SYNGENEIC MONOCLONAL ANTIIDIOTYPE CAN INDUCE CELLULAR IMMUNITY TO REOVIRUS

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Viruses attach to cells via specialized attachment proteins that interact with specific cell surface receptors (1–5). An understanding of such virus–host cell interactions may provide new insights into the pathogenesis and prevention of infectious diseases (6).

The mammalian reoviruses, segmented, double-stranded RNA viruses include three serotypes (7). The segmented nature of the reovirus genome has permitted a genetic approach for the identification of the role of individual viral components in pathogenesis, making the study of virus-host interactions more amenable to direct experimentation (8). These studies have revealed that each of the three outer capsid proteins (μ1c, σ1, σ3) plays critical, distinct roles in viral virulence. One of the outer capsid proteins, σ1, the reovirus hemagglutinin (HA),[1] has been shown to be the reovirus cell attachment protein (9–12). Studies utilizing monoclonal antibodies have revealed that the HA is divided into three distinct domains (13, 14), one of which governs the important biologic properties of receptor interaction, recognition by cytotoxic T lymphocytes (CTL) and recognition by neutralizing antibody (15, 17). Reovirus mutants at this site, selected for resistance to neutralization, are altered in cell tropism (in the nervous system) (16) and in recognition by CTL (17). Therefore, monoclonal antibodies directed to this site, block a number of important biologic properties of reovirus.

That such diverse biologic properties as CTL recognition and viral neutralization localize to one part of the HA suggests that binding is restricted by a unique conformation or, alternatively, by a set of epitopes on the HA. This implies that the receptor for virus on immune CTL, the natural receptor found on neurons, and the binding moiety of neutralizing monoclonal antibodies (idiotype) share a common configuration as well.

To test this possibility, syngeneic monoclonal antibodies were raised against the idiotype portion of a prototypic neutralizing monoclonal antibody, 9BG5 (18, 19). The antiidiotype obtained has been shown to block both viral binding

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[1] *Abbreviations used in this paper:* C, complement; CTL, cytotoxic T lymphocytes; DME, Dulbecco's modified Eagle's medium; DTH, delayed type hypersensitivity; ELISA, enzyme-linked immunosorbent assay; HA, hemagglutinin; IEF, isoelectric focusing; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; pfu, plaque-forming units; RIA, radioimmunoassay.

to neuronal cells (18) and CTL lysis of virally infected target cells.[2] These findings further reinforced the hypothesis that the antiidiotype functionally resembles the viral HA. To further explore the similarities of this antiidiotypic antibody to HA, we have asked whether the antiidiotype can directly stimulate antiviral immunity, serving as a noninfectious mimic of virus in vivo. In this paper we show that purified monoclonal antiidiotypic antibodies can stimulate reovirus-specific delayed type hypersensitivity (DTH) and CTL responses in a dose-dependent and hemagglutinin-restricted fashion.

Materials and Methods

Mice. BALB/c (H-2^d; Igh-1^a) and C3H/HeJ (H-2^k; Igh-1^j) female mice, 6–8 wk of age, were obtained from The Jackson Laboratory, Bar Harbor, ME, and maintained on standard lab chow and water ad libitum. Mice were tested for antireovirus antibodies before immunization and were found to be free of antibodies by enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and neutralization testing (11).

Virus. Reovirus type 1 (strain Lang), type 3 (strain Dearing), and reassortant viruses 1.HA3 and 3.HA1 have been described previously (12, 20). Reassortant 1.HA3 contains nine dsRNA segments from reovirus type 1 and the S1 genome segment from reovirus type 3. Reassortant 3.HA1 contains nine dsRNA segments from reovirus type 3 and the S1 dsRNA segment from type 1. The variant virus K, selected by resistance to neutralization with the 9BG5 monoclonal antibody, has been described previously (15). All viruses were grown and titered in mouse L929 cells as described previously (21) and purified according to the method of Drayna and Fields (22). All purified viral stocks were administered to animals in gelatin-containing buffered saline as described previously (23).

Cells. P815 (H-2^d) mastocytoma cells were maintained in vivo by serial passage as ascites in BALB/c mice and in vitro in Dulbecco's modified Eagle's medium (DME) supplemented with 10% fetal calf serum (M. A. Bioproducts, Walkersville, MD), 10 mM glutamine (Irvine Scientific, Santa Ana, CA).

87.92.6 is a B cell hybridoma that is specific for the idiotype of the antireovirus type 3 HA hybridoma 9BG5 (18). The 87.92.6 cell line, which secretes an IgM, kappa monoclonal antibody, was maintained in HB101 synthetic media plus HB101 serum-free supplement (Hanna Labs, Berkeley, CA).

Purification of Monoclonal Antiidiotype. Cell-free supernatants of 87.92.6 cells grown in HB101 synthetic media were first pooled and concentrated on an Amicon ultrafiltration cell (Amicon Corporation, Danvers, MA) with a Type C-100 membrane (Nucleopore, Pleasanton, CA) and then sequentially precipitated three times at 30–50% ammonium sulfate. The resulting precipitate was dissolved in phosphate-buffered saline (PBS) and extensively dialyzed against PBS at 4°C. After dialysis was complete, antibody was adjusted to 20 mM phosphate (pH 7.2), 0.5 M NaCl, and 1% *n*-butanol and IgM was purified by Sephadex G-200 column chromatography. Eluate fractions from the void volume were shown to contain >95% antiidiotype IgM by SDS-PAGE, isoelectric focusing (IEF), and Ouchterlony analysis. Protein concentration of purified antiidiotype was determined by the method of Bradford (24).

Immunization of Mice. For DTH experiments, mice were inoculated subcutaneously (s.c.) in two separate sites on the dorsal flanks of the animal (over each hind limb). For immunizations with virus, animals were inoculated with 10^7 particles of virus/0.2 ml given as two separate injections of 0.1 ml. For immunization with antiidiotype, animals were inoculated with varying amounts of antiidiotype given in two separate injections of 0.1 ml on day 0 and day 2. All animals were challenged 6 d after initial immunization in the left footpad with 25 μl of gelatin saline containing a total of 3×10^7 viral particles. Footpad swelling was measured 24 h later (except when time course studies were performed) in a

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blind fashion using a Fowler micrometer (Schelinger's, Brooklyn, NY). Four or five animals per group were studied and the magnitude of the response was expressed as the increment of thickness of the challenged left footpad, as compared to the untreated right footpad.

For CTL experiments, animals were immunized intraperitoneally with 10^7 plaque-forming units (pfu) of reovirus type 3 or with antiidiotype (~100 μg) or with gamma-irradiated 87.92.6 cells (3×10^5 cells/animal) given to each animal twice (on day 0 and day 2). 1 wk later, splenocytes were harvested and incubated in 24-well Costar dishes (Costar, Cambridge, MA) with 10^7 pfu of virus per well in a total volume of 1.6 ml of DME supplemented with 2% fetal calf serum, glutamine, and 2-mercaptoethanol (7×10^6 splenocytes/well). Cells were harvested 5 d later, purified over Ficoll-Isopaque (Litton Bionetics, Kensington, MD), and tested in a 5-h ^{51}Cr-release assay on ^{51}Cr-labeled P815 cells, reovirus type 3-infected P815 cells, and 87.92.6 cells as targets, as described previously (25).

Adoptive Transfer Experiments. For transfer experiments, mice were inoculated with 10^7 viral particles/0.4 ml or with antiidiotype (~100 μg protein), given in four separate sites on the back of the animal (over hind limbs and forelimbs). 5 d after subcutaneous immunization, the draining lymph nodes were removed and a single cell suspension was prepared as described previously (21). Syngeneic recipients received 1×10^8 cells injected intravenously and were simultaneously primed with 3×10^7 particles of virus.

Anti–Thy-1.2 Treatment. Anti-mouse Thy-1.2 antisera (source) and guinea pig complement (C) treatment was performed on sensitized lymph node cells before transfer as reported previously (23).

Results

Monoclonal Antiidiotype Can Elicit a DTH Response to Reovirus. To determine whether immunization of mice with purified monoclonal antiidiotype could elicit an antiviral immune response for DTH, animals were immunized with 100 μg s.c. of antiidiotype or saline and challenged in the footpad with 3×10^7 particles of reovirus type 3 or type 1. After preliminary studies demonstrated that antiidiotype could elicit footpad swelling in response to reovirus type 3, footpad swelling was measured every 4 h from 4 to 32 h after challenge and at 48 and 72 h after challenge. As seen in Fig. 1, footpad swelling began by 12 h after challenge, peaked at 28 h after challenge, and disappeared by 72 h after challenge. The kinetics of this response are, thus, characteristic of the delayed hypersensitivity reaction and identical in mice primed with either reovirus type 3 or antiidiotype and challenged with reovirus type 3. Furthermore, the peak response observed in mice immunized with antiidiotype was 75% of that of mice primed with reovirus type 3. Characteristic findings of DTH response were observed histologically in animals exhibiting footpad swelling.

Adoptive Transfer of Antiidiotype-elected DTH to Reovirus by Thymus-derived Cells. To define the cellular nature of the antiidiotype-induced DTH response to reovirus, adoptive transfer experiments were performed. Draining lymph nodes were removed from BALB/c mice 6 d after repeated immunization with antiidiotype and single-cell suspensions were prepared and transferred to naive BALB/c recipients intravenously (10^8 cell/mouse). Recipient mice were challenged in the footpad at the time of transfer and footpad swelling was measured 24 h later. Thy-1.2 cells were removed by treatment with anti–Thy-1.2 monoclonal antibody plus complement before transfer (27).

As shown in Fig. 2, complement-treated lymph node cells from animals immunized with antiidiotype transferred DTH reactivity for reovirus type 3 to

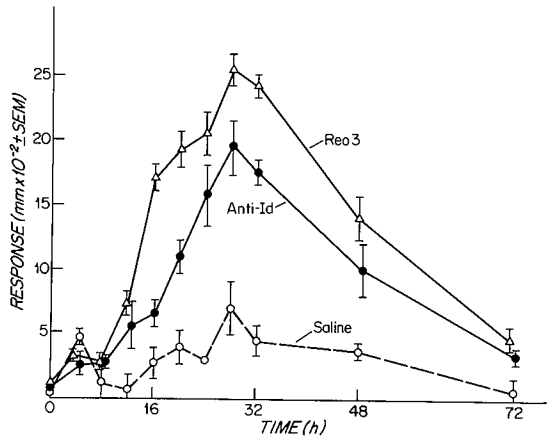

FIGURE 1. Time course of DTH with reovirus type 3 and the antiidiotypic antibody. BALB/c mice were immunized subcutaneously with 10^7 particles of reovirus type 3 or 100 μg of purified monoclonal antiidiotypic antibody on day 0 and challenged with reovirus 6 d later. Footpad swelling was measured at various times after challenge.

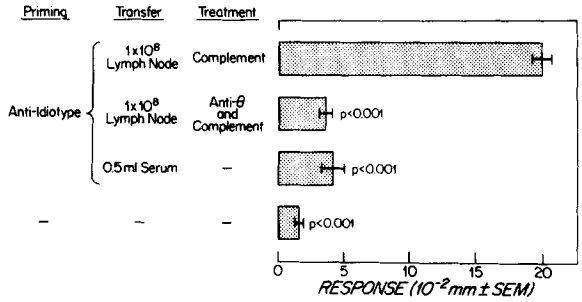

FIGURE 2. Adoptive transfer of antiidiotype-induced DTH by T cells. BALB/c mice were immunized subcutaneously with 100 μg of purified monoclonal antiidiotypic antibody and lymph nodes were removed 6 d after immunization. Naive BALB/c mice received either complement-treated immune lymph node cells, immune lymph node cells treated with anti-Thy-1.2 plus complement, antiidiotype-immune serum, or saline. Immediately after transfer, animals were challenged with reovirus type 3. Foodpad swelling was measured 24 h later.

secondary recipients. The immune cells responsible for adoptive transfer of DTH are T cells, since the ability to transfer immune reactivity was abrogated by anti-Thy-1.2 plus C treatment. The inability of reovirus type 3 hyperimmune sera to transfer responses is also shown. Thus, monoclonal antiidiotype induces a T cell–dependent immune response that recognizes reovirus type 3.

DTH Response Elicited by the Monoclonal Antiidiotype Is Specific for the Neutralization Domain of the Reovirus Type 3 HA. Previous studies have shown that the DTH response to reovirus is serotype-specific and that the viral HA determines serotype specificity (23). To determine the specificity of the DTH response elicited by the monoclonal antiidiotype, animals were immunized with 100 μg of antiidiotype, boosted with an additional 100 μg 2 d later, and challenged in the footpad on day 6 with reovirus type 1, type 3, the reassortant viruses 1.HA 3 and 3.HA 1, and the variant virus, K.

The results presented in Fig. 3 indicate that the DTH response elicited by

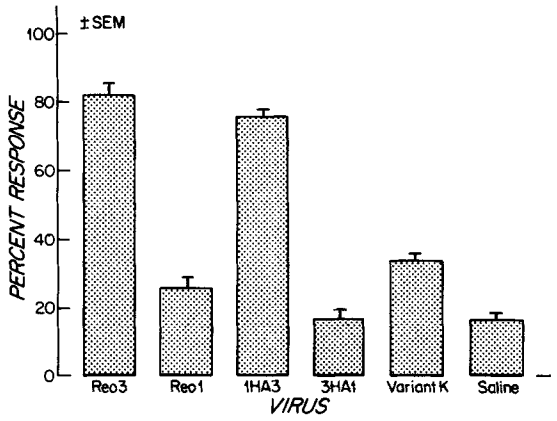

FIGURE 3. Specificity of the DTH response elicited by the monoclonal antiidiotype. BALB/c mice were immunized with 100 μg s.c. of antiidiotype, boosted with an additional 100 μg 2 d later, and challenged in the foodpad on day 6 with either reovirus type 3, type 1, reassortant 1.HA3, reassortant 3.HA1, variant virus K, or saline. Footpad swelling was measured 24 h later. Results are expressed as a percentage of the DTH response (±SEM) in mice primed and challenged with reovirus type 3. For Reo 1, 3HA1, variant K, and saline, $P < 0.001$. For Reo 3 and 1HA3, $P < 0.01$.

monoclonal antiidiotype is serotype specific and is mediated by the viral HA. A DTH response was observed in animals challenged with reovirus type 3 and with the reassortant 1.HA3 that contains the type 3 S1 dsRNA segment (HA) and the remaining nine dsRNA segments from type 1 reovirus. In both instances, the magnitude of the DTH response was ~80% of the response elicited in mice primed and challenged with reovirus type 3. In contrast, mice challenged with type 1 reovirus and the reciprocal reassortant 3.HA 1 exhibited little or no DTH response. For these mice, footpad swelling was similar to that observed in control mice immunized with gelatin-saline and challenged with type 3 reovirus.

Recently, reovirus type 3 viruses with antigenically altered HA proteins have been generated. These viruses, selected by their capacity to grow in the presence of the neutralizing monoclonal antibody 9BG5, have been designated viral "variants." Because the antiidiotype is directed against the 9BG5 neutralizing monoclonal antibody, it was of interest to determine the HA domain specificity of the antiidiotype-induced response. When antiidiotype-primed mice were challenged with reovirus type 3 or the K variant, the magnitude of the DTH response was much lower in mice challenged with the K variant (Fig. 3). The magnitude of the DTH response to type 3 was ~75% of the response elicited in mice primed and challenged with reovirus type 3, whereas the response to variant k was only 20%. Thus, antiidiotype induces an immune response that discriminates the type 3 HA neutralization domain from the variant HA epitope. Interestingly, the DTH response observed in mice primed with type 3 and challenged with the variant virus K is 61% of the response seen in mice immunized and challenged with reovirus type 3.

Effect of Dose of Antiidiotype on the Magnitude of the DTH Response. Animals were immunized subcutaneously with varying amounts of purified antiidiotype (0.1–10 μg) on day 0 and day 2 and challenged 6 d after initial immunization

with reovirus type 3. Control mice were immunized with equivalent amounts of an irrelevant purified IgM Kappa monoclonal antibody (HO 13.4) or type 3 reovirus. A reovirus-specific DTH response was elicited even with 0.1 μg of antiidiotype (8×10^8 molecules). The magnitude of the DTH response was dependent on the dose of antiidiotype in this concentration range (Fig. 4). No response was observed in either immunization (HO 13.4) or challenge (reovirus type 1) controls. Thus, the purified antiidiotype induces a specific, potent immune response to reovirus type 3.

Effect of Allotype on the DTH Response. To evaluate whether the Igh allotype of mouse strain was important for the antiidiotype-elicited DTH response, C3H/HeJ (Igh-1^j) were primed with antiidiotype. As shown in Table I, C3H/HeJ and BALB/c mice were equivalently primed by antiidiotype. Thus, the Igh-1 allotype of recipients is not relevant to the ability of antiidiotype to elicit an immune response.

Antiidiotype-bearing Hybridoma Cells Can Elicit a CTL Response to Reovirus Type 3. To determine whether immunization of mice with antiidiotype could elicit an antiviral CTL response, animals were immunized subcutaneously with anti-

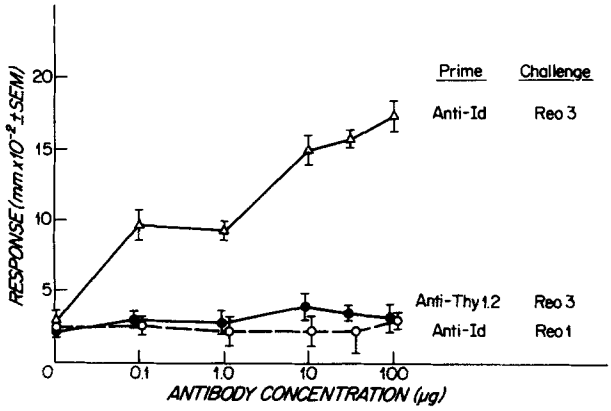

FIGURE 4. Dose response of antiidiotype on magnitude of DTH response. BALB/c mice were immunized s.c. with varying amounts of purified monoclonal antiidiotype or control and challenged in the footpad 6 d later with reovirus type 3 or type 1.

TABLE I

Delayed Type Hypersensitivity Responses to the 87.92.6 Monoclonal Antiidiotype in C3H/HeJ Mice

Immunization	Challenge	Footpad swelling
Type 3	Type 3	34.25
87.92.6	Type 3	23.5
87.92.6	Type 1	8.5
87.92.6	3HA1	23.5
87.92.6	1HA3	8.5
Saline	Type 3	6.5

The DTH experiments were carried out as described in Materials and Methods, using either reovirus type 3 or the syngeneic monoclonal antiidiotype to immunize mice and reovirus type 1 or 3 or reassortants 1HA3 or 3HA1 to challenge the mice.

idiotypic antibody or with irradiated 87.92.6 hybridoma cells. The 87.92.6 hybridoma cell, which is an early B cell and expresses significant amounts of immunoglobulin on its surface, was utilized as a potential vehicle for immunization. Initial attempts to immunize optimally with 87.92.6 antibody led to only minimal activation of antireovirus CTL activity. Immunization of animals with irradiated 87.92.6 cells elicited vigorous CTL activity against reovirus. 1 wk after in vivo immunization with 3×10^5 irradiated 87.92.6 cells, immune spleen cells were removed and incubated in vitro with reovirus type 3. Five days later, spleen cells were assayed for their capacity to kill reovirus-infected P815 cells. As seen in Fig. 5, 87.92.6-induced T cells generated vigorous cytotoxic activity against reovirus-infected target cells, but not against noninfected target cells, mirroring the effects of reovirus immunization.

Discussion

These studies show that purified monoclonal antiidiotypic antibody (87.92.6) induced by monoclonal antibodies directed to the reovirus HA neutralization domain, can induce potent reovirus-specific T cell immunity in the absence of adjuvant. Syngeneic antiidiotype priming elicits potent transferable T cell immunity to reovirus type 3 as measured by DTH responses in vivo. The magnitude of the DTH response elicited by syngeneic monoclonal antiidiotype is dose-dependent, and is specific for the neutralization domain of the reovirus type 3 HA. In addition, the extent of immune responsiveness to antiidiotype is not restricted by the IgH locus. Antiidiotype also induces potent cytolytic T cell activity when animals are first primed in vivo with monoclonal antiidiotype-bearing hybridoma cells. The failure to efficiently prime for cytolytic activity with soluble protein contrasts with the effective stimulation of inflammatory cells

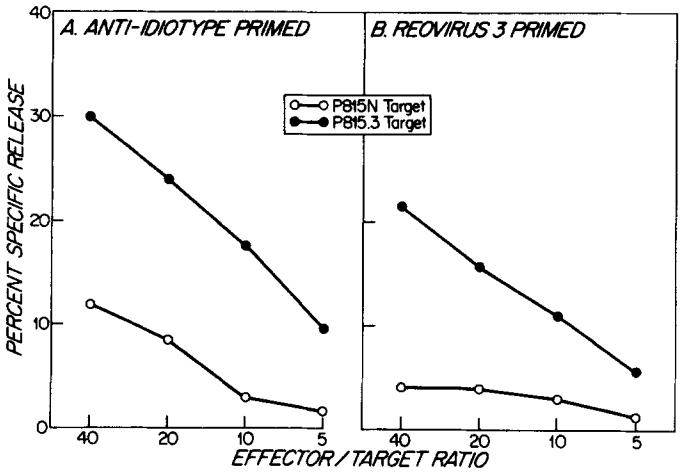

FIGURE 5. CTL response elicited by the antiidiotype-bearing hybridoma cells 87.92.6, to reovirus type 3. BALB/c mice were primed subcutaneously with (*A*) 87.92.6 cells or (*B*) reovirus type 3. Immune spleen cells were removed 1 wk later and incubated in vitro with reovirus type 3 for 5 d. The capacity of splenocytes to kill reovirus type 3–infected P815 cells (P815.3) versus noninfected P815 cells (P815N) were compared using a 5-h ^{51}Cr-release assay. Results are presented as the mean of triplicate experiments.

by antibody alone. This may relate to the different type of histocompatibility-associated signals required for activation of these distinct sets of cells. Cytolytic T cell precursors are stimulated by antigen in conjunction with major histocompatibility complex (MHC) components. The antiidiotype-bearing hybridomas express both antiidiotype and both class I and II MHC components on the cell surface, and thereby may be more effective stimulators of CTL than antibody alone. Alternatively, the affinity of the antiidiotypic antibody for reovirus-specific receptors on the cytolytic cells may be different from the receptors used by T cells that mediate inflammation. This latter possibility is difficult to reconcile with the efficiency of priming for either DTH or CTL activities by virus (17, 23). Thus, monoclonal anti-HA antiidiotype mimics the reovirus HA in both the extent and specificity of immune responsiveness. These results are consistent with previously observed antiidiotype mimicry of viral binding and cellular activities (18–20, 28).

Manipulation of the immune response by injection of antibodies to idiotypes has been used in several systems (28–31). In these studies exposure to antigen after the injection of antiidiotypic reagents resulted in either suppression of the idiotype-bearing antigen-binding molecule or increased idiotype expression and antigen-binding activity, depending upon the route of injection. Recently, Kennedy and Dreesman (32) have shown that rabbit antiidiotypic antibody could prime BALB/c mice for the generation of antibody to hepatitis B surface antigen (HbsAg) if the mice were secondarily boosted with HbsAg or antiidiotype. The evaluation of cellular responses was, however, not reported. Ertl et al. (33) have used a crude monoclonal anti–T cell receptor antibody supernatant to prime mice for reactivity to Sendai virus. However, the idiotypic specificity of the antiviral response is not well-characterized in that system. In contrast, the purified monoclonal antiidiotype in the reovirus system detects idiotopes on anti-HA antibodies specific for the neutralization domain of the HA. The 87.92.6 monoclonal antiidiotype was generated by priming BALB/c mice with a BALB/c-induced monoclonal antibody 9BG5. Hence, the use of the monoclonal antiidiotype 87.92.6 in inducing immune responses in BALB/c mice is equivalent to priming with self structures as suggested originally by Jerne (34). Using antiidiotypic antibody protein purified to homogeneity we showed that 8×10^8 molecules were able to prime for cellular responses to reovirus. To our knowledge, this is the first demonstration of the effectiveness of pure antibody protein in the absence of adjuvant in eliciting antiviral immunity.

Taken together, these results indicate that antiidiotypic reagents provide an attractive means for modulating the immune response. The strategy of using antiidiotypes for inducing or augmenting immunity to viral diseases without direct exposure of the host to the pathogen is advantageous in the development of an immunologic vaccine for protection against lethal viruses. Further studies with antiidiotypic reagents should provide insight into the uses of these reagents to manipulate immune responses in order to provide protection of hosts from infectious agents without the risks of current vaccine approaches. Preliminary experiments indicate that polymerized syngeneic monoclonal antiidiotype protein can induce neutralizing antibodies to the mammalian reovirus.

Summary

A syngeneic monoclonal antiidiotypic antibody was generated in BALB/c mice after repeated immunization with a BALB/c monoclonal anti-reovirus hemagglutinin (HA) antibody. The resultant syngeneic monoclonal antiidiotypic antibody, in the absence of adjuvant, was found to be capable of priming both BALB/c ($H\text{-}2^d$, $Igh\text{-}1^a$) and C3H/Hej ($H\text{-}2^k$, $Igh\text{-}1^j$) mice for $Lyt\text{-}1^+$- and $Lyt\text{-}2^+$-dependent responses against the mammalian reovirus. By the use of intertypic reassortants and variant virus analysis, the specificity of the response was finely mapped to the neutralization domain of the viral hemagglutinin (HA). Using purified monoclonal antiidiotype, we were able to compare the potency of antiidiotype to virus in terms of induction of immunity. 8×10^8 protein molecules were able to prime for cellular responses to reovirus. These studies indicate that in the reovirus system, T cells and B cells share idiotypic configurations, and that antiidiotypic antibodies of the type described herein may be useful in the development of vaccines against certain viral infections.

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