Antibody Responses to Virion Polypeptides in Gnotobiotic Dogs Infected with Canine Distemper Virus

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A radioimmunoprecipitation-polyacrylamide gel electrophoresis technique was applied to sera from canine distemper virus-infected dogs. Sera from fatally infected dogs precipitated only the nucleoprotein, the matrix protein, and trace amounts of fusion glycoprotein. Sera from normal convalescent dogs precipitated all five major polypeptides. In contrast, sera from persistently infected dogs were characterized by a modest overall response compared with sera from convalescent dogs and by no or little response to the matrix and phosphorylated proteins until 5 to 7 weeks after infection.

Canine distemper virus (CDV), a morbillivirus closely related to measles virus, is a serious pantropic viral disease of dogs. The virus is ordinarily spread from infected to uninfected dogs by aerosols. The early events of pathogenesis are well documented (1, 2, 6, 12, 24). The magnitude of the humoral immune response to CDV (1, 2, 16) correlates with the eventual outcome of the infection. A delayed or diminished complement-fixing antibody response to virion envelope antigens occurs in animals with persistent neurological disease (16). More precise delineation of the immune response to individual virion polypeptides in these canine sera can be made by radioimmunoprecipitation-polyacrylamide gel electrophoresis (RIP-PAGE).

For this study, serum samples were obtained (15, 16, 18, 19) before inoculation and at each post-inoculation week (PIW) from 16 R252-CDV-infected gnotobiotic dogs (14). The dogs were assigned to one of three groups based on the outcome of the infection. Group 1 (four dogs) died of acute noninflammtory viral encephalitis at PIW 4 to 5. Group 2 (eight dogs) developed subclinical yet persistent disseminated nonsuppurative encephalitis with demyelination after infection. Group 3 (four dogs) did not develop clinical signs or pathological evidence of encephalitis and were considered to be normal convalescent animals. As technical controls, both known-positive (CDV-immune) and known-negative (CDV-nonimmune) gnotobiotic dog sera were also included in each gel run.

A cloned pool of R252-CDV adapted to growth in Vero cells (5) was used for preparation of $^{35}$S-methionine-labeled viral antigen (S. Krakowka, J. A. Miele, L. E. Mathes, and A. E. Metzler, Ann. Neurol., in press). Whole cell lysates clarified by high-speed centrifugation (16,000 x g) were used as antigen. All sera were reacted with $^{35}$S-methionine-labeled CDV in a RIP-PAGE assay as previously described (Kra-kowka et al.; in press). Viral polypeptides in developed fluorographs (Fig. 1) were identified by published data (3, 9, 11, 22, 23) and semiquantitated by using an arbitrary visual scale (0 [absent] to 5+ [strong]) based on the width and intensity of the relevant polypeptide compared with that of the positive control.

Dogs that develop acute fatal encephalomyelitis (group 1) after infection have little or no virus-neutralizing antibody in their sera (16). Analysis of the serum samples by RIP-PAGE revealed that they were devoid of antibody to CDV hemagglutinin-equivalent (H) and phosphorylated (P) proteins and contained only limited amounts of antibody to CDV nucleocapsid (NP) protein, fusion (F) glycoprotein, and matrix (M) protein from PIW 1 until death (Fig. 2). Modest responses to the CDV NP and F proteins were first noted at PIW 1 in the persistently infected dogs of group 2 (Fig. 3). By PIW 4, antibody to these two proteins reached peak levels. Antibody to the three remaining virion polypeptides, H, P, and M, was first detected at PIW 3. Modest levels of anti-H protein persisted throughout the observation period, whereas increases beyond trace levels for the P and M proteins were noted from PIWs 5 through 7 onward. In contrast to the results for groups 1 and 2, sera collected from the convalescent group 3 dogs reacted promptly and strongly (PIW 2 onward) with al of the polypeptides (Fig. 4).
FIG. 1. Representative RIP-PAGE fluorograph of serial serum samples from an R252-CDV-infected convalescent dog (group 3). Lanes 1 through 4, Samples taken at PIWs 9 through 12, respectively. Lane 5, Negative (nonimmune) control canine serum. The faint nonspecific binding of this serum with the F protein was obscured in the photographic reproduction process. Lane 6, Major viral polypeptides precipitated by the positive serum. Additional minor bands detected are probably fusion glycoprotein glycosylation or cleavage products F1 and F2 (4, 11).

Previous studies in this laboratory (12, 13, 15, 16, 18, 19) and elsewhere (1, 2, 6) delineated the essential features of disease in CDV-infected dogs and identified variables which influence both the course and nature of CDV-associated lesions in the brain. The rapid and fatal course of disease that occurred in the group 1 dogs corre-

FIG. 2. Humoral immune response to radiolabeled CDV structural polypeptides in fatally infected group 1 dogs. Antibody to the CDV H and P proteins was not detected. Only modest levels of antibody (0 to 2+) to the other virion polypeptides were documented.

FIG. 3. Humoral immune response to radiolabeled CDV structural polypeptides in persistently infected group 2 dogs. Antibody to the CDV H, P, and M proteins was not detected in any of the samples until PIW 3. Moderate (P protein) to strong (M protein) responses to these virion polypeptides did not occur until PIW 6 or 7.

FIG. 4. Humoral immune response to radiolabeled CDV structural polypeptides in convalescent group 3 dogs. Response to all of the virion polypeptides except H was immediate (PIW 1 or 2) and strong (4 to 5+) in this group.
persistently infected group 2 dogs. Study of these serum samples by the complement-fixing antibody test suggested that the relative lack of antibody to ether-sensitive envelope antigen is a characteristic serological marker of persistence (16). The paucity of response to the H and F proteins by this group early in the course of the disease compared with that of group 3 dogs supported this hypothesis. More intriguing than this, however, was the delay in the appearance of significant quantities of antibody to the M protein (and to a lesser extent the P protein) until PIW 5 to 7 and after at least 4 weeks of CDV residence and replication within the brain (24). In this respect the pattern of antibody responses during this time resembled the pattern of response in subacute sclerosing panencephalitis (SSPE), a measles virus-associated chronic fatal neurological disease of humans (20, 21). SSPE patients apparently lack antibody to the measles virus M protein in spite of showing exaggerated responses to other measles virus virion proteins (7, 10). Although several explanations for this have been offered, it seems likely that the measles virus variants of SSPE lack the ability to synthesize the M protein (7, 8, 17). Dogs destined to develop persistent infection apparently go through a phase of infection serologically resembling SSPE. This infection differs from SSPE in the ultimate development by the dogs of antibodies to all of the virion proteins, including M. This distinction between SSPE and persistent CDV infection has been made by others (9).

Thus, our data suggest that either a transient lack of production of the M protein by the virus in vivo or, more likely, a lack of prompt development of antibody to the M protein is characteristic of persistent infection and may be a critical event in the in vivo evolution of persistent paramyxovirus infection in the brain. If this hypothesis is true, then further studies on the role of this protein in the establishment or maintenance of persistent infection by CDV seem to be warranted.

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LITERATURE CITED