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Aleutian Disease Parvovirus Infection of Mink and Ferrets Elicits an Antibody Response to a Second Nonstructural Viral Protein

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A second nonstructural protein of the Aleutian disease parvovirus was predicted from nucleotide sequence analysis and a detailed transcription map. Western immunoblotting analysis showed that infected mink and ferrets show an antibody response to this predicted protein.

Aleutian disease virus (ADV), an autonomous parvovirus, infects mink and ferrets. Adult mink infected with virulent strains of mink ADV generally develop a progressive, persistent infection with plasmacytosis, hypergammaglobulinemia, glomerulonephritis, and arteritis (for a review, see reference 9). Less-virulent strains of mink ADV produce a progressive, persistent infection with tissue lesions mainly in mink of the Aleutian genotype; non-Aleutian mink infected with such viral strains may become persistently infected with few or no lesions or may clear the viral infection (for a review, see reference 7). In contrast, newborn mink from antibody-free mothers develop an acute and fatal interstitial pneumonia when infected with virulent ADV, and the virus replicates in type II pneumocytes (1). Ferrets infected as adults with the immunologically similar but biologically distinct ferret ADV typically develop a persistent infection without disease (15).

Mink and ferrets infected with ADV show prompt and huge antibody responses to viral-specific proteins, and most of the animals develop hypergammaglobulinemia (14). In some examples of progressive, persistent infection in mink, as much as 80% of a 10-fold-elevated immunoglobulin G level may be viral-specific antibody (11). Neutralization of ADV by antibody is difficult to demonstrate (17), and it is unknown whether neutralizing antibody plays any protective role in this infection. Immune complexes are responsible for the glomerulonephritis and arteritis of Aleutian disease (9). Antiviral antibody given to neonatal mink infected with ADV restricts viral replication and converts an otherwise-lethal acute pneumonia into the chronic form of Aleutian disease (4). Viral replication in adult mink appears to be restricted by possibly nonimmunologic mechanisms (3). Analysis of the antibody response to ADV in mink and ferrets by immunoprecipitation and Western immunoblotting has shown that essentially all infected animals with progressive or nonprogressive disease develop antibody to 86,000- and 78,000-dalton (Da) virion proteins (VP-1 and VP-2, respectively) and to a 71,000-Da nonvirion protein (NS-1) (6, 13). Some infected ferrets develop antibody only to detergent-sensitive determinants on VP-1 and VP-2 (12). A 143,000-Da protein demonstrable only by Western blotting appears to be a dimer of NS-1.

Nucleotide sequence analysis (5) and a detailed transcription map (2) of ADV indicate that the virus should encode a second nonstructural protein (NS-2) with a predicted size of 13,400 Da. Review of the Western blotting strips from previously reported experiments (10, 12, 13), which used 6 and 10% separating polyacrylamide gels, failed to show unequivocal visual evidence of a viral protein of this size. However, sera from uninfected mink gave a moderately high level of background staining on bands in this size range. Using a glucose oxidase-labeled-antibody method, ADV antigen prepared from lysed cells infected with ADV strain G for 68 h (as used in previous experiments [10, 12, 13]), and 12 to 20% separating polyacrylamide gels, we were able to demonstrate antibody reactive with the predicted NS-2 in the sera of ADV-infected mink and ferrets (Fig. 1). The time of the appearance of NS-2 during ADV infection of the cell culture was not studied.

Optimal separation of NS-2 from an intense, nonspecific band of 15,000 Da was found at a gel concentration of 13.5%. The measured molecular weight of NS-2 was 13,800 ± 1,000 Da (nine determinations), in close agreement with the predicted value of 13,400 Da. A typical reaction of serum from an infected mink is shown in Fig. 1, lane C, where there is intense staining of viral proteins of 143,000, 86,000, 78,000, 71,000, and 14,000 Da; the same serum reacted with uninfected cell extract is shown in Fig. 1, lane D. Serum from an uninfected mink failed to react with proteins of these sizes in lysates of infected (Fig. 1, lane A) or uninfected (Fig. 1, lane B) cell cultures. Serum from an infected ferret which did not react with the detergent-treated virion proteins (Fig. 1, lane E) showed a strong reaction to both NS-1 and NS-2. Rabbit antisera to purified ADV virions reacted strongly with VP-1 and VP-2 but gave no reaction with NS-1 or NS-2 (not illustrated). A total of 39 serum samples from ADV-infected mink and ferrets were examined for antibody to NS-2, and with only one exception, all sera with antibody to any of the ADV proteins had antibody to NS-2. The exception was that three of six serum samples available from transplacentally infected mink (8) reacted strongly with the two virion proteins and NS-1 but failed to react with NS-2. The other three serum samples from transplacentally infected mink did have antibody reactive with NS-2. Antibodies to all the ADV proteins appeared at the same time during the experimental infection of mink. No qualitative differences in the immune responses to NS-2 or the other viral proteins were found between mink with progressive and nonprogressive Aleutian disease. The transcription map of ADV suggests that there could be a third ADV nonstructural protein of approximately 10,000 Da (2), but we found no evidence of a reactive band of this approximate size.

This study demonstrates that ADV, like most other autonomous parvoviruses, expresses two nonstructural proteins

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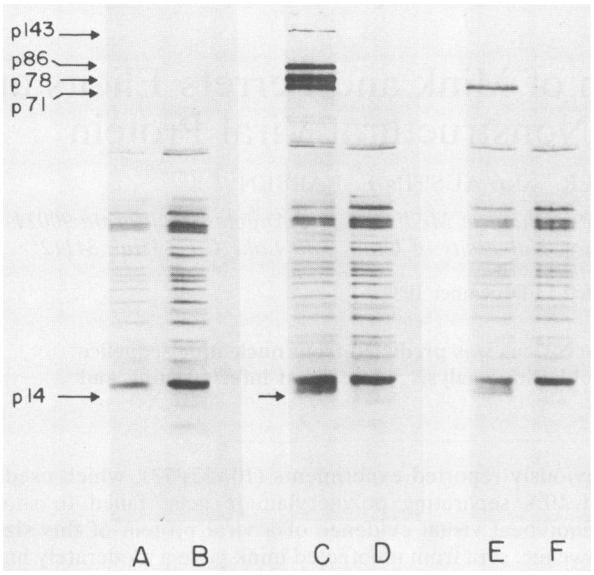

FIG. 1. Protein transfer blots of ADV-infected (lanes A, C, and E) or uninfected (lanes B, D, and F) cell lysates reacted with mink or ferret serum. Lanes A and B, Serum from an uninfected mink, showing only nonspecific background staining; lanes C and D, serum for an ADV-infected mink, which in lane C reacted with ADV proteins of 143,000 Da (p143; NS-1 dimer), 86,000 Da (p86; VP-1), 78,000 Da (p78; VP-2), 71,000 Da (p71; NS-1), and 14,000 Da (p14; NS-2); lanes E and F, ADV-infected-ferret serum which was known to react only with detergent-sensitive determinants on VP-1 and VP-2 and which in lane E reacted only with NS-1 and NS-2. The separating gel was 13.5%, and the nitrocellulose strips were developed with glucose oxidase-labeled goat anti-mink immunoglobulin G and then subjected to a disclosing reaction. The sera were diluted 1:20 for this test.

and that the smaller NS-2 is expressed sufficiently in vivo to result in a strong humoral immune response to NS-2. The function of the nonstructural proteins of ADV is unknown. The functions of the nonstructural proteins of other parvoviruses are to mediate viral DNA replication and regulate the temporal expression between viral transcription units (16, 18). The finding that three of six mink infected transplacentally with ADV failed to develop antibody to NS-2 suggests that either the regulation of viral replication or the immune response is altered in this milder form of chronic Aleutian disease.

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