Impaired Phagocytosis by the Mononuclear Phagocytic System in Sapphire Mink Affected with Aleutian Disease (43121)

DONALD L. LODMELL, ROBERT K. BERGMAN, MARSHALL E. BLOOM, LARRY C. EWALT, WILLIAM J. HADLOW, AND RICHARD E. RACE

National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, Hamilton, Montana 59840

Abstract. **The phagocytic function of the mononuclear phagocytic system (MPS) in normal sapphire mink and in sapphire mink affected with experimental Aleutian disease was compared. Clearance from blood of carbon particles or ^{125}I-labeled microaggregated human serum albumin, and subsequent measurement of radioactivity in phagocytic organs indicated profound MPS blockade in mink affected with advanced Aleutian disease. In contrast, MPS activity in mink in the early stage of the disease was comparable to that of normal mink. It is suggested that the MPS blockade may be responsible for some pathologic changes in Aleutian disease.** [P.S.E.B.M. 1990, Vol 195]

Aleutian disease (AD) is a progressive disease of ranch mink (*Mustela vison*) that is induced by the Aleutian mink disease parvovirus (ADV) (1, 2). Adult mink affected with typical AD have a persistent infection, extreme hypergammaglobulinemia, high titers of antiviral antibody, and immune-mediated lesions (3). The cause of death is usually uremia thought to result from immune-complex mediated glomerulonephritis or hemorrhage due to cerebral arteritis (3).

Studies on the pathogenesis of ADV infections have suggested that the mononuclear phagocytic system (MPS) (4) may have a potential role in the evolution of the disease. Immunohistochemical and *in situ* hybridization analyses of infected adult mink have revealed that large amounts of virion DNA and virion antigens are associated with phagocytic cells located in the liver, spleen, and mesenteric lymph node (1, 5). Although it is uncertain if the ADV is replicating in or is merely sequestered by these cells, the presence of virus may interfere with MPS function. Furthermore, mink immunoglobulin can be identified in a similar distribution in spleen and mesenteric lymph node (6). If this immunoglobulin is antiviral antibody participating in immune complexes, immune complexes containing virus may also be associated with cells of the MPS.

It is widely recognized that formation of immune complexes is a common immunological response to viral antigens (7). Furthermore, immune complexes have been shown to induce phagocytic defects in macrophages (8) and polymorphonuclear leukocytes (9). Because of the considerations mentioned above and because immune complexes may play an important pathogenetic role in AD, we initiated studies to examine the function of the MPS in AD-affected mink. We found that the phagocytic activity of the MPS was greatly impaired late in the course of disease.

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Materials and Methods

Mink. One- to 2-year-old male sapphire (Aleutian genotype) mink were acquired from a herd free of AD and maintained at the Rocky Mountain Laboratories (10–12). Experimentally infected mink were inoculated intraperitoneally with 0.5 ml of 10^{-2} dilution of a suspension of 10th passage of the Pullman isolate of ADV, or about 10,000 LD_{50} of virus. The presence of AD was confirmed by necropsy. Three groups of animals were studied: 1—seven normal mink that had not been inoculated with ADV; 2—five mink in the early stage of the experimental disease (4–6 weeks after inoculation) as determined by a recent rise in serum γ-globulin to at least 1.5 g/100 ml; 3—nine mink in the late stage (18–45 weeks after inoculation) of disease.

Phagocytosis Studies. Phagocytic activity of the

MPS was assayed by determining the clearance from blood of ^{125}I-labeled microaggregated human serum albumin (13) (^{125}I-HSA) (Albumatope H I-125; Squibb) and carbon particles (14) (Pelikan Carbon Black Suspension C 11/143/a; John Henschel and Co., Inc., Long Island, NY). Mink were inoculated with the ^{125}I-HSA and carbon via a catheter placed in the jugular vein while they were under pentobarbital anesthesia (15). Disappearance of colloids from the blood was expressed as the time for the colloidal level in the blood to be decreased by half ($t_{1/2}$).

Before ^{125}I-HSA was administered, two blood samples were obtained (1 ml for a total serum protein determination and electrophoretic analysis and 0.2 ml for a preinoculation background sample). Each mink received 1 ml of a solution containing 2.5 mg/ml of HSA with an approximate activity of 50 μCi of ^{125}I/ml. Blood samples (0.2 ml) were collected via the catheter at 3-min intervals starting 3 min after inoculation and extending to 30 min. Each sample was diluted with 2 ml of distilled water. After each blood sample was obtained, the catheter was purged with 0.2 ml of physiologic saline to prevent it from becoming plugged with clotted blood. Radioactivity in the diluted blood samples was measured and a semilogarithmic plot of radioactivity against time was made. A regression line was fitted to the data by the principle of least squares and the $t_{1/2}$ time in minutes was calculated from the regression line.

Five minutes after the last blood sample was obtained to measure the ^{125}I-HSA clearance, 15 mg of carbon/100 g body wt was injected via the catheter. Blood samples (0.2 ml) collected at 3, 4, 5, 6, 7, 9, 12, 15, 20, 25, and 30 min after inoculation were diluted with 3 ml of 0.1 N Na_2CO_3. The catheter was purged with saline as before. Optical densities at 650 nm were determined against a blood blank taken immediately before the carbon injection. Optical densities were plotted against time, and $t_{1/2}$ values were determined as mentioned previously.

After the last blood sample for carbon clearance had been obtained, each anesthetized mink was exsanguinated via the catheter. The spleen, liver, lungs, femoral bone marrow, and popliteal, axillary, and mesenteric lymph nodes were removed, rinsed in distilled water, blotted dry, and weighed. Triplicate samples of each organ were tested for radioactivity, as was done for blood samples.

Significance of differences between the means of the normal mink and AD-affected mink was determined by Student's t test (16).

Results

Clearance Rates of Carbon and Microaggregated ^{125}I-HSA. Colloidal sized particles such as carbon or microaggregated ^{125}I-HSA are normally cleared very rapidly from the circulation by the MPS. If a defect or block exists in the MPS, the disappearance from the blood of such substances is delayed. The clearance of carbon from the blood of AD-affected mink in the late stage of disease was greatly impaired (Table I), implying a MPS blockade. In extreme cases, $t_{1/2}$ values could not be determined, suggesting that in these mink there was loss of water from the circulation and an increase in the concentration of carbon in the remaining intravascular fluid volume. The impaired ability of late AD-affected mink to clear carbon from the circulation was highly significant ($P < 0.005$), even when the test for significance did not include data from the three mink in which $t_{1/2}$ values could not be determined because blood carbon concentrations increased during the test interval. In contrast to the results obtained with mink in the late stage of the disease, no significant differences in $t_{1/2}$ values for carbon clearance were detected between the normal mink and those with early AD.

Table I. Carbon and Microaggregated ^{125}I-HSA Clearance Rates ($t_{1/2}$ [Minutes]) for Individual Normal and Aleutian Disease-Affected Sapphire Mink

	$t_{1/2}$ (min)	
	Carbon	^{125}I-HSA
Normal		
T-1894	13.8	12.9
T-2087	9.0	10.2
Q-177	9.7	13.3
T-1438	9.1	9.4
T-2064	15.6	13.6
T-2048	7.9	16.9
T-2069	11.0	12.0
	Mean 10.87 ± 0.99[a]	12.6 ± 0.89
Early AD		
AD-1546	7.7	13.9
AD-1111	8.1	13.7
AD-1056	24.7	12.2
AD-1536	10.6	11.6
AD-1549	7.8	12.5
	Mean 11.8 ± 2.9	12.8 ± 0.39
Late AD		
AD-1376	51.9	14.2
AD-1448	—[b]	14.3
AD-850	49.0	11.1
AD-1184	68.7	16.5
AD-1403	52.1	19.5
AD-1532	99.3	15.2
AD-1396	—[b]	17.0
AD-1384	Died	34.4
AD-1390	—[b]	22.1
	Mean 64.2[c] ± 1.78	18.3 ± 0.73

[a] SEM.
[b] $t_{1/2}$ could not be determined because concentrations of carbon in the blood increased during the test interval.
[c] Significantly different from normal mink at 0.5% level (t test). Mean does not include results from three mink with severe MPS blockade in which the concentration of carbon in the circulation increased during the test interval.

Mean and individual $t_{1/2}$ values for clearance of ^{125}I-HSA also are shown in Table I. The means of the normal mink and early AD-affected mink were essentially identical. In contrast, the probability of the difference between the means of the normal group and mink in the late stages of disease approached significance ($0.10 > P > 0.05$).

Distribution of ^{125}I-HSA Colloid in Tissues. The quantity of ^{125}I-HSA present per mg of phagocytic tissue in the three groups of mink is shown in Figure 1. No differences in the amount of radioactivity were detected in the lymph nodes and lungs of the three groups. In contrast, significantly more ^{125}I-HSA per mg of tissue was present in spleen, liver, and bone marrow of normal mink than in these organs of mink tested either early or late in the disease. It is unclear why livers of late AD-affected mice contained more ^{125}I-HSA per mg of tissue than livers of early AD-affected mink. Nevertheless, using this measurement, impairment of MPS function could be demonstrated even early in the course of disease.

Table II shows that the spleen and liver weights of both early and late AD-affected animals exceeded those of normal mink, yet the increased size of these organs appeared to have no affect on the removal of the test colloids from the circulation.

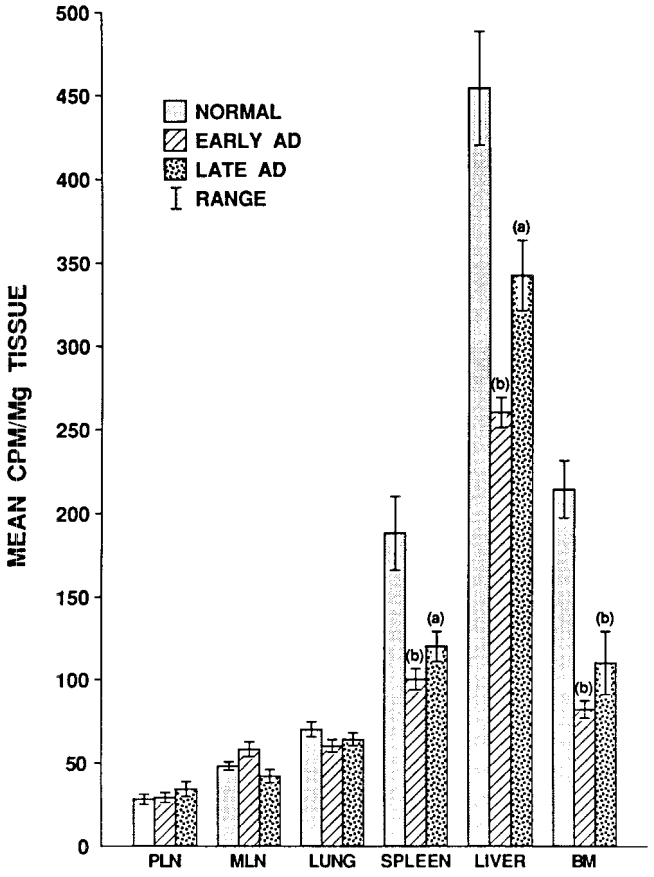

Figure 1. Mean counts per minute of ^{125}I-HSA colloid per mg of tissue in normal and AD-affected mink. (a) Significantly different from normal mink, $P \leq 0.05$. (b) Significantly different from normal mink, $P \leq 0.01$. PLN, popliteal lymph nodes; MLN, mesenteric lymph node; BM, bone marrow.

Discussion

The results of this study have shown that mink suffering from protracted AD have a significant MPS blockade as assayed by the clearance of carbon particles or ^{125}I-HSA from the blood. In the early stages of the disease, by contrast, clearance rates were similar to those of normal mink. Thus, it appeared that impairment of MPS function correlated with the clinical severity of the disease. The MPS blockade of late AD-affected mink was demonstrated most dramatically in the carbon clearance studies in which the carbon concentration increased and $t_{1/2}$ values could not be demonstrated in three of the nine severely impaired animals. The inability to determine $t_{1/2}$ values during clearance studies also has been shown in lipid-treated mice whose MPS function was severely depressed (17).

Results reported herein with ^{125}I-HSA captured by the MPS largely corroborated those of the blood clearance studies. That is to say, lesser amounts of radioactivity per mg of tissue were present in the major phagocytic organs of AD-affected mink than in those organs of normal mink. However, using the amount of ^{125}I-HSA sequestered as an index of phagocytic function, even mink early in the course of AD were abnormal, suggesting that the defect could be detected before advanced AD was present. Nevertheless, the increased size of spleens and livers of AD-affected mink (Table II) appeared to have minimal bearing on the ability of these animals to remove test colloids from the circulation.

The impaired clearance of colloids by the MPS observed in this study was probably a consequence of a phagocytic cell defect which reduced the capacity of cells to phagocytize additional particulate material (18). This impaired phagocytosis or blockade of the MPS might have resulted from the sequestration of virus-induced immune complexes which are known to inhibit macrophage function (19). Although current evidence suggests that most of the ADV within these cells is in fact sequestered, it remains possible that very low level, restrictive replication of ADV may be occurring in cells of the MPS (20, 21) and interfering with normal phagocytic function, as has been shown for other viruses (22).

On the basis of indirect evidence, Karstad (23) postulated that MPS blockade supervenes in AD-affected mink. This impedes fibrin clearance and results in intravascular coagulation. Furthermore, the intravascular coagulation is believed to be directly responsible for some pathologic changes in AD, such as focal hepatic necrosis and hyalin glomerular deposits (23). The excessive phagocytosis of antigen-antibody com-

Table II. Organ Weights in Normal and Aleutian Disease-Affected Sapphire Mink

Mink	*n*	Average tissue weight		
		Spleen (g)	Lung (g)	Liver (g)
Normal	7	4.1 (2.6–5.9)	11.0 (9.3–12.9)	37.0 (28.3–45.0)
Early AD	5	11.6 (10.0–14.1)[a]	11.6 (8.3–14.7)	49.9 (41.5–56.8)[b]
Late AD	9	14.2 (6.6–25.3)[c]	10.0 (7.1–13.9)	44.4 (31.3–55.1)[a]

[a] Significantly different from normal mink at 0.1% level, $P \leq 0.001$.
[b] Significantly different from normal mink at 1.0% level, $P \leq 0.01$.
[c] Significantly different from normal mink at 5.0% level, $P \leq 0.05$.

plexes by the MPS also may predispose to a sequence of intracytoplasmic release of lysosome enzymes, denaturation of native proteins, and creation of autoantigens (23), all of which may contribute to the pathogenesis and fatal course of the disease. Furthermore, loss of normal macrophage function may contribute to the increased susceptibility of AD-affected mink to various bacterial infections (11).

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