NOTES

Carbohydrate Side Chains of Rauscher Leukemia Virus Envelope Glycoproteins Are Not Required to Elicit a Neutralizing Antibody Response†

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Antisera raised against Rauscher leukemia virus (R-MuLV) contain a preponderance of antibodies against glycoprotein gp70 that are dependent on the presence of carbohydrate side chains for reactivity, as judged by immunoprecipitation or Western blotting. However, the majority of neutralizing antibodies were not dependent on the presence of carbohydrate, as indicated by (i) the ability of deglycosylated R-MuLV to adsorb neutralizing antibody from sera as efficiently as glycosylated R-MuLV and (ii) the ability of deglycosylated R-MuLV to induce neutralizing antibody responses when injected into rabbits. Moreover, a faster response was obtained with deglycosylated R-MuLV than with untreated control virus in the latter experiments. The results indicate that the neutralizing antibodies are a discrete subpopulation of the total antibody response. Furthermore, the carbohydrate moieties appear to afford protection to the virion during infection, rather than serve as a target for neutralization.

Oligosaccharides are an integral part of the structure of many viral and eucaryotic cell surface proteins. The precise function(s) of the carbohydrate moieties of these proteins remains unknown, although they may play significant roles in specific recognition phenomena such as the interaction of cells with other cells or the extracellular matrix (7, 8, 11, 16). In the case of the retroviruses, interest has focused on the envelope glycoproteins (gp70s), which contain multiple oligosaccharide side chains, because of the role of these glycoproteins in target cell recognition and the determination of host range of the virus (3, 13). gp70 is the primary target for neutralizing antibodies and contains group, type, and interspecies determinants which allow delineation of subtypes *in vitro* (5, 9, 13).

We have been interested in exploring the relative contribution of the carbohydrate and protein portions of these gp70 molecules (as well as other viral glycoproteins) in generating the neutralizing immune response to these viruses (1). Until recently, these studies have been hampered by the lack of reagents for removing carbohydrate from the glycoproteins without proteolysis or modification of the polypeptide backbone. The discovery of an endoglycosidase (endo F [4]) that efficiently cleaves both high-mannose and complex asparagine-linked carbohydrates from glycoproteins has allowed us to test directly certain aspects of this problem. We previously reported that the carbohydrate side chains of retroviral and influenza virus glycoproteins have a dramatic influence on the immune reactivity of antisera prepared to these antigens (1). Antisera assayed by Western blotting or immunoprecipitation were not reactive with gp70 after deglycosylation. The results of this study were consistent with the interpretation that carbohydrate side chains may serve as immune decoys to direct the immune response

toward epitopes influenced directly or indirectly by oligosaccharides. In addition, the carbohydrate side chains can effectively block the binding of antibodies to polypeptide sequences around their site of attachment. Thus the carbohydrate of the viral glycoproteins may play a role in the survival of the virus during infection.

The present studies specifically investigate the involvement of the carbohydrate side chains of retrovirus gp70 molecules in the generation of a neutralizing response to these viruses. First, we found that Rauscher murine leukemia virus (R-MuLV), deglycosylated with endo F, was as capable as untreated virus in adsorbing neutralizing antibodies from hyperimmune antisera. This is a direct demonstration that only the protein part of the envelope glycoprotein of the virus elicits the humoral neutralizing immune response. Second, we found that deglycosylated R-MuLV, when used as an immunogen, was more efficient at eliciting a virus-neutralizing response in rabbits than was the respective untreated virus.

Figure 1 shows the results obtained from adsorption experiments with control and deglycosylated R-MuLV. Deglycosylated virus was generated with endo F (4). Complete deglycosylation was verified by Western blotting of the control and deglycosylated virus preparations (data not shown). All gp70 was reduced to a molecular weight of 49,000, consistent with the removal of virtually all carbohydrate (1, 4). Goat hyperimmune serum prepared to intact R-MuLV (obtained from the Resources Branch of the National Cancer Institute) was then sequentially adsorbed by four treatments with either control or deglycosylated R-MuLV (Fig. 1). The neutralizing activity of the serum was assayed after each adsorption step. Both the control and deglycosylated virus preparations were able to adsorb neutralizing activity with equal efficiency, even though the majority of the antibody response to the surface glycoprotein gp70 in this antiserum failed to recognize the deglycosylated antigen (1). These findings indicate that the virus-

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FIG. 1. Neutralization of R-MuLV by antiserum adsorbed with control and deglycosylated virus. R-MuLV (500 μ g, obtained from the Resources Branch of the National Cancer Institute) was treated with endo F (10 U) in phosphate-buffered saline at 37°C for 16 h as described previously (4). An identical, untreated sample served as a control. Samples were taken and analyzed to show that the endo F sample was completely deglycosylated, whereas the control was unchanged (data not shown). The viruses were inactivated with UV light for 5 min and were shown to be incapable of infection and replication in tissue culture cells. Goat antibodies to R-MuLV (1) were diluted in tissue culture medium (1:100) and were adsorbed for 1 h at room temperature with 50- μ g samples of control or deglycosylated R-MuLV. Serum samples were removed, and the adsorption process was repeated four additional times. Each of the adsorbed serum samples was tested for the presence of neutralizing antibodies with a plaque reduction assay (6, 10) on mouse SC-1 cells. Panels: 1X, first time adsorbed with either control (○) or deglycosylated (×) virus; 2X, 3X and 4X, sequential adsorptions. The unadsorbed serum (▲) is shown in panel 1X.

neutralizing antibodies in this antiserum are a discrete subpopulation of the total antibody response to the viral glycoprotein. They do not constitute an appreciable portion of the antibodies detected by Western blotting. Furthermore, these neutralizing antibodies are not directed against carbohydrate, or polypeptide epitopes influenced by the presence of carbohydrate, since they were adsorbed by both untreated and deglycosylated R-MuLV.

As an additional approach to examining the role of carbohydrate in the antiviral neutralizing response, we directly tested the relative efficacy of glycosylated and deglycosylated viral antigens to elicit neutralizing antibodies when used as immunogens (Fig. 2). Rabbits were immunized with either control or deglycosylated R-MuLV. Sera were collected after the initial immunization and a series of boosts, and the neutralizing titers of these samples were determined as described previously (6). The neutralizing titer of the antiserum produced against the deglycosylated R-MuLV preparations was significant after the first immunization and slowly increased in titer through three additional boosts. In contrast, sera obtained after immunization with glycosylated (control) R-MuLV had no detectable neutralizing activity after the initial immunization. Subsequent boosts with the glycosylated R-MuLV raised the neutralizing titers of the antisera until they were essentially identical to those from the animals immunized with deglycosylated R-MuLV. We have obtained similar results in experiments where control and deglycosylated feline leukemia virus were used as immunogens (data not shown). Thus, the primary effect observed with deglycosylated R-MuLV was a much more rapidly mobilized neutralizing response. Moreover, the data demonstrate that the carbohydrate moieties of the R-MuLV gp70 antigens are not required to elicit a neutralizing immune response.

The data presented in this report, taken together with our previous observations (1), support the idea that the carbohydrate moieties of viral glycoproteins have a major influence on the immune response to these molecules. Specifically, we now have shown that the carbohydrate side chains of the R-MuLV gp70 molecules act as immune decoys in the sense that the antibodies directed toward the carbohydrate side chains of the R-MuLV gp70 molecules (which account

FIG. 2. Neutralizing antibodies generated by immunization with control and deglycosylated R-MuLV. R-MuLV virions were disrupted using 1% Nonidet P-40–1% 2-mercaptoethanol–50 ml of EDTA–0.1% sodium phosphate buffer (pH 6.1). Half of the preparation was deglycosylated with endo F (4) overnight at 37°C. The samples were tested by sodium dodecyl sulfate-polyacrylamide gel electrophoresis to confirm that deglycosylation was complete. Rabbits were immunized with 50 μ g of control or deglycosylated virus in complete Freund adjuvant. The rabbits were boosted at 8-week intervals with incomplete Freund adjuvant. Serum samples were taken after boosts and tested for their ability to neutralize R-MuLV by using a plaque reduction assay (6, 10). The results are representative of the two independent experiments and demonstrate that neutralizing antibodies can be raised to deglycosylated antigens and that the response to the deglycosylated antigen appears more rapidly than that toward the corresponding glycosylated antigen.

for the majority of the antibody response to R-MuLV gp70) are not responsible for neutralizing the virus on which these glycoproteins reside. Instead, the neutralizing response is apparently mediated by a relatively small subpopulation of antibodies directed toward polypeptide determinants. These results are supported by the studies of others (17), where monoclonal antibodies were used to select variants of influenza virus and these variant viruses were protected from neutralization by the addition of an additional carbohydrate side chain at a newly formed site of glycosylation. Thus, the immune response to the virus can be influenced by attached carbohydrate without the direct involvement of the oligosaccharide as an epitope (17). In addition, partial deglycosylation with either neuraminidase or a mixture of glycosidases did not reduce the ability of treated Friend murine leukemia virus to adsorb neutralizing activity from antiserum (14).

Studies of bovine leukemia virus (2, 12, 15) have reported similar observations to the results reported here with murine leukemia viruses. The major immune reactivity in neutralizing sera of infected cattle, as monitored by radioimmunoassay, was heavily dependent on carbohydrate for reactivity, leading to the conclusion that carbohydrate was necessary for virus neutralization (12, 15). However, further studies with monoclonal antibodies against the surface glycoprotein gp51 (2) revealed that the neutralizing epitopes reside on a nonglycosylated 15,000-molecular-weight proteolytic fragment. Antibodies to epitopes on the glycosylated 35,000-molecular-weight fragment had no influence on viral infectivity. Thus, as we report here for the murine viruses, the major immunological response in cattle to bovine leukemia virus is against carbohydrate moieties or epitopes under their influence. However, the neutralizing antibodies are a discrete subset which is independent of carbohydrate.

Our findings support the idea that the carbohydrate side chains on the surface glycoproteins of the retroviruses may afford a significant degree of protection from immune surveillance during a primary virus infection.

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