Syngeneic monoclonal anti-idiotypic antibody Ab2,2-17C3SCC was raised against an idiotope on a protective monoclonal antibody with specificity for poliovirus type II. Ab2,2-17C3SCC detects a paratope-related interspecies IdX. Ab2,2-17C3SCC purified from supernatant fluids of hybridoma cells by protein A-Sepharose was injected into 4- to 6-wk-old BALB/c mice. The sera of the mice were screened for the expression of antibodies bearing the corresponding idiotope. Immunization of mice with Ab2,2-17C3SCC induced antibodies of complementary specificity. Furthermore, micro VN tests suggest that Ab2,2-17C3SCC can substitute for antigen in the induction of anti-polio neutralizing antibodies, and hence can function as a monoclonal anti-idiotypic vaccine.

In a number of systems, anti-idiotypic antibody (Ab2) exogenously administered in animals has been able to replace antigen for the induction of specific immune responses by priming idotype-positive, antigen-specific clones (1, 2). The most fascinating observation in this connection is the demonstration that immunization of mice against infection with Trypanosoma rhodesiense could be accomplished by the administration of polyclonal allogeneic Ab2 raised against the idiotypes of a protective monoclonal antibody with specificity for the major glycoprotein of a clone of this parasite (3). In this system, the Ab2 did not appear to represent an internal image of a parasite epitope, because the immunity induced by Ab2 was genetically restricted (4). An example of Ab2 functioning as a true internal image of an epitope specificity. Furthermore, we were able to demonstrate that a single inoculation of neutralizing anti-poliovirus monoclonal antibody could protect mice against a lethal challenge with live poliovirus (manuscript in preparation). The results of this study indicate primarily that Ab2,2-17C3SCC can substitute for antigen for the induction of specific immune responses by priming idiotype-positive, antigen-specific clones (1, 2). The most fascinating observation in this connection is the demonstration that immunization of mice against infection with Trypanosoma rhodesiense could be accomplished by the administration of polyclonal allogeneic Ab2 raised against the idiotypes of a protective monoclonal antibody with specificity for the major glycoprotein of a clone of this parasite (3). In this system, the Ab2 did not appear to represent an internal image of a parasite epitope, because the immunity induced by Ab2 was genetically restricted (4). An example of Ab2 functioning as a true internal image of an epitope.

In principle, however, the use of polyclonal xenogeneic or allogeneic Ab2 preparations for this purpose has several disadvantages. First, it would be very difficult to establish production of polyclonal Ab2 preparations of high consistency and identity. Furthermore, a polyclonal Ab2 preparation will contain a heterogeneous population of anti-idiotypic antibodies in which only a small fraction induces idotype-bearing molecules that will also bind antigen (3). Monoclonal Ab2 possessing a related epitope would represent the ideal anti-idiotypic vaccine. On the other hand, the unique specificity of monoclonal Ab2 could be a major drawback for its use as a vaccine, because such a vaccine will induce antibody of unique epitope specificity.

The present study describes the induction of neutralizing antibody against poliovirus type II in mice by treatment with a syngeneic monoclonal Ab2. A series of monoclonal Ab2 was raised against various murine monoclonal Ab1 with specificity for neutralization-inducing epitopes of the MEF1 strain of poliovirus type II (6, 7). This poliovirus system was chosen because the assumed limited number of neutralization-inducing epitopes on polioviruses would overcome the theoretical drawback of using monoclonal Ab2 as a vaccine (8, 9). Furthermore, antibody-mediated immunity is probably the major component of the protective immune response against infection. Additionally, we were able to demonstrate that a single inoculation of neutralizing anti-poliovirus monoclonal antibody could protect mice against a lethal challenge with live poliovirus (manuscript in preparation). The results of this study indicate primarily that Ab2,2-17C3SCC can substitute for antigen for the induction of specific immune responses by priming idiotype-positive, antigen-specific clones (1, 2). The most fascinating observation in this connection is the demonstration that immunization of mice against T. rhodesiense could be accomplished by the administration of polyclonal allogeneic Ab2 raised against the idiotypes of a protective monoclonal antibody with specificity for the major glycoprotein of a clone of this parasite (3). In this system, the Ab2 did not appear to represent an internal image of a parasite epitope, because the immunity induced by Ab2 was genetically restricted (4). An example of Ab2 functioning as a true internal image of an epitope specificity. Furthermore, we were able to demonstrate that a single inoculation of neutralizing anti-poliovirus monoclonal antibody could protect mice against a lethal challenge with live poliovirus (manuscript in preparation). The results of this study indicate primarily that Ab2,2-17C3SCC can substitute for antigen for the induction of specific immune responses by priming idiotype-positive, antigen-specific clones (1, 2). The most fascinating observation in this connection is the demonstration that immunization of mice against infection with T. rhodesiense could be accomplished by the administration of polyclonal allogeneic Ab2 raised against the idiotypes of a protective monoclonal antibody with specificity for the major glycoprotein of a clone of this parasite (3). In this system, the Ab2 did not appear to represent an internal image of a parasite epitope, because the immunity induced by Ab2 was genetically restricted (4). An example of Ab2 functioning as a true internal image of an epitope specificity.
ELISA for the detection of cross-reactivities with monoclonal Ab1. ELISA plates (Titertek type III; Flow Laboratories Inc, McLean, VA) were coated with 150 μl poliovirus type II, strain MEF1 antibodies (45 μg/ml) diluted in carbonate buffer, pH 9.6, by incubation at 20°C for 1 hr. Phosphate-buffered saline (PBS)-Tween 0.05% (Tween-80; Merck, Darmstadt, F.R.G.) was used for washing between each step. One hundred microliters/well of saline was added, and the plates were incubated at 37°C for 2 hr. After washing, monoclonal anti-poliovirus type II antibodies or control monoclonal BALB/c IgG2a,κ was added to the plates and the plates were incubated for 2 hr at 37°C. The plates were washed and then incubated with horseradish peroxidase-conjugated anti-poliovirus type II monoclonal Ab1.1-10C9E8 and tetramethyl-benzidine substrate. The reaction was stopped with 2 N H₂SO₄ (14). Absorbance at 450 nm was read with a Titertek Multiskan (Flow Laboratories).

Inhibition of idiotypic cross-linking ELISA. The detection of monoclonal anti-idiotypic antibody, Fab′2 preparations were prepared from protein-A-Sepharose-purified Ab1.1-10C9E8 (IgG2a,κ) control murine monoclonal IgG2a,κ (T1), and Ab2.2-17C3SCC (IgG1) by pepsin digestion. Purified IgG was dialyzed against saline overnight at 4°C. The solution was adjusted to pH 3.8 with 0.1 N HCl. Fifty units of pepsin (Worthington Inc., Freehold, NJ) were added, and the plates were incubated at 37°C for 2 hr. After washing, monoclonal anti-poliovirus type II antibodies or control monoclonal BALB/c IgG2a,κ was added to the plates and the plates were incubated for 2 hr at 37°C. The plates were washed and then incubated with horseradish peroxidase-conjugated anti-poliovirus type II monoclonal Ab1.1-10C9E8 and tetramethyl-benzidine substrate. The reaction was stopped with 2 N H₂SO₄ (14). Absorbance at 450 nm was read with a Titertek Multiskan (Flow Laboratories).

Detection of paratope-related idiotopes. Inhibition of the binding of horseradish peroxidase-labeled Ab2.2-17C3SCC (16) to its homologous plate-bound Ab1.1-10C9E8 by poliovirus was considered to be indicative for site specificity of the Ab1.1-10C9E8 idiotypic. Serial dilutions of poliovirus types I, II, or III were transferred to Ab1.1-10C9E8 Fab′2-coated ELISA plates. Controls were performed by incubation with diluent without virus. After incubation, 100 μl/well of horseradish peroxidase-labeled Ab2.2-17C3SCC were added. Plates were finally developed as described. The percentage inhibition was expressed as indicated above.

Measurement of neutralizing anti-poliovirus antibodies. Mouse sera were incubated in wells of microtiter plates (Titertek type III, Flow Laboratories) linked to peroxidase (16). Plates were developed with anti-mouse IgG, with Fc fragment-specific IgG, and with protein-A (Titertek type III. Flow Laboratories) for 2 hr at 37°C. The plates were washed and the remaining binding sites were blocked as described above. Fab′2 preparations were determined by peroxidase-conjugated sheep anti-mouse Ig (Amersham, Inc., Buckinghamshire, England), whereas the undegraded IgG response was determined with peroxidase-linked goat anti-mouse Ig, with Fc fragment-specific IgG, and with protein-A (Pharmacia) linked to peroxidase (16). Plates were developed with tetramethyl-benzidine substrate, and the reaction was stopped with 2 N H₂SO₄. Absorbance was read at 450 nm in a Titertek Multiskan (Flow Laboratories) using all of the Fab′2 preparations used in the present study. Undegraded IgG was not detectable.

RESULTS

Monoclonal antibodies against polioviruses. Monoclonal antibodies against polioviruses (Ab1) were selected from a large panel on the basis of their broad reactivities with poliovirus type II strains as tested in the micro VN test (6, 7). Reaction patterns of these monoclonal antibodies in the VN test are shown in Table 1. The results suggested that these monoclonal antibodies react with the same viral epitope. This was confirmed by the observation that the binding of one monoclonal inhibits the binding of another to the virus (Fig. 1). One of these monoclonal antibodies, Ab1.1-10C9E8, which has the isotype IgG2a,κ, was used subsequently to generate syngeneic monoclonal Ab2 in BALB/c mice.

Preparation of monoclonal Ab2. In three independent experiments, spleen cells of mice immunized against Ab1.1-10C9E8 were fused. Five clones were obtained that showed reactivities in the idiotopic cross-linking ELISA. Culture supernatant fluids of these hybridomas.
cloned by limiting dilution, contained antibody activity against idiotopes of Ab1,1-1OC9E8, and none showed binding to normal BALB/c Ig-coated plates. Clone 2-17C3, in particular, reacted strongly with Ab1,1-1OC9E8, and appeared to be a stable producer. 2-17C3 was recloned by manual isolation of single cells (SCC). Subsequent studies were performed with F(ab')2 of protein A-purified culture supernatant fluids of Ab2,2-17C3SCC, the iso-type that was found to be IgG1. The results of these experiments show conclusively that Ab2,2-17C3SCC bound only to Ab1,1-1OC9E8 and not to control BALB/c IgG2a,x (Fig. 2A). Furthermore, no binding of control BALB/c IgG1 to Ab1,1-1OC9E8 or to control BALB/c IgG2a,x was observed.

Characterization of Ab2,2-17C3SCC. To determine whether Ab2,2-17C3SCC was capable of interacting with the Ab1 paratope-related idiotope, the inhibition of the horseradish peroxidase-conjugated Ab2,2-17C3SCC [16] to the purified F(ab')2 of protein A-purified poliovirus type I, II, and III was carried out. Figure 2B shows that binding could indeed be inhibited by 5 μg of intact 155S particles of poliovirus type II, whereas comparable amounts of 155S particles of poliovirus types I or III did not affect the binding of Ab2,2-17C3SCC to Ab1,1-1OC9E8. Although on the basis of these results the possible influence of steric hindrance cannot be ruled out completely, the data suggest that Ab1,1-1OC9E8 possesses a paratope-related idiotope with complementarity for Ab2,2-17C3SCC.

Competitive inhibition studies were also carried out to determine whether Ab2,2-17C3SCC could recognize a structure uniquely associated with Ab1,1-1OC9E8 or a cross-reactive determinant. The results are shown in Figure 2C. Preincubation of Ab2,2-17C3SCC with its homologous Ab1,1-1OC9E8 idiotope induced a dose-related inhibition pattern in the idiotope cross-linking ELISA, as did two other anti-poliovirus type II monoclonal antibodies. Other anti-poliovirus type I, II, and III monoclonal antibodies and a control monoclonal antibody (IgG2a,x) failed to inhibit the binding of Ab2,2-17C3SCC to its homologous idiotope Ab1,1-1OC9E8 (Fig. 2C). These results suggest that Ab2,2-17C3SCC may identify a cross-reactive idiotope. In experiments with the use of Ab2,2-17C3SCC as a probe to screen conventional poliovirus-neutralizing antisera of several species including man, evidence was obtained that Ab2,2-17C3SCC detects an interspecies cross-reactive idiotope. The results of these experiments are depicted in Figure 2D. Cross-linking of Ab1,1-1OC9E8 by Ab2,2-17C3SCC could be inhibited by previous incubation of Ab2,2-17C3SCC with the sera of poliovirus type II-immune sera, mice, guinea pigs, and humans. Preimmune sera showed no inhibition in this assay. The reactivity of monoclonal Ab2 with immune sera from different species including humans can also.

**Figure 1.** Inhibition of the binding of peroxidase-conjugated Ab1,1-1OC9E8 to plate-bound poliovirus type II by the homologous and two other heterologous monoclonal anti-poliovirus type II antibody preparations. Samples Ab1,1-1OC9E8 (A), Ab1,1,11E7 (B), Ab1,4-15D4D8 (C), and control BALB/c IgG2a,x (D) were reacted with plate-bound poliovirus, as described in Materials and Methods. Control BALB/c IgG2a,x, used as an inhibitor, resulted in OD 450 nm values comparable with values obtained with PBS-Tween 0.05% as an inhibitor.

**Figure 2.** (A) Detection of anti-idiotope in idiotope cross-linking ELISA. F(ab')2 of protein A-purified supernatant fluids of anti-idiotope-producing hybridoma cell line 2-17C3SCC (O) or control BALB/c IgG2a,x (D) were incubated on ELISA plates coated with either idiotope Ab1,1-1OC9E8 (open symbols) or control BALB/c IgG2a,x (closed symbols). Plates were additionally developed with the horseradish peroxidase-labeled idiotope Ab1,1-1OC9E8. Similar results were obtained in seven separate experiments. (B) Detection of paratope-related idiotope. Inhibition of binding of horseradish peroxidase-labeled anti-idiotope 2-17C3SCC to plate-bound idiotope 1-1OC9E8 by poliovirus type I (O), type II (D), and type III (D). Data are presented as the mean ± SE of triplicate determinations. (C) Detection of cross-reactive idiotope. Inhibition of anti-idiotope cross-linking ELISA by incubation of anti-idiotope 2-17C3SCC with neutralizing anti-poliovirus type II monoclonal antibodies; 1-1OC9E8 (O), 11E7 (D), 4-15D4D8 (D), or other monoclonal antibodies; and nonneutralizing anti-poliovirus type II (O), neutralizing anti-poliovirus type I (S), neutralizing anti-poliovirus type III (O), and control BALB/c IgG2a,x (D). Similar results were obtained in three separate experiments. (D) Detection of interspecies cross-reactive idiotope. Inhibition of idiotope cross-linking ELISA by incubation of anti-idiotope 2-17C3 with anti-poliovirus type II hyperimmune sera (closed symbols) of rats (A), guinea pigs (B), mice (C), and humans (D), or their respective preimmune sera (open symbols). One panel of representative data is given from experiments in which sera were tested from 12 rats, two guinea pigs, 12 mice, and 12 humans.
be interpreted to mean that these molecules possess a true internal image of a viral epitope (2, 18, 19).

Generation of Ab3 in BALB/c mice by immunization with Ab2,2-17C3SCC. Because Ab2,2-17C3SCC possibly bears a poliovirus-related epitope, experiments were performed to induce protective immunity against poliovirus by immunizing mice with different doses of Ab2,2-17C3SCC. Screening of sera from mice after Ab2,2-17C3SCC treatment but before challenge with poliovirus revealed evidence for the presence of Ab3 in all sera tested in the Ab2,2-17C3SCC cross-linking ELISA (Table II). The sera of control mice that had been treated with the protein equivalent of normal BALB/c IgG showed no detectable reactivity. The antigen-binding capacity of all sera was investigated by using the micro VN assay. As shown in Table II, four of eight mice, all expressing Ab3 after Ab2,2-17C3SCC treatment, were able to neutralize the MEFl strain of poliovirus type II. The sera of control mice did not exert neutralizing activity. Taken together, these results strongly indicate that treatment of BALB/c mice with Ab2,2-17C3SCC induced the expression of the Ab1,1-10C9E8 idiotype.

Although Ab2,2-17C3SCC was capable of inducing poliovirus-neutralizing antibody in mice, it failed to protect BALB/c mice against a lethal challenge with the MEFl strain of poliovirus type II. In contrast, a single inoculation of Ab1,1-10C9E8 in BALB/c mice proved to be protective against a lethal challenge with poliovirus (Table II). The relatively low levels of serum-neutralizing antibody in Ab2,2-17C3SCC-treated mice as compared with Ab1,1-10C9E8-treated animals may explain this observation. Studies are in progress to improve the immune response to Ab2,2-17C3SCC in mice by using newly developed methods of antigen presentation.

**DISCUSSION**

We have demonstrated that immunization of mice with a monoclonal syngeneic Ab2,2-17C3SCC directed against a neutralizing anti-poliovirus type II monoclonal antibody, Ab1,1-10C9E8, without antigen, induces an Ab3 response, of which at least part consists of a population of antibody that shares two properties with Ab1,1-10C9E8: the binding of poliovirus type II strain MEFl (Table II) and the binding to Ab2,2-17C3SCC (Table II). Ab2,2-17C3SCC defines not only a paratope-related idiotope on Ab1,1-10C9E8 but also idiotopes on two other neutralizing anti-poliovirus type II monoclonal antibodies (Fig. 2C). This finding is in agreement with the results of the inhibition studies shown in Figure 1, which indicate that all three neutralizing monoclonal antibodies react with the same epitope on poliovirus type II. Thus, Ab2,2-17C3SCC probably defines an idX that constitutes a major component of the immune response of mice to poliovirus type II. Tesch et al. (20) and Takekoshi et al. (21) suggested that if Ab2 recognizes an idiotope on Ab1 that is formed by many germline-encoded Vh-D-Vk combinations, immunization with Ab2 would induce antibodies that are idiotypically related to Ab1 preferentially. In addition, these authors emphasized that immunization with Ab2 recognizing a paratope-related idiotope would generate antibodies that are indistinguishable from Ab1 also in terms of antigen binding. Therefore, it is reasonable to assume that the paratope-related idiotope of Ab1,1-10C9E8 is encoded by Vh and Vk germline genes. The outcome of immunization with Ab2,2-17C3SCC is in agreement with this assumption.

On the other hand, the screening of hyperimmune sera from several species (including man) revealed a striking association between the Ab2,2-17C3SCC-binding Idiotope and poliovirus type II-specific antibody (Fig. 2D). Such an association (that is related to the present reactivity of Ab2 with Ab1 from different species could be interpreted as interspecies idiotope cross-reactivity. The existence of interspecies idiotope cross-reactivity has been reported in the murine GAT idiotype (22-24) and the T15 idiotype (25), as well as in the human CHBs idiotype (26) system. This interspecies idiotype cross-reactivity has been explained on the basis of a common Vh germline gene coding for IdX that has been maintained through extended periods of evolution (22, 24-26).

However, the interspecies reactivity (as in the case of Ab2,2-17C3SCC and Ab1,1-10C9E8) might also reflect that a related epitope, i.e., internal image, is present (1, 2, 18, 19). Whatever the actual mechanism is, the present results are important in terms of an anti-idiotypic vaccine that is monoclonal, and hence predictable and available in large quantities. Furthermore, in terms of internal image such an anti-idiotypic vaccine against viral infection could be useful in any species.

The generation of idiotype antibody of predefined antigen specificity by immunization with Ab2 in the absence of antigen has been reported for a number of experimental systems (3, 5, 27-31). Recently Ertl and Finberg (32) reported the induction of a protective cytolytic T cell response in mice against a lethal Sendai virus...
infection by preimmunization of mice with a monoclonal anti-idiotypic antibody directed against a Sendai virus-specific helper T cell clone. To our knowledge, this is the first report demonstrating that monoclonal Ab2 can be used to induce specific virus-neutralizing antibody responses.

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