), both the primary and anamnestic responses to Keyhole limpet hemocyanin (KLH) were severely impaired in sapphire mink infected with AD at the time of primary stimulation with the antigen. When ADV was given concomitantly or after KLH, however, the primary anti-KLH response was depressed after onset of disease, but the anamnestic response was not.

This paper presents observations on the effect of ADV injection on serum hemolysin titers and antibody-forming cells in spleen and lymph nodes of sapphire and pastel mink to G-RBC at various times after inoculation with virus.

**MATERIALS AND METHODS**

**Mink.** Male and female mink, 1 to 2 years old unless stated otherwise, were obtained from a local herd known to be free of AD for the past 9 years. They were of the same breeding as those used in our previously reported studies and were similarly housed (5, 10, 11). None had received previous vaccines or other inoculations. Serum protein analysis by cellulose-acetate electrophoresis indicated that none was affected with AD before the start of the investigation. Mink were considered positive for AD if their serum gamma globulin was greater than 1.5 g/100 ml or if it had increased twofold after inoculation with ADV. The presence of AD was confirmed by finding characteristic macroscopic and microscopic lesions after a complete necropsy.

**Virus.** Each experimentally infected mink was inoculated intraperitoneally (i.p.) with 0.5 ml of a 10^−4 dilution of a splenic suspension of 10th passage ADV (Pullman isolate) which contained about 10^4 mean lethal dose of virus as titrated in sapphire mink.

**Antigen and antigen administration.** Erythrocytes, obtained from a single goat (G-RBC), were washed four times in 0.85% saline and adjusted to a 10% suspension containing 6.0 × 10^6 cells/ml. Mink were inoculated i.p. with 1.0 ml of the 10% suspension for study of the primary response and a second, similar dose for study of the anamnestic response.

**Serum antibody determinations.** Blood was obtained by an exsanguinating cardiac puncture while the mink were under ether anaesthesia. Sera were inactivated at 56°C for 30 min and individually titrated by the 50% hemolysis method (9). Sensitivity
of hemolysins to 2-mercaptoethanol, (2-ME), to estimate 19S and 7S antibody, was determined as previously described (10).

Amplifying antiserum. Guinea pig 7Sγ, antimink globulin was prepared and used as previously described (10).

Lymphoid cells. The spleen and two pools of lymph nodes were collected. The pooled, peripheral lymph nodes included the mandibularis, retropharyngeal, prescapular, axillaries, prefemorals, popliteals, and superficial inguinals; the pooled abdominal lymph nodes (ALN) included the mesenteric, gastrics, colics, pancreatic, renales, and internal iliacas. The cells were prepared for plaque assay as described previously (10).

Plaque assay. The Jerne hemolytic plaque assay (7, 8) was used with the modifications of Golub et al. (6). Direct plaques detected after incubation with complement alone were considered to be produced by cells releasing 19S hemolysins. Indirect plaques detected after treatment with amplifying antiserum were considered to be produced by cells releasing low-efficiency 7S antibody. Calculation of direct (19S) and indirect (7S) hemolytic plaques and counts of lymphoid cells in the suspensions were done as previously described (10).

Experimental design. Three groups of mink were studied. Group A was comprised of 8 sapphires and 8 pastels that were tested for “background responses” at various intervals after inoculation with ADV. Group B was comprised of 20 sapphires and 20 pastels that were inoculated with ADV 1 day after a primary sensitizing dose of G-RBC. Two mink of each color phase were studied at 2, 4, 6, and 10 days after the primary inoculation with G-RBC and at 2, 4, 6, 10, 14, and 20 days after a booster inoculation given 7 days after the primary one. One sapphire inoculated with ADV was included with groups A and B to verify that a sufficient amount of virus had been given to cause disease. Group C was comprised of 20 mink of each color phase, and they were studied in the same manner as those in group B, except that the primary dose of G-RBC was given 47 days after inoculation with ADV.

Statistical analysis. To determine if there was a significant difference between the responses of pastel and sapphire mink, median tests (4) were used to determine the significance of the differences between the two color phases.

RESULTS

Background plaque-forming cells and hemolysin titers of mink after inoculation with ADV (group A). Plaque-forming cells (PFC) and hemolysin titers were determined with two mink of each color phase at 3, 9, 17, and 23 days after inoculation with ADV. None of the sapphire mink tissues had more than five direct or indirect PFC/10⁶ cells. Two sapphires had low hemolysin titers (<1:10). Although pastel mink PFC responses were somewhat higher than those of sapphires, only one tissue (ALN) of one pastel had >10 PFC/10⁶ cells. Seven of eight pastels had low hemolysin titers (<1:10).

PFC responses and hemolysin titers of mink inoculated with ADV 1 day after primary sensitization with G-RBC (group B). To determine the effect of ADV on PFC responses and hemolysin titers before onset of disease, pastel and sapphire mink were examined at various intervals through 26 days after inoculation with ADV. Throughout this period, PFC and hemolysin responses of both color phases were essentially similar to those observed in normal mink (10). The number of primary and anamnestic direct and indirect PFC of both color phases were similar in comparable lymphoid tissues at most test intervals. At the same intervals, ALN of both color phases usually contained greater numbers of direct and indirect PFC than did the spleen. These differences were most apparent in anamnestic responses. Primary and anamnestic total and 2-ME resistant hemolysin responses also were comparable in the two color phases at each test interval.

PFC responses and hemolysin titers of mink inoculated with ADV 47 days before receiving G-RBC (group C). As observed in the previous experiment, ADV had no effect on PFC and hemolysin responses of either pastel or sapphire mink for intervals up to 26 days after inoculation with virus. Hence, this experiment was done to determine the effect of ADV on these responses of mink inoculated with virus 47 days before primary antigenic stimulation. At the time of testing, all 20 sapphire mink, but only 7 of 20 (35%) pastels, were considered positive for AD (see Materials and Methods).

None of the 20 sapphire mink had primary or anamnestic direct or indirect splenic PFC (Fig. 1). In contrast, 10 pastels, 2 of which were positive for AD, had direct, indirect, or both types of splenic PFC. Neither type of PFC, however, was detected in several unaffected and AD-affected pastels.

In contrast to the absence of splenic PFC in AD-affected sapphire mink, the ALN of 15 of 20 (75%) of these same mink had primary or anamnestic direct PFC; indirect PFC were detected in 5 of 20 (25%) (Fig. 2). Substantial differences between PFC responses of spleen and ALN also were detected in pastels inoculated with ADV. Both plaque types were detected in ALN of all but one of them. In general, ALN of sick pastel mink contained fewer PFC than did ALN of pastels that were inoculated with ADV, but did not become sick.

In both color phases, responses of peripheral lymph nodes were similar to those of the respective spleens. PFC were detected in only 3 of 20 (15%) sapphire mink, whereas 10 of 20 (50%)
pastel mink, 5 of which were positive for AD, had direct, indirect, or both types of PFC.

Serum hemolysins were present in non-2-ME-treated sera of 9 of 20 (45%) sapphire mink; low titers of 2-ME-resistant hemolysins were detected in several of these mink (Fig. 3). In contrast, 18 of 20 (90%) of the non-2-ME-treated pastel mink sera contained hemolysins, and all pastel mink sera contained 2-ME-resistant hemolysins. In general, hemolysin titers of AD-affected pastel and sapphire mink were similar at comparable intervals. However, the hemolysin titers of AD-affected pastel mink were usually lower than those of pastels that were inoculated with ADV but that did not become affected with the disease.

Statistical evaluation of these results demonstrated that there was a significant difference in the immune responses between sapphire and pastel mink inoculated with ADV 47 days before receiving G-RBC. In every instance (Table 1), the immune response of the pastel mink, based on their overall response, was greater than that of the sapphire mink.

**DISCUSSION**

A wide variety of viruses are known to depress the immune response of the host (1-3, 13, 14). Many hypotheses have been proposed to explain how this occurs. Viruses that infect cells of the immune system could depress antibody production by depressing cellular protein (antibody) synthesis, by altering the uptake and processing of antigens and/or by destroying antibody-producing cells or their precursors (14). Furthermore, competition between the infecting virus and immunizing antigen for antibody-producing cells could also play a role in immunodepression (14).

In the present study, ADV injected 1 day after primary sensitization with G-RBC did not significantly alter the immune response of either
sapphire or pastel mink to G-RBC for the first 26 days. During this period, the primary and anamnestic direct and indirect PFC responses and hemolysin titers of both color phases were essentially similar.

In contrast, the immune response to G-RBC was greatly altered in mink that had been inoculated 47 days earlier with ADV. This was most apparent in AD-affected sapphire mink; PFC were not detected in their spleens, lymph node PFC responses were much inferior to those of pastel mink, and only 9 of 20 (45%) had hemolysin titers. The immune responses of pastel mink also were depressed, but usually not as severely as those of sapphires. Furthermore, the responses of pastel mink to G-RBC were extremely variable; PFC were not detected in spleens of several unaffected pastel mink, and in other instances PFC and hemolysin responses of AD-affected pastel mink were equal to or greater than those of pastels that had been inoculated with ADV but in which disease did not occur.

From these results it appears that the depressed immune response of sapphire mink to G-RBC correlates with the presence of lesions of AD and with increased amounts of serum gamma globulin. The immune impairment also correlates with the intervals after inoculation of ADV when high titers of serum anti-ADV antibody are detected (16). In this regard, it has been shown that the increased gamma globulin
in AD-affected mink consists of extremely high antibody titers specific for the AD viral antigen and that a high positive correlation coefficient exists between anti-ADV titer and the percentage of serum gamma globulin (12).

The extremely variable immune responses of pastel mink that received G-RBC 47 days after inoculation with ADV are difficult to interpret, but might be reflections of the varied responses of this color phase to the Pullman isolate of ADV. For example, in a group of 20 pastel mink studied for 2 years after inoculation subcutaneously with this isolate of ADV, four distinct groups were recognized on the basis of their response to the virus (W. J. Hadlow, unpublished data): (i) no viremia and no rise in gamma globulin; (ii) fleeting viremia 2 to 4 weeks postinoculation without a significant rise in gamma globulin; (iii) viremia 2 to 8 or 12 weeks postinoculation with a slight, but variable, rise in gamma globulin; and (iv) continued viremia, accompanied by a sustained rise in gamma globulin, and disease.

Thus, if a similar heterogeneity were present in the group C pastel mink of this study, the normal immune response may be found in group 1 (above), whereas the depressed immune response in clinically normal animals may be found in groups 2 and 3, and the depressed immune response in AD-affected pastel mink in group 4. If so, this would suggest that the presence (replication) of ADV was the actual
cause of the immunodepression to the heterologous antigen. Thus, in the absence of replication of ADV, the animal responds more efficiently to the heterologous antigen. In contrast, viral replication appears to result in persistent stimulation of the immune system by ADV and viral antigens that results in antigenic competition and a depressed response to the heterologous antigen.

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