Immunization against Multiple Viruses by Using Solid-Matrix–Antibody–Antigen Complexes

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We have previously shown that mice immunized with solid-matrix–antibody–antigen (SMAA) complexes in the absence of adjuvants show vigorous humoral and cell-mediated immune responses to the immunizing antigen. Here we report that various proteins involved in inducing protective immune responses to different viruses can easily and simply be incorporated into SMAA complexes and that such complexes act as powerful multivalent immunogens. Construction of such SMAA complexes may be one of the most practical and effective ways of producing multivalent subunit vaccines for use in humans and animals.

There are numerous advantages in developing subunit vaccines based on proteins capable of eliciting protective immune responses. However, a number of major problems need to be overcome before such vaccines can be successfully produced. The antigens involved in inducing protective immunity need to be identified, a good source of these antigens is required, and then procedures need to be developed to purify the antigens. In addition, immunization schemes need to be devised for presenting the antigens to the immune system in such a way as to induce protective immunity. To produce successful vaccines to some viruses, it may be necessary to induce both virus-neutralizing antibody and specific T-cell responses, and since the major target antigens for B and T cells may reside on different virus proteins, multiple virus antigens may need to be incorporated into such vaccines. Furthermore, since the major target antigens for T cells may be different from individual to individual, depending on major histocompatibility complex status (1, 3, 5, 7), different proteins from the same virus may need to be incorporated into the vaccine to ensure appropriate immune responses in all individuals of a heterozygous population. Also, since vaccines tend to protect against disease rather than infection, it may be beneficial to induce a broad immune response, thereby ensuring a rapid secondary immune response upon subsequent infection with a virulent virus. Thus, for example, induction of a broad T-helper cell response may be advantageous, particularly since it has been demonstrated that T-helper cells which recognize antigenic determinants on internal virus proteins may help B-cell responses to surface proteins present on the same virus particle through the mechanism of B-cell antigen presentation (8, 15, 17). Besides these arguments for multivalent vaccines, it would, if possible, be both economically desirable and practically convenient to incorporate into subunit vaccines the appropriate proteins from a variety of infectious agents. In our study of immunity to paramyxoviruses, we have been developing an immunization scheme which may permit the development of such multivalent subunit vaccines (11, 12). The basis of the immunization scheme is to attach monoclonal antibodies to particulate solid matrices; the solid-matrix–antibody complexes are then used to purify the proteins of interest (ideally in such a way as to saturate the antibody with the antigen), and the resulting solid-matrix–

antibody–antigen (SMAA) complexes are used as immunogens. We have shown that immunization of mice with such SMAA complexes in the absence of adjuvants results in induction of vigorous humoral and cell-mediated immune responses (11).

Here we report that SMAA complexes can be very easily constructed to produce multivalent immunogens containing various virus glycoproteins known to be involved in inducing protective immunity from a number of different viruses. The protein A-immunoglobulin Fc-binding sites on fixed and killed suspensions of *Staphylococcus aureus* Cowan strain A (6) were saturated with a mixture of protein A-binding monoclonal antibodies (in approximately equimolar ratios) specific for glycoprotein D of herpes simplex virus (HSV) (LP14; 9), the HA proteins of influenza virus (HC245) and measles virus (B10; 16), and the HN proteins of simian virus 5 (SV5) (SV5-HN-4a; 13) and parainfluenza virus type 2 (PIV2) (PIV2-HN-3a; 14). The unbound antibody was removed by suspension and sedimentation ($2,500 \times g$ for 3 min) of these complexes three times in phosphate-buffered saline. These strain A-antibody complexes were used to purify the respective proteins from lysates of virus (measles virus, HSV, PIV2, and SV5)-infected tissue culture cells and from detergent extracts of purified influenza virus. Soluble antigen extracts were made from SV5-, PIV2-, or HSV-infected BHK cells and from measles virus-infected Vero cells as previously described (13). A detergent extract was also made from purified influenza virus by incubating the virus with extraction buffer (20 mM Tris hydrochloride [pH 7.2], 5 mM EDTA, 0.5% Nonidet P-40, 0.1% sodium dodecyl sulfate, 0.65 M NaCl) for 30 min at $+4^{\circ}\text{C}$. The particulate material was then removed by centrifugation at $100,000 \times g$ for 30 min. These extracts were then mixed together before being incubated with the solid-matrix–antibody complexes (20 μl of a 10% [wt/vol] suspension of solid-matrix–antibody complexes per 2×10^8 cell equivalents of each antigen extract or ~ 1 mg of influenza virus extract) for 4 to 6 h at $+4^{\circ}\text{C}$. The resulting SMAA complexes were washed three times by sedimentation from ($3,300 \times g$ for 3 to 10 min) and resuspension in extraction buffer and washed twice in phosphate-buffered saline. Estimations of the amounts of virus proteins in SMAA complexes were made by sodium dodecyl sulfate-polyacrylamide electrophoretic analysis of the complexes as previously described (11). Groups of three mice were immunized twice intraperitoneally without adjuvants

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FIG. 1. Analysis of [^{35}S]methionine-labeled polypeptides from precipitates formed by the reaction of soluble antigen extracts of SV5 (lanes 2 and 6)-, PIV2 (lanes 3 and 7)-, measles virus (MV) (lanes 4 and 8)-, and HSV (lanes 5 and 9)-infected tissue culture cells with monoclonal antibodies specific for the HN protein of SV5 (lane 2), the HN protein of PIV2 (lane 3), the HA protein of measles virus (lane 4), and glycoprotein D (gD) of HSV (track 5) or with polyclonal sera from mice immunized with SMAA complexes that contained HSV glycoprotein D, the HN proteins of SV5 and PIV2, and the HA proteins of measles virus and influenza virus (lane 6 to 9). Lane 1 shows the polypeptides present in the precipitate formed by the reaction of a mixture of SV5, PIV2, measles virus, and HSV soluble antigen extracts and the mouse polyclonal sera. The polypeptides present in the precipitates were separated by electrophoresis through a 12% polyacrylamide slab gel and the radioactively labeled polypeptides visualized by autoradiography. Soluble antigen extracts were made from [^{35}S]methionine-labeled infected-cell extracts, and immune precipitates were formed as previously described (13). The positions of the virus proteins are indicated on the left.

with the resulting SMAA complexes that contained 1 to 5 μg of each of the various virus proteins with a gap of 3 to 4 weeks between immunizations 1 and 2. At 10 days after immunization 2, the mice were bled and the sera were tested for the ability to precipitate virus proteins from infected tissue culture cells (Fig. 1). The sera from mice immunized with such complexes precipitated only the appropriate virus proteins. These sera were also tested for the ability either to neutralize virus infectivity (HSV, measles virus, PIV2, and SV5; 14) or to react with purified influenza virus attached to sheets of nitrocellulose ($\sim 100 \mu\text{g}$ of purified virus per sheet [7 by 5 cm]) in a radioimmunoassay (11). These results showed that sera from immunized mice had high titers of antibodies to all of these viruses (Table 1).

TABLE 1. Virus neutralization by sera from immune and nonimmune mice and anti-influenza virus antibody titers of these sera

Serum ^a	Neutralization titer				Anti-influenza virus antibody titer ^b
	HSV	Measles virus	PIV2	SV5	
1	>1/1,280	>1/1,280	1/2,560	>1/1,280	1/640
2	1/1,280	1/2,560	1/2,560	1/1,280	1/640
3	1/1,280	1/2,560	1/2,560	1/1,280	1/640
4	<1/20	<1/20	<1/20	<1/20	<1/20

^a Sera 1 to 3 were from immune mice, and serum 4 was from a nonimmune mouse.

^b Determined by radioimmunoassay.

Antigen-antibody complexes have been previously used as immunogens for the preparation of antisera of defined specificity and to enhance immune responses to specific proteins in experimental animals (4, 10, 18). In addition, it has been recently reported that there is an enhanced immune response in human volunteers to the gp120 protein of human immunodeficiency virus when a fragment of this protein is coupled to a specific mouse monoclonal antibody before immunization (2). However, the use of SMAA complexes as immunogens may have a number of practical advantages over the use of antibody-antigen complexes as immunogens. Thus, the solid-matrix-antibody complexes are used to purify the proteins of interest, as well as being part of the immunogen. We have also previously shown that immunization of mice with SMAA complexes without adjuvants results in induction of vigorous humoral and cytotoxic T-cell responses (11) and that mice immunized in this manner with either internal or external structural proteins of SV5 are protected from subsequent infection with this virus (12). Here we report that by coupling different monoclonal antibodies to the same solid matrix and by using the solid-matrix-antibody complex to purify the appropriate proteins it is a relatively simple procedure to produce multivalent immunogens that contain antigens involved in inducing protective immunity against a variety of different pathogenic viruses. Furthermore, by incorporating different antigens from the same microbe into the complex it is possible to produce immunogens that contain major antigens involved in inducing T-cell responses together with conformational determinants required for the induction of an appropriate antibody response (11, 12). However, before the efficacy of SMAA complexes as subunit vaccines can be tested in humans, biodegradable microcarriers other than strain A may need to be designed. The use of human or human-rodent chimeric monoclonal antibodies as the linking antibodies between the solid matrix and the antigen may also be desirable. The specific design and manufacture of SMAA complexes containing the appropriate proteins from various infectious agents may be one of the most practical and effective methods of producing multivalent subunit vaccines for use in humans and animals.

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