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Idiotypes and Biological Activity of Murine Monoclonal Antibodies Against the Hemagglutinin of Measles Virus

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Three hybridomas, designated C2, V17, and B2, were produced from BALB/c mice after immunization with measles virus. All three were directed against the virus hemagglutinin (HA). The HA is a structural peptide of the virus and constitutes a major target for the host immune response during measles infection. The monoclonal anti-HA antibodies have biological functions such as (i) measles virus neutralization in vitro, (ii) binding to acutely and persistently infected cells, and (iii) inhibition of HA-mediated Rhesus monkey erythrocyte agglutination. Different idiotypes, designated HAMM-1, HAMM-2, and HAMM-3, were defined on C2, V17, and B2, respectively, by syngeneic anti-idiotype sera against those three monoclonal antibodies. A limited cross-reactivity with the HAMM-1 idiotype was detected in sera from some BALB/c mice immunized with measles virus. The anti-idiotype sera could significantly inhibit the biological functions of the HAMM-1 and HAMM-3 idiotypes bearing monoclonal anti-HA-antibodies. This suggests a possible role for auto-anti-idiotypes in the immune response after infection with measles virus.

Measles virus hemagglutinin (HA), one of the six major structural polypeptides of measles virus (4, 20), is a major target for the host immune response after measles infection or immunization. This glycoprotein (molecular weight, 76,000) is expressed on the surface of the virion and on the membrane of acutely and persistently infected cells (3, 18). Cell-mediated immunity as measured by lymphocyte proliferation is directed against this component of the virus (3), and a major part of the virus neutralizing activity in serum is associated with anti-HA antibodies (22, 23).

The role of idiotypes (Id's) and anti-Id's in the immune response to HA and to measles virus in general is unknown. Id's have been described in patients with subacute sclerosing panencephalitis, a disease presumably caused by chronic measles infection (7, 26). In one of these reports, it was demonstrated that the Id was associated with anti-measles virus specificity (7). Studies of Id's in the human anti-measles response have been limited, mainly because it has not been possible until now to affinity purify sufficient quantities of anti-measles antibodies. Therefore, in the current investigation, a different approach was taken.

First, murine monoclonal antibodies against the HA of measles virus were produced. Subsequently, these were purified, characterized chemically, immunochemically, and biologically, and used to raise syngeneic anti-Id's. These antisera defined different Id's on the biologically active monoclonal anti-HA antibodies. A limited cross-reactivity with the Id of one of those monoclonal antibodies was detected in serum from BALB/c mice that were immunized with measles virus. Other experiments with this system demonstrated that anti-Id can significantly inhibit the biological function of the antibody at the level of the virus.

MATERIALS AND METHODS

Hybridomas against the HA of measles virus. Three different hybridomas secreting antibody to the HA peptide of measles virus were developed. The 79 XI C2 hybridoma (hereafter designated C2) was obtained through fusion between spleen cells of a BALB/c mouse that was immunized with purified Edmonston strain of measles virus and immunoglobulin G1 κ chain (IgG1κ)-secreting P3/X63/Ag8 myeloma cells (hereafter designated X63) (18). The 79 XV V17 and 80 III B2 hybridomas (hereafter designated V17 and B2, respectively) were derived from fusion between the κ chain-synthesizing P3/NS-1/1Ag4-1 myeloma cells (hereafter designated NS-1) and spleen cells from BALB/c mice immunized with Edmonston measles virus. The same methodology as for C2 was used except that 35% polyethylene glycol was used to induce fusion and that clones were obtained by the limiting dilution technique. The specificity of each of the three hybridomas for the HA peptide was established by

immunoprecipitation as described previously (18). The monoclonal antibody was purified from hybridoma culture supernatant or from ascites induced in Pristane-primed BALB/c mice by intraperitoneal injection of 5×10^6 hybridoma cells.

Ion-exchange chromatography. Samples were dialyzed against 0.025 M tris(hydroxymethyl)aminomethane hydrochloride buffer, pH 7.5 (conductivity, 1.8 mMho), and applied to a diethylaminoethyl (DEAE)-cellulose (DE 52, Whatman) column which had been equilibrated with the same buffer. Approximately 1 ml of DEAE-cellulose was used per mg of protein sample. The monoclonal antibody was eluted with a linear gradient from 0 to 0.1 M sodium chloride in this same buffer.

SPA chromatography. For *Staphylococcus aureus* protein A (SPA) chromatography, supernatants from hybridoma cultures were passed once through Sepharose-CL-4B-SPA (Pharmacia) columns (bed volume, 8 ml) equilibrated in borate-buffered saline, pH 8.0. The bound monoclonal antibody was eluted either with 3 M potassium thiocyanate or 0.2 M phosphate-citric acid buffer, pH 4.5.

Characterization of monoclonal antibodies. The binding of monoclonal antibody to measles antigen was measured with a solid-phase radioimmunoassay (RIA) (17). Hemagglutination inhibition (HAI) and virus neutralization assays were done as described previously (4). The immunolgobulin class, subclass, and type of the monoclonal antibodies were determined by double immunodiffusion (24) against specific antisera (Litton Bionetics). Thin-layer isoelectric focusing was done on Ampholine PAG plates, pH 3.5 to 9.5, as described by the manufacturer (LKB Instruments). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 10% gels was done as described previously (15).

Production of syngeneic anti-Id sera to monoclonal anti-HA antibody. BALB/c mice, 10 to 12 weeks of age, were immunized by the method described by Bona et al. (5). Purified monoclonal anti-HA antibody was conjugated to keyhole limpet hemocyanin with gluteraldehyde. The resulting anti-HA-keyhole limpet hemocyanin conjugate was emulsified in complete Freund adjuvant to give 75 μg of conjugate and 100 μg of *Mycobacterium tuberculosis* H37 Ra (Difco) in 100 μl of emulsion. Each mouse was given 50 μl of this emulsion intraperitoneally, and another 50 μl was distributed among the hind leg foot pads and the inguinal and axillary regions. Five days after the first immunization, 75 μg of anti-HA-keyhole limpet hemocyanin conjugate in 100 μl of incomplete Freund adjuvant was injected over the axillary and inguinal regions. The mice were boosted once weekly with 75 μg of conjugate in saline given in the axillary and inguinal regions. The mice were first bled 6 days after injection 6 and subsequently every 2 weeks.

Assays for antibody activity to monoclonal anti-HA antibody. The sera were tested for antibody activity to monoclonal anti-HA antibody by agglutination of sheep erythrocytes (SRBC) coated with monoclonal anti-HA antibody. Antigen controls consisted of SRBC coated with purified X63 myeloma protein or with RPC5, an IgG2a κ myeloma protein of BALB/c origin (Litton Bionetics). Chromium chloride was used to prepare the coated SRBC (8). Each putative antiserum was diluted serially in microtiter plates. An equal volume of a 0.2% suspension of coated SRBC was added. The agglutination titer was the $\log_2$ of the reciprocal of the highest dilution of antiserum giving agglutination.

Inhibition RIA for Id determinants. The gamma globulin fraction of anti-Id sera was coupled to activated cellulose (21). For the assay, 500 μl of an appropriate dilution of this immunoadsorbent in bovine serum albumin buffer (21) was incubated overnight at 4°C with 100 μl of sample and 100 μl of ^{125}I-labeled monoclonal anti-HA antibody (20,000 to 30,000 cpm). The immunoadsorbent was then washed twice with bovine serum albumin buffer and counted. Standard curves were established by using known amounts of unlabeled monoclonal anti-HA antibody as samples in the assay.

Results were expressed as percent inhibition = $100 \times \{1-[(\text{cpm} - \text{cpm}^\infty)/(\text{cpm}_0 - \text{cpm}^\infty)]\}$, where cpm is the number of counts bound to the immunoadsorbent in the presence of the sample, cpm^∞ is the number of counts bound with a large dose (50 μg) of unlabeled monoclonal anti-HA antibody, and cpm_0 is the number of counts bound with 100 μl of diluent as sample.

To obtain an RIA specific for the Id's of the anti-HA antibody of the C2 hybridoma, syngeneic anti-C2 antiserum was absorbed over Sepharose-4B–coupled X63 myeloma protein before coupling to activated cellulose.

Effect of anti-Id sera on binding of Id's bearing monoclonal anti-HA antibody to measles antigen. The influence of anti-Id sera on the binding of the monoclonal anti-HA antibody to measles antigen was assessed by a modification of the solid-phase anti-measles RIA (17). Serial 10-fold dilutions of anti-Id sera or normal BALB/c serum were mixed in wells containing either measles or control antigen and a constant amount of the respective monoclonal anti-HA antibody known to give a specific binding of approximately 900 cpm (Δcpm). After 1 h, the wells were thoroughly washed, and ^{125}I-labeled rabbit anti-mouse Fc immunoglobulin (25,000 cpm) was added. After 1 h, the wells were washed again and counted. The effect of anti-Id sera was expressed as the percent change of the Δcpm obtained with each dilution of anti-Id serum (x) compared with the Δcpm obtained with the same dilution of normal BALB/c serum (y) for each individual monoclonal anti-HA antibody [percent change $= (x - y)100/y$].

In other experiments, the monoclonal anti-HA antibody was incubated with the antigen before the addition of anti-Id or control sera. The effect of anti-Id sera on the binding of ^{125}I-labeled anti-HA antibody was also determined.

Effect of anti-Id sera on HAI by Id's bearing monoclonal anti-HA antibody. Twofold serial dilutions of anti-Id sera were mixed with the least amount of each of the purified monoclonal anti-HA antibodies known to have complete HAI activity. After 1 h of incubation, these mixtures were used as samples in classical HAI (4).

Effect of anti-Id sera on virus neutralization. After establishing the measles neutralization titers for the three monoclonal anti-HA antibodies, the effect of

anti-Id sera on this activity was assessed. A constant amount of monoclonal anti-HA antibody known to give between 60 and 85% neutralization was incubated with serial dilutions of anti-Id sera for 1 h and then used to neutralize measles virus (4). The results were expressed as the percent change in neutralization by anti-HA antibody mixed with anti-Id sera (x), as compared with the neutralization by the anti-HA antibody alone (y) [percent change = $(x-y)100/y$].

Other methods. Radiolabeling with ^{125}I was done by a modified (19) chloramine T method (12). The X63 myeloma protein was coupled to cyanogen bromide-activated Sepharose-4B (Pharmacia) (2). Goat antiserum against bovine serum proteins and rabbit anti-bovine IgG antisera (Cappel) were used in double immunodiffusion (24) to detect bovine antigens in SPA-purified monoclonal anti-HA antibody.

RESULTS

Monoclonal anti-HA antibodies. The characteristics of the three monoclonal anti-HA antibodies C2, V17, and B2 are summarized in Table 1. Tumor ascites from Pristane-primed mice injected with each of the anti-HA hybridomas had significant anti-measles activity. To prepare anti-Id sera, each monoclonal antibody was purified. Chemical procedures were used because the HA peptide of measles virus is not available in sufficient quantities to allow affinity purification of anti-HA hybridoma products.

(i) C2 monoclonal anti-HA antibody. The 50% saturation ammonium sulfate precipitate of 20 ml of C2 anti-HA immune ascites was fractionated by DEAE-cellulose chromatography. The fraction which showed highest specific activity in the anti-measles RIA were pooled, yielding 49 mg of protein. Because C2 was obtained from fusion with the IgG1κ-secreting X63 myeloma, the X63 myeloma protein was also purified by ion-exchange chromatography and compared with purified C2 in thin-layer isoelectric focusing (Fig. 1). Purified C2 contained the parent myeloma protein and, in addition, some unique bands which presumably contained the anti-HA activity. In sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reduced conditions, two bands were seen in purified C2 and X63 corresponding to the molecular weights of γ and L chains.

TABLE 1. *Characteristics of the anti-HA hybridomas C2, V17, and B2*

Hybridoma	Class, subclass, and type	SPA reactivity[a]	pI[b]	Biological activity[c] (titer)	
				HAI	Neutralization
C2	IgG1κ	±	6.3	1/3,200	1/4,000
V17	IgG2aκ	++	7.0	1/3,200	1/2,000
B2	IgG2aκ	++	8.0	1/100,000	1/16,000

[a] ±, Weak; ++, strong.
[b] Approximate (see Fig. 1).
[c] Tumor ascites induced by intraperitoneal injection of 5×10^6 hybridoma cells in Pristane-primed BALB/c mice were used.

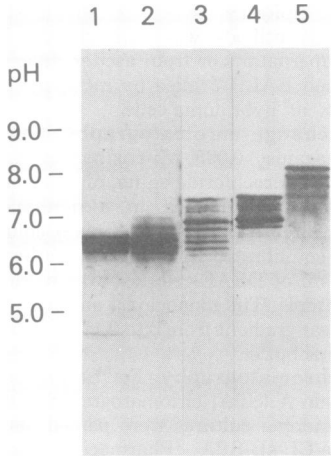

FIG. 1. *Thin-layer isoelectric focusing of DEAE-cellulose-purified X63 myeloma protein (lane 1) and of the following monoclonal anti-HA antibodies: DEAE-cellulose-purified C2 (lane 2), SPA-purified V17 (lane 3), SPA- and DEAE-cellulose-purified, high anti-HA activity V17 (lane 4), and SPA-purified B2 (lane 5).*

(ii) V17 monoclonal anti-HA antibody. Twenty milligrams of V17 monoclonal anti-HA antibody was purified from 1 liter of V17 hybridoma culture supernatant by SPA chromatography. The preparation contained only murine IgG when analyzed by double immunodiffusion. Specifically, no bovine serum antigens were detected. In sodium dodecyl sulfate-polyacrylamide gel electrophoresis, SPA-purified V17 showed two L chains of slightly different apparent molecular weights. It showed a relatively complex pattern (Fig. 1) in thin-layer isoelectric focusing. Because of this, the monoclonal nature of V17 was questioned. This possibility was discounted because an identical pattern was obtained with SPA-purified V27 monoclonal antibody, which was derived from recloning the parent hybridoma.

SPA-purified V17 was then further fractionated on DEAE-cellulose, using the same conditions as those for C2. The L chain with the lower molecular weight (L1) was associated with the fractions with a higher isoelectric point (pI) in thin-layer isoelectric focusing and with greater specific activity in the anti-measles RIA. The other L chain (L2) was associated with fractions showing lower specific anti-measles activity and a lower pI. Intermediate fractions with compa-

rable amounts of L1 and L2 and intermediate anti-measles activity were also obtained.

These findings suggested that L1 originates from the donor lymphocyte, that L2 was the NS-1 L chain, and that hybrid molecules (14), containing one or two myeloma L chains, were secreted by the hybridoma, in addition to the donor lymphocyte immunoglobulin. Fractions which had higher specific activity for measles and a higher pI (approximately 7.0) (Fig. 1) and which contained predominantly the L1 chain were used for subsequent experiments.

(iii) B2 monoclonal anti-HA antibody. Fifteen milligrams of B2 monoclonal antibody was purified from 1 liter of tissue culture supernatant by SPA chromatography. The thin-layer isoelectric focusing pattern of SPA-purified B2 was consistent with that of a single immunoglobulin (Fig. 1) (1). The approximate pI was 8.0. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reduced conditions showed one band with the molecular weight of γ chain and one band with the molecular weight of L chain.

The three purified anti-HA monoclonal antibodies retained biological activity. Specific binding (Δcpm) of 900 cpm in the anti-measles RIA was obtained with 10 ng of purified C2, 2.5 ng of V17, and 1.25 ng of purified B2. The minimal amounts of purified anti-HA antibodies which gave complete HAI were 800 ng of C2, 200 ng of V17, and 10 ng of B2. This indicated that, biologically, B2 was the most efficient of the three anti-HA antibodies. This was already suggested by the results obtained with hybridoma ascites (Table 1).

Idiotypes of the monoclonal anti-HA antibodies. (i) Syngeneic antisera define different Id's on the monoclonal anti-HA antibodies. All mice immunized with C2 developed antisera which agglutinated both C2-coated SRBC and X63-coated SRBC. After absorption over Sepharose-4B-coupled X63, these antisera agglutinated only C2-coated SRBC (Table 2). This agglutination was completely inhibited by C2, but not by normal BALB/c serum, X63, V17, or B2. The antibody response continued to rise until injection 10, when a plateau was reached. This was maintained over several months by weekly injections of anti-HA-keyhole limpet hemocyanin conjugate. The mice immunized with V17 or B2 each developed antisera which agglutinated V17- or B2-coated SRBC, respectively (Table 2). These agglutinations were completely inhibited by the autologous anti-HA antibody but not by normal BALB/c serum, RPC5 (a BALB/c myeloma, IgG2aκ, like V17 and B2), or heterologous anti-HA antibody.

TABLE 2. *Syngeneic antibody response to monoclonal anti-HA*

Antigen[a]	SRBC coupled with:	Titer[b] ($\log_2$ U)
C2	C2	13.8 ± 1.6[c]
	X63	0
V17	V17	11.0 ± 2.0
	RPC5	0
B2	B2	17.4 ± 0.5
	RPC5	0

[a] BALB/c mice (five each) immunized with BALB/c anti-HA-keyhole limpet hemocyanin conjugate.

[b] Mean ± standard error of individual titers in each group after six immunizations.

[c] After absorption of anti-C2 sera over Sepharose-4B-coupled X63.

These results demonstrated that a syngeneic antibody response had occurred, that these antisera were directed against the Id's of the anti-HA antibodies, and that they defined different Id's on the anti-HA antibodies against which they were raised.

This was confirmed by the RIAs. Figure 2 shows a standard RIA for C2 anti-HA antibody. Normal BALB/c serum, diluted 1:20, did not significantly affect this curve. Comparable curves were obtained for the Id's of the V17 and B2 monoclonal anti-HA antibody. Table 3 shows that BALB/c myeloma proteins of the same class, subclass, and type as the monoclonal anti-HA antibodies did not inhibit the RIAs. This demonstrated that the RIAs were specific for the Id's of the anti-HA antibodies. In each of the RIAs, inhibition was obtained by 200 ng of autologous monoclonal anti-HA; heterologous monoclonal anti-HA did not inhibit, even when 5 μg was used. Thus, there was no evidence of shared Id's among the three monoclonal antibodies.

For easy reference, the Id's of these murine antibodies with specificity for the HA of measles virus have been termed HAMM Id's. The C2 anti-HA bears the HAMM-1 Id; the Id's of V17 and of B2 anti-HA are called HAMM-2 and HAMM-3, respectively.

In each of the three systems, supernatants of the uncloned cell culture from which the hybridomas were derived and of sister clones of the hybridomas completely inhibited the RIA and therefore seemed to express a complete set of HAMM Id's.

(ii) Cross-reacting Id's in hyperimmune anti-measles antisera. Sera from 12 BALB/c mice, hyperimmunized with the Edmonston strain of measles virus, were tested. Sera from two of those animals at a 1:20 dilution gave 34

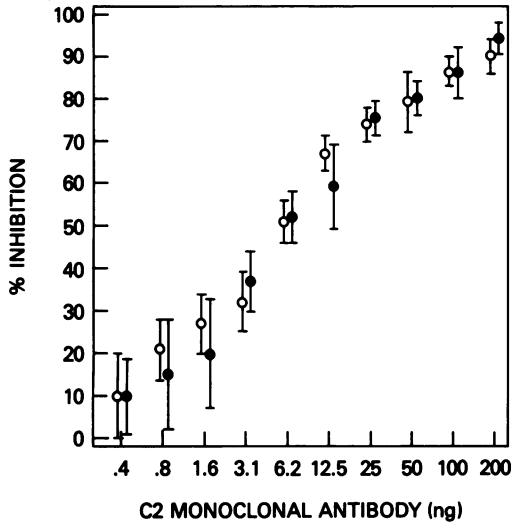

FIG. 2. *Standard curve in bovine serum albumin buffer of the RIA for the Id's of C2 monoclonal anti-HA antibody* (●). *The curve was not displaced significantly when normal BALB/c serum diluted 1:20 was used as the diluent for the standards* (○). *Each point represents the mean ± standard error (vertical bars) of triplicate determinations.*

TABLE 3. *Inhibition of HAMM Id's in RIA*

Inhibitor	Amt (ng)	% Inhibition obtained in RIA for following Id:		
		HAMM-1	HAMM-2	HAMM-3
C2	200	93 ± 2[a]	ns[b]	ns
C2	5,000	97 ± 2	ns	ns
V17	200	ns	92 ± 3	ns
V17	5,000	ns	97 ± 4	ns
B2	200	ns	ns	81 ± 3
B2	5,000	ns	ns	99 ± 1
X63	200	ns	ns	ns
X63	5,000	ns	ns	ns
RPC5	200	ns	ns	ns
RPC5	5,000	ns	ns	ns

[a] Values are the mean ± standard error of assays in triplicate.
[b] ns, Not significant.

± 8 and 46 ± 4% inhibition of the HAMM-1 Id RIA. In addition, a pool of BALB/c hyperimmune anti-measles (Edmonston) sera diluted 1:20 gave 47 ± 6% inhibition. Inhibition of approximately the same magnitude was observed at a 1:100 dilution of these sera. This indicated that some, but not all, of the HAMM-1 Id's present on the C2 monoclonal anti-HA antibody and recognized by the syngeneic antiserum were also expressed in those immune sera. None of the 13 sera significantly inhibited the HAMM-2 or HAMM-3 Id RIA.

Hyperimmune anti-measles (Edmonston) antisera from two rabbits did not inhibit any of the RIAs. Sera from 8 subacute sclerosing panencephalitis patients, from 8 individuals during convalescence after acute measles infection, and from 16 normal adults were tested in HAMM Id RIAs. None of these sera showed significant inhibition in any of the three Id systems.

Effect of anti-Id sera on the biological functions of HAMM Id's bearing antibody. (i) Anti-Id sera can inhibit binding of Id's bearing antibody to measles virus-infected cells. Figure 3 shows that anti-HAMM-1 Id serum specifically inhibited the binding of HAMM-1 Id bearing C2 antibody to measles antigen. The other anti-Id sera had no effect on C2. Binding of B2 monoclonal antibody to measles antigen was inhibited specifically by anti-HAMM-3 Id serum. However, anti-HAMM-2 Id serum did not inhibit V17 in this manner. Inhibition of C2 and B2 by the respective anti-Id was also obtained when monoclonal antibodies were incubated with the antigen before the addition of anti-Id or control sera. Furthermore, the binding of ^{125}I-labeled C2 and B2 to measles antigen was blocked by the respective anti-Id sera.

(ii) Anti-Id sera can inhibit HAI. The HAI activity of C2 monoclonal anti-HA antibody was reduced by anti-HAMM-1 Id serum. The endpoint of the effect was at a 1:64 dilution of anti-Id serum. This was specific for the anti-HAMM-1 Id serum and was not seen with either the anti-HAMM-2 or anti-HAMM-3 Id serum. Similarly, HAI by B2 anti-HA was specifically inhibited by anti-HAMM-3 Id serum to a dilution of 1:128. However, HAI by V17 anti-HA antibody was blocked neither by any of the anti-HAMM-2 Id sera nor by any of the other anti-Id sera.

Neither normal BALB/c serum nor the anti-Id sera showed HAI activity. Normal BALB/c serum also did not have HAI activity. It is significant that none of the anti-Id sera agglutinated Rhesus monkey erythrocytes; i.e., these anti-Id sera did not mimic this biological function of the antigen.

(iii) Anti-Id sera can inhibit measles neutralization. As shown in Fig. 4, anti-HAMM-1 Id serum substantially inhibited in vitro neutralization of measles virus by C2 monoclonal anti-HA antibody, whereas anti-HAMM-2 or anti-HAMM-3 Id serum had no significant effect.

Likewise, measles neutralization by B2 monoclonal anti-HA antibody could be specifically inhibited by anti-HAMM-3 Id serum. Anti-HAMM-2 Id serum, however, did not inhibit measles neutralization by V17.

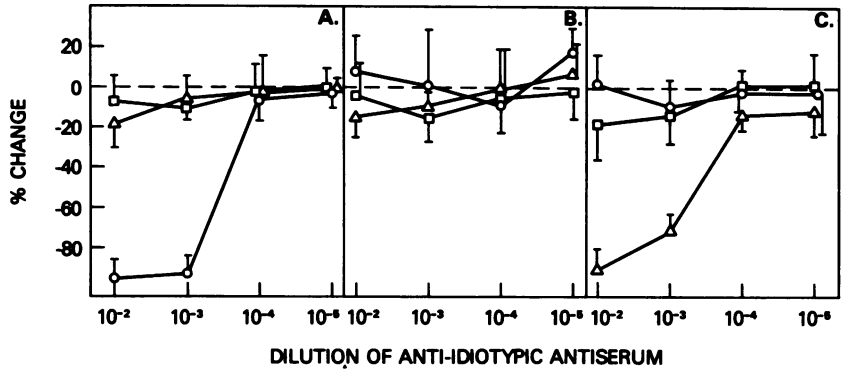

FIG. 3. *Effect of anti-Id sera on the binding of monoclonal anti-HA to measles antigens. (A) C2, 10 ng per well; (B) V17, 2.5 ng per well; (C) B2, 1.25 ng per well. Symbols: ○, anti-HAMM-1 Id serum; □, anti-HAMM-2 Id serum; △, anti--HAMM-3 Id serum. Anti-HAMM-1 Id serum inhibited the binding of C2 (A), and anti-HAMM-3 Id serum inhibited the binding of B2 to measles antigen (C). Each point represents the mean ± standard error (vertical bars) of triplicate determinations.*

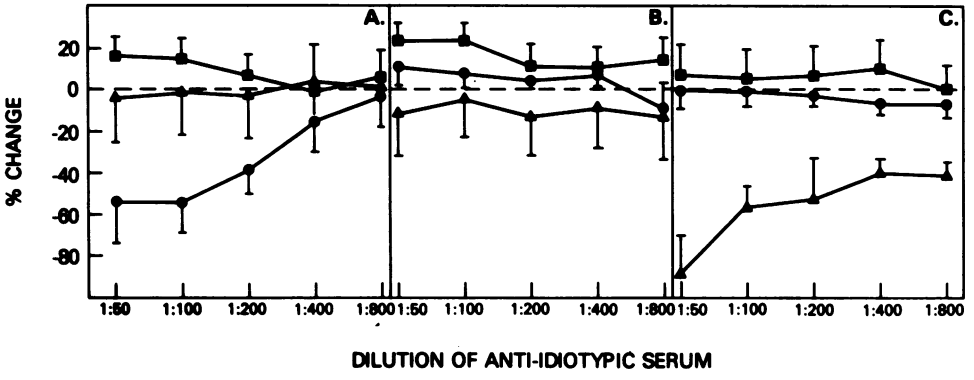

FIG. 4. *Effect of anti-Id sera on measles virus neutralization. (A) C2; (B) V17; (C) B2. The amount of each monoclonal anti-HA antibody used was known to give between 60 and 85% neutralization. Symbols: ●, anti-HAMM-1 Id serum; ■, anti-HAMM-2 Id serum; ▲, anti-HAMM-3 Id serum. The anti-HAMM-1 and anti-HAMM-3 Id sera significantly inhibited measles virus neutralization by C2 and B2, respectively. Each point represents the mean ± standard error (vertical bars) of triplicate determinations.*

Normal BALB/c serum did not neutralize measles virus and did not have an effect on measles neutralization by any of the monoclonal anti-HA antibodies. The anti-Id sera did not neutralize measles virus.

DISCUSSION

Because measles virus contains six major structural polypeptides which are immunogenic in various degrees (10, 27, 28), the immune response to the virus is highly complex and heterogeneous. Our initial studies of this complex immune response have focused on one component of the virus, the HA. Because this polypeptide is expressed on the virion surface and on infected host cells, it is important in the recognition of the virus by the immune system (3). Although the three monoclonal anti-HA antibodies that were used in this study had the same biological effects, such as HAI and virus neutralization, there were considerable quantitative differences. For example, purified B2 was twice as effective as V17 in reacting with measles by RIA, but was 20 times as effective in HAI. Such differences in biological activity are possibly related to the position of the determinant on HA against which a particular anti-HA antibody is directed. There is evidence that C2, V17, and B2 recognize different regions of HA (W. J. Bellini, G. D. Silver, and D. E. McFarlin, Fed. Proc. **40**: 1065, 1981).

Because C2, V17, and B2 have biological activity, it was possible to evalute the potential role of anti-Id in the anti-HA response and the immune response to measles virus in general. An important finding was that anti-Id could specif-

ically inhibit the biological function of the antibody in both the HAMM-1 and HAMM-3 Id systems. The observations that anti-Id could displace the bound antibody from infected cells suggest that the effect was because of competition between anti-Id and an epitope on measles HA for the binding site of the antibody, rather than agglutination of the antibody by the anti-Id. Competition between Id, anti-Id, and antigen has been described in certain myeloma systems (25, 29) and anti-hapten immune responses (6). The present results extend this to a biologically active immune response directed at a surface component of a pathogenic virus. The reason why such an effect was not observed in the HAMM-2 Id system is not known. One possiblity is that in this system the anti-Id sera were not directed against determinants in the binding site. Another possibility is that the affinity of the antibody for the antigen was higher than the affinity of the anti-Id for the antibody.

Each of the syngeneic anti-Id sera defined a different Id or set of Id's on the respective monoclonal anti-Ha antibodies. Although this is consistent with the view that there is considerable heterogeneity of the immune response to HA, the existence of a major cross-reacting Id in this immune response cannot be excluded. A limited cross-reactivity with the HAMM-1 Id was detected in some sera from BALB/c mice immunized with the Edmonston strain of measles virus. Apart from the sensitivity of the RIAs, there are three explanations why the hybridoma Id's were not detected in the sera to a greater extent. First, immunoglobulins may exist which share only part of a set of Id's and do not inhibit significantly the reaction between an anti-Id serum and the monoclonal antibody bearing the complete set of Id's. This has been shown with monoclonal antibodies expressing a well-defined cross-reacting Id in addition to private Id's (16). A second possibility is that the lymphocyte that gave rise to the monoclonal anti-HA antibody does not lead to circulating antibody because it is present in a suppressive immunoregulatory environment in vivo. Third, it is possible that myeloma and donor lymphocyte-encoded immunoglobulin chains are assembled to hybrid molecules (14) which give rise to a private Id in the monoclonal anti-HA antibody. Such an Id would not be expressed on serum immunoglobulins or on the myeloma protein.

It is currently believed that auto-anti-Id can occur during an immune response and play a role in immunoregulation (9, 11, 13). It is possible that this occurs in the immune response after infection with a pathogenic virus, such as measles, and particularly in the long-standing immune response that accompanies a chronic or persistent infection. The syngeneic conditions used to obtain an anti-Id response in the present study indirectly support this hypothesis. In addition, the data imply that such an auto-anti-Id could significantly interfere with the biological effects of Id-bearing antibodies or immunocompetent cells. In this respect, Id heterogeneity would seem to be beneficial to an efficient antiviral immune response. If a dominant Id existed, an auto-anti-Id could undermine the host defense against the virus and contribute directly to pathogenesis.

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LITERATURE CITED

1. **Awdeh, Z. L., A. R. Williamson, and B. A. Askonas.** 1970. One cell—one immunoglobulin. Origin of limited heterogeneity of myeloma proteins. Biochem. J. **116:** 241-248.
2. **Axen, R., J. Porath, and S. Ernback.** 1967. Chemical coupling of peptide and proteins to polysaccharides by means of cyanogen halides. Nature (London) **214:**1302-1304.
3. **Bellini, W. J., D. E. McFarlin, G. D. Silver, E. S. Mingioli, and H. F. McFarland.** 1981. Immune reactivity of the purified hemagglutinin of measles virus. Infect. Immun. **32:**1051-1057.
4. **Bellini, W. J., A. Trudgett, and D. E. McFarlin.** 1979. Purification of measles virus with preservation of infectivity and antigenicity. J. Gen. Virol. **43:**633-639.
5. **Bona, C., R. Hooghe, P. A. Cazenave, C. Leguern, and W. E. Paul.** 1979. Cellular basis of regulation of expression of idiotype. II. Immunity to anti-MOPC-460 idiotype antibodies increases the level of anti-trinitrophenyl-antibodies bearing 460 idiotypes. J. Exp. Med. **149:**815-823.
6. **Brient, B. W., and A. Nisonoff.** 1970. Quantitative investigations of idiotypic antibodies. IV. Inhibition by specific haptens of the reaction of anti-hapten antibody with its anti-idiotypic antibody. J. Exp. Med. **132:**951-962.
7. **Ebers, G. C., J. B. Zabriskie, and H. G. Kunkel.** 1979. Oligoclonal immunoglobulins in subacute sclerosing panencephalitis and multiple sclerosis: a study of idiotypic determinants. Clin. Exp. Immunol. **35:**67-75.
8. **Goding, J. W.** 1976. The chromic chloride method of coupling antigens to erythrocytes: definition of some important parameters. J. Immunol. Methods **10:**61-66.
9. **Golub, E. S.** 1980. Idiotypes and the network hypothesis. Cell **22:**641-642.
10. **Hall, W. W., R. A. Lamb, and P. W. Choppin.** 1979. Measles and subacute sclerosing panencephalitis virus proteins. Lack of antibodies to the matrix proteins with subacute sclerosing panencephalitis. Proc. Natl. Acad. Sci. U.S.A. **76:**2047-2051.
11. **Herzenberg, L. A., S. J. Black, and L. A. Herzenberg.** 1980. Regulatory circuits and antibody responses. Eur. J. Immunol. **10:**1-11.
12. **Hunter, W. M., and F. C. Greenwood.** 1962. Preparation of iodine-131 labeled human growth hormone of high specific activity. Nature (London) **194:**495-496.

13. **Jerne, N. K.** 1974. Towards a network theory of the immune system. Ann. Immunol. (Inst. Pasteur) **125:** 373–389.
14. **Kohler, G., and M. J. Shulman.** 1978. Cellular and molecular restrictions of the lymphocyte fusion. Curr. Top. Microbiol. Immunol. **81:**143–148.
15. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
16. **Lamoyi, E., P. Estess, J. D. Capra, and A. Nisonoff.** 1980. Heterogeneity of an intra-strain cross-reactive idiotype associated with anti-*p*-azophenylarsonate antibodies of A/J mice. J. Immunol. **124:**2834–2840.
17. **McFarland, H. F., and D. E. McFarlin.** 1979. Cellular immune responses to measles, mumps, and vaccinia viruses in multiple sclerosis. Ann. Neurol. **6:**101–106.
18. **McFarlin, D. E., W. J. Bellini, E. S. Mingioli, T. N. Behar, and A. Trudgett.** 1980. Monospecific antibody to the hemagglutinin of measles virus. J. Gen. Virol. **48:** 425–429.
19. **Mingioli, E. S., W. Strober, W. W. Tourtelotte, J. N. Whitaker, and D. E. McFarlin.** 1978. Quantitation of IgG, IgA and IgM in the CSF by radioimmunoassay. Neurology **28:**991–995.
20. **Mountcastle, W. E., and P. W. Choppin.** 1977. A comparison of the polypeptides of four measles virus strains. Virology **78:**463–474.
21. **Noppe, M., A. Lowenthal, D. Karcher, and J. Gheuens.** 1979. Two-site immunoradiometric assay for the determination of α-albumin (GFA). J. Immunol. Methods **27:**75–81.
22. **Norrby, E., and Y. Gollmar.** 1975. Identification of measles virus-specific hemolysis-inhibiting antibodies separate from hemagglutination-inhibiting antibodies. Infect. Immun. **11:**231–239.
23. **Norrby, E., and B. Hammarskjold.** 1972. Structural components of measles virus. Microbios **5:**17–29.
24. **Ouchterlony, O.** 1958. Diffusion-in-gel methods for immunological analysis. Prog. Allergy **5:**1–78.
25. **Sher, A., and M. Cohn.**1972. Effects of haptens on the reaction of anti-idiotypic antibody with a mouse antiphosphorylcholine plasmacytoma protein. J. Immunol. **109:**176–178.
26. **Strosberg, A. D., B. Marescau, K. Thielemans, B. Vray, D. Karcher, and A. Lowenthal.** 1979. Cross-idiotypic specificity among immunoglobulins in subacute sclerosing panencephalitis and multiple sclerosis, p. 97–103. *In* D. Karcher, A. Lowenthal, and A. D. Strosberg (ed.), Humoral immunity in neurological diseases. Plenum Publishing Corp., New York.
27. **Trudgett, A. G., W. J. Bellini, and D. E. McFarlin.** 1980. Antibodies to the structural polypeptides of measles virus following acute infection and in SSPE. Clin. Exp. Immunol. **39:**652–656.
28. **Wechsler, S. L., H. L. Weiner, and B. N. Fields.** 1979. Immune response in subacute sclerosing panencephalitis: reduced antibody response to the matrix protein of measles virus. J. Immunol. **123:**884–889.
29. **Weigert, M., W. C. Raschke, D. Carson, and M. Cohn.** 1974. Immunochemical analysis of the idiotypes of mouse myeloma proteins with specificity for levan or dextran. J. Exp. Med. **139:**137–147.