IDENTIFICATION OF A HEMAGGLUTININ-SPECIFIC IDIOTYPE ASSOCIATED WITH REOVIRUS RECOGNITION SHARED BY LYMPHOID AND NEURAL CELLS*

By JERRY T. NEPOM, HOWARD L. WEINER, MARC A. DICHTER, MARC TARDIEU, DALE R. SPRIGGS, COLETTE F. GRAMM, M. LINDA POWERS, BERNARD N. FIELDS, AND MARK I. GREENE‡

From the Department of Pathology, Harvard Medical School, the Department of Neuroscience, Children's Hospital Medical Center, the Department of Microbiology, Harvard Medical School, and the Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts 02115

Cell surface receptors function as specific binding structures for a wide variety of ligands, including antigens, hormones, toxins, and viruses. For recognition of antigen by B lymphocytes, the basis of receptor binding to ligand relates to the arrangement of variable regions of heavy and light immunoglobulin (Ig) chains, creating novel conformational structures in and around the binding site that are termed idiotypic determinants (1, 2). Antibodies (Ab)[1] to such Ab binding sites are termed anti-idiotypic. Indeed, it is thought that anti-idiotypic Ab interact with the idiotypic elements in the binding site in a manner similar to ligand binding to those same idiotypic structures.

Other types of cell surface receptors such as those used to bind hormones also are highly specific. For example, the binding of insulin to its target receptor can be blocked in some cases by antireceptor Ab (3). Moreover, it has been shown that antibody to anti-insulin Ab can mimic the effect of insulin itself (4). This observation may imply a similarity in the structural conformation of the insulin binding site to insulin binding sites on specific Ig.

We have begun an investigation of viral receptor interactions using reovirus as a model in an effort to determine whether viral receptors share idiotypic determinants with Ab to the virus. Two reovirus serotypes have been defined that differ in their ability to bind to lymphoid and neuronal tissues. Specifically, reovirus type 3 causes a fatal encephalitis in newborn mice with damage to neuronal cells but sparing of ependymal cells. Reovirus type 1 causes ependymal cell damage with a resultant hydrocephalus but no neuronal damage. Using recombinant viral clones, it has been shown that this in vivo tropism is determined by the viral hemagglutinin, a product of the S1 gene (5). Identical specificity has been shown for reovirus binding to neuronal cell cultures in vitro (Dichter and Weiner, manuscript submitted for

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‡ Address all correspondence to Dr. Mark I. Greene, Department of Pathology, Harvard Medical School.

[1] *Abbreviations used in this paper:* Ab, antibody; FACS, fluorescence-activated cell sorter; FI, fluorescein isothiocyanate; HA, hemagglutinin; Id3, idiotype selective for hemagglutinin of type 3 reovirus; PBS, phosphate-buffered saline; RIA, radioimmunoassay.

publication). In addition, reovirus type 3 but not type 1 binds to a subset of nonimmune T and B lymphocytes (6). The present study describes the creation and characterization of anti-idiotypic Ab to an idiotype of antihemagglutinin Ab. A population of nonimmune reovirus-binding lymphocytes as well as neurones are shown to share an idiotype characteristic of antihemagglutinin type 3 Ab. The genetic and pathophysiologic significance of this observation is discussed.

Materials and Methods

Animals. BALB/c mice, obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass., were maintained on standard lab chow and water ad lib. New Zealand white rabbits for immunizations were obtained from Microbiological Associates, Walkersville, Md., and used at 4–6 mo of age.

Virus. Virus was grown in mouse L cells maintained in suspension culture and purified on cesium chloride (7). Reovirus type 1 (Lang strain), type 3 (Dearing strain), and the two recombinant clones used have been previously described (8). 1.HA3 is a recombinant virus containing the hemagglutinin (HA) from type 3 on a background of type 1. 3.HA1 is the reciprocal recombinant.

Ab to Reovirus. Adult BALB/c mice were inoculated intraperitoneally with 10^9 particles of purified reovirus type 1 or 3, bled 2 wk later, then boosted with the same amount of virus and bled again 2 wk after the boost. Antisera were twice precipitated with 40% saturated ammonium sulfate followed by Sephacryl-200 gel filtration to obtain the Ig fraction. These antisera contained Ab to several viral proteins. To enrich for anti-HA Ab, each serum was absorbed on a recombinant reovirus that differed only in the gene encoding the HA protein. Absorption on ultraviolet inactivated reovirus was performed by mixing the antireovirus 3 Ig with 10^{11} particles of reovirus 1 or 3.HA1 for 1 h at 4°C, followed by ultracentrifugation at 30,000 *g* to remove virus. Antireovirus 1 was similarly absorbed with reovirus 3 and 1.HA3. As described in Results, a series of such absorptions yielded Ab with high but not absolute specificity or the HA of type 3 in the antireovirus 3 and type 1 in the antireovirus 1 antisera.

Anti-Idiotypic Antisera. New Zealand white rabbits were immunized with an initial injection of 500 μg absorbed antireovirus Ab (either anti-3 or anti-1) in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) intramuscularly and in the footpads. Rabbits were immunized at 3 wk and 5 wk with the same emulsion and were bled 7 d after the last injection. All sera were stored at −20°C until use. Serum obtained from each rabbit before immunization served as a normal control.

Anti-idiotypic Ab were identified by affinity for HA-specific Ig. Antisera selective for an HA type 3 idiotype $(Id3)^1$ were purified by affinity chromatography on a Sepharose 4B immunoadsorbent containing a monoclonal Ab from a hybridoma to HA type 3 (9BG5). Detailed characterization of the antireovirus hybridomas will be presented elsewhere. 10 mg purified monoclonal Ab was coupled to 5 ml packed Sepharose and washed extensively. Anti-idiotypic Ig bound to this immunoadsorbent was recovered by elution with 0.2 M glycine, pH 2.8, followed by dialysis against phosphate-buffered saline (PBS), pH 7.2.

Radioimmunoassay (RIA). Two types of RIA were developed: (*a*) direct RIA measured Ab binding activity to viral particles and was used to identify Ig with HA specificity; (*b*) indirect RIA measured idiotype on Ig molecules by competitive binding to the anti-idiotypic antiserum. The direct RIA was modified from standard methods: purified viral particles at 100 μg/ml were plated in 20 μl PBS onto polyvinyl microtiter plates (Cooke Engineering Co., Dynatech Corp., Alexandria, Va.) overnight at 4°C. After washing with PBS containing 10 mg/ml bovine serum albumin (PBSA), mouse Ab were added at 20 μl/well for 60 min at room temperature. After washing, ^{125}I rabbit anti-mouse Ig was added for a further 90 min at room temperature. Plates were then extensively washed; well bottoms were removed with a hot wire cutter and counted in a Beckman Auto Gamma 4000 counter (Beckman Instruments, Inc., Fullerton, Calif.). All determinations were performed in duplicate or triplicate wells.

Indirect RIA measured the binding of rabbit anti-idiotypic antiserum to ^{125}I radiolabeled antireovirus Ab, either monoclonal Ab or absorbed anti-HA Ig. Polyvinyl microtiter plates were coated overnight at 4°C with 100 μg/ml protein A from *Staphylococcus aureus* (Sigma Chemical

Company, St. Louis, Mo.) in PBS. After washing and incubating with PBSA as above, 20 μl rabbit antisera to antireovirus Ig was added at a concentration determined by titration to be limiting for binding of the ^{125}I-radiolabeled ligands. After 60 min at room temperature, wells were washed with PBSA and incubated for a further 30 min with 2% normal rabbit sera to saturate any unoccupied protein A binding sites. These preparative steps permit highly specific Ab-binding capacity in microwells suitable for competitive RIA. 30 min before addition of the ^{125}I-labeled ligand, 20 μl of an appropriate dilution of the test competitive antisera was added to each well in the presence of 0.5% normal mouse serum. 10 ng of ^{125}I-labeled hybridoma protein or 100 ng of ^{125}I-labeled antireovirus Ig was then added in a volume of 20 μl. After 90 min continuous shaking at room temperature, wells were washed, harvested, and counted as before.

All ^{125}I-labeled Ab were prepared by the chloramine T method (9) using Na^{125}I (New England Nuclear, Boston, Mass.) and stored at −20°C until use.

Isoelectric Focusing (IEF). Analytical flatbed IEF with a 4% acrylamide gel containing 6 M urea and 1% NP40 was run at 4 watts continuous power until voltage exceeded 1100 V. The gel was dried and exposed for 4 d on Kodak X-Omat film to generate the autoradiograph shown.

Immunofluorescence. Lymphoid cell lines R1.1 and YAC were stained in suspension for analysis on a fluorescence-activated cell sorter (FACS). Cells from tissue culture were spun over a Ficoll-Hypaque cushion to remove dead cells and debris, and pelleted after washing. 15 μl of anti-idiotypic antisera diluted 1:5 in PBS was added to cell pellets containing 10^6 cells, which was then mixed and incubated on ice at 0°C for 30 min. After washing, 15 μl of FITC-conjugated goat anti-rabbit Ig or FITC protein A was added to each pellet for a further 30 min incubation on ice. Cells were fixed with 1% paraformaldehyde before FACS analysis (B-D FACS Systems, Becton, Dickinson & Co., Sunnyvale, Calif.). Monolayer cultures of rat cortex and murine spinal cord were prepared as described by Dichter (10) and Ransom et al. (11). For staining of neuronal cells with anti-idiotype antiserum, cells adherent to cover slips were gently dipped in PBS before staining in a similar two-step protocol, except that all washes were performed by dipping the cover slips in PBS. Neuronal cells were fixed after staining with 5% acetic acid in ethanol. Ciliated ependymal cell suspensions were prepared from adult C57 black mice by a procedure modified from Manthorpe, et al. (2) and stained in suspension as described above.

Cells. R1.1 is a cell line derived from a spontaneous thymoma of a C58 (H-2^k) mouse and bears serologically detectable H-2^k TL and Lyt antigens. The cell line was originally obtained from Dr. R. Hyman and maintained in RPMI 1640 supplemented with 10% fetal calf serum (FCS), glutamine, penicillin, and streptomycin. The YAC Moloney virus-induced lymphoma of A(H-2^a) strain mice was obtained from Dr. G. Klein, Karolinska Institute, and maintained in continuous culture in RPMI 1640 with 5% FCS.

Results

Ab to Reovirus Bind HA Determinants. To prepare an anti-idiotypic antiserum with specificity for HA-binding molecules, we first prepared antireovirus Ab that selectively bound the appropriate HA. The IG fractions from BALB/c antisera to reovirus type 1 and reovirus type 3 were serially absorbed on the opposite serotype (antireovirus 1 on reovirus 3 and antireovirus 3 on reovirus 1 to remove Ab recognizing common determinants. Titers of reovirus-binding Ig were monitored with an RIA measuring total bound IG. Figs. 1 A and B illustrate these data: after three absorptions on reovirus type 1, antireovirus 3 antibody titers against reovirus 3 were substantially higher than against reovirus 1 (Fig. 1 A). The reciprocal data after absorptions of antireovirus 1 Ig on reovirus type 3 are shown in Fig. 1 B. To further enrich for HA-specific Ig, absorptions were then performed using recombinant clone 1.HA3, containing the HA of type 3 on a background of reovirus 1 and clone 3.HA1, the reciprocal recombinant. Antireovirus 3 Ig was absorbed on 3. HA1 until the titer of

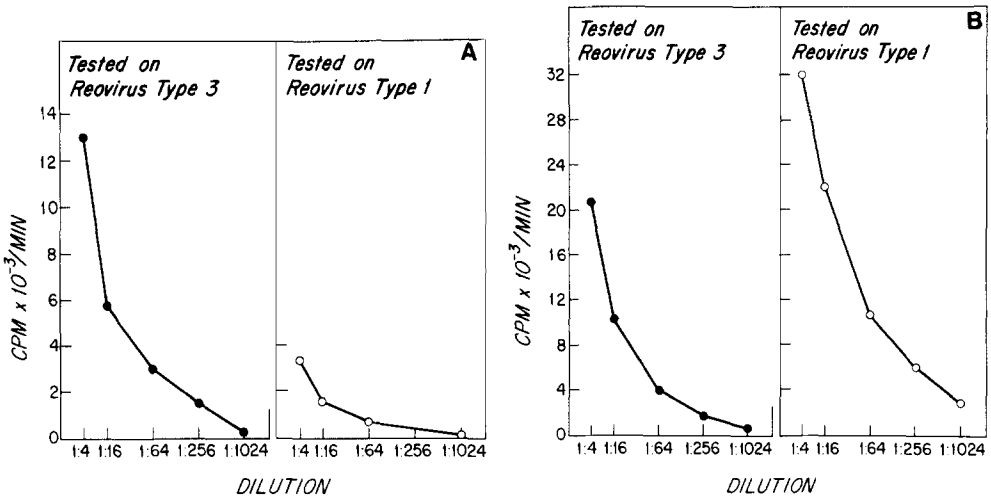

FIG. 1. Binding assays of antireovirus Ab. (A), Ig from antireovirus 3 antisera was extensively absorbed with reovirus 1 and tested for binding to reovirus type 3 or type 1 to determine serotype selectivity. (B), Ig from antireovirus 1 antisera after absorption on reovirus 3 tested on the same virus serotypes demonstrates reciprocal selectivity.

Ig bound on 1.HA3 was 2^4 higher than on reovirus 1. Antireovirus 1 Ig was similarly absorbed on 1.HA3.

These Ig fractions selected for type 1 or type 3 HA-binding activity were then used as immunogens to raise anti-idiotypic Ab. Each Ig fraction was also radiolabeled with ^{125}I[NaI] for use in the competitive binding RIA described below. Fig. 2 is a tracing of an autoradiogram obtained from the IF of each radiolabeled Ig fraction after binding to its target HA. Thus, the ^{125}I-labeled antireovirus 3 Ig that was bound and eluted from reovirus 1.HA3 is predominantly IgG_2 isotype, as is the antireovirus Ig that was bound and eluted from reovirus 3.HA1. The third gel channel contained ^{125}I-labeled Ig from A/J mice immune to azobenzenearsonate, demonstrating for comparison the restricted IgG_1 isotype characteristic of the major cross-reactive idiotype of Ig in that model.

Rabbit Antisera to Antireovirus 3 Ig Recognize HA-binding Determinants. The competitive binding RIA described below was used to identify variable-region idiotypic determinants present on antireovirus Ig recognized by rabbit antisera raised against the absorbed, anti-HA type 3 Ig. All measurements were performed in the presence of 0.5% normal mouse sera because some of the activity of the rabbit antisera was directed against common mouse Ig determinants. Our initial screening RIA evaluated the ability of various Ig to inhibit the binding of ^{125}I-labeled absorbed antireovirus Ig to the rabbit antisera. As shown in lines one and two of Table I, both antireovirus 1 and antireovirus 3 Ig inhibited this interaction, suggesting that a large number of antireovirus specificities were present that cross-reacted in this type of inhibition assay. Because our objective was to identify HA-specific binding structures, we screened a series of antireovirus HA hybridoma Ab for their ability to inhibit this interaction, postulating that inhibition would be observed if a significant amount of the rabbit antisera recognized a major determinant shared between the hybridoma Ig and the HA-selective absorbed antireovirus Ig. Six monoclonal Ab to reovirus HA

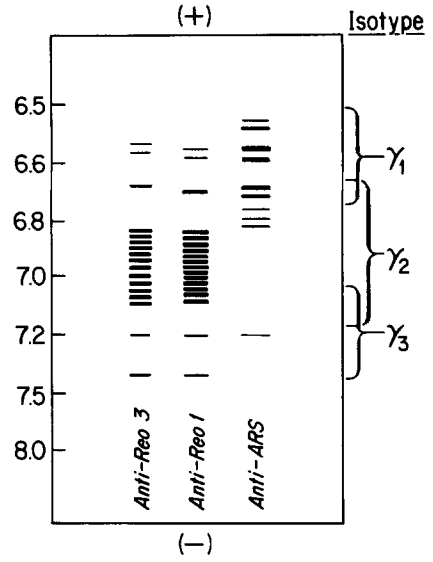

FIG. 2. IEF pattern of antireovirus Ab. Radiolabeled Ig from antireovirus antisera with HA-selective binding was compared to the Ig from anti-azobenzenearsonate atisera. Tracing of the resultant autoradiograph shows a predominance of IgG_2 in both the antireovirus 3 and antireovirus 1 preparations.

TABLE I

Identification of a Distinct Antireovirus 3 Determinant Shared between Antisera to Reovirus 3 and Hybridoma Ab to the Reovirus 3 HA

Experiment	Rabbit antisera	^{125}I Antireovirus Ab	Inhibiting Ab	Ab concentration at 50% inhibition	Percent inhibition at 200 μg/ml
				μg/ml	
I	Anti-anti-3	III	III	12	92
			I	15	90
			F4	>200	38
			A2	>200	46
			B2	>200	34
			D2	175	55
			G5	35	83
			F7	>200	40
II	Anti-anti-3	III	G5	18	82
	Anti-anti-1	I	G5	50	78
	Anti-anti-1		III	50	80
	Anti-anti-1		I	12	90

type 3 tested for inhibition are shown in Table I. One of these, designated G5, significantly inhibited the binding of the rabbit antisera to the anti-type 3 Ig. The G5 hybridoma Ab is known to bind to a domain of the HA responsible for neutralization of the virus (Spriggs and Fields, unpublished observations). Four monoclonal Ab to

reovirus type 1 HA were also tesed in this same interaction and did not inhibit. The high degree of inhibition observed with the G5 monoclonal Ab was not due to nonspecific anti-Ig or antiallotype or isotype reactivity of the rabbit antisera because all other monoclonal Ab tested to date have not inhibited the binding.

Experiment II in Table I further demonstrates the specificity of inhibition by the G5 monoclonal Ab. A competitive binding RIA using rabbit antisera to antireovirus type 1 Ab instead of to type 3 was developed, and inhibition by monoclonal Ab G5 was compared in the two assays. A threefold higher concentration of G5 was necessary to inhibit the anti-type 1 binding compared with the anti-type 3; indeed, the concentration of G5 necessary for 50% inhibition of the type 1 binding was comparable with the concentration of antireovirus 3 Ig, which inhibited the type 1 interaction. The last line of Table I depicts a positive control for the type 1 binding and shows that anti-type 1 Ig inhibits four times better than either G5 or the anti-type 3 Ig. Thus, the G5 determinant appeared to be preferentially recognized by the antisera raised against antirevirus type 3 Ig; because the G5 monoclonal Ab is directed against the viral HA and the absorbed Ig used in the inhibition assays was selected for HA-binding activity, we postulated that we had identified a shared HA-binding determinant, with specificity for the type 3 protein. Subsequent attempts to investigate this determinant, which we have designated Id3, have used the G5 monoclonal Ab both to assay for presence of this shared determinant and to enrich by affinity purification the rabbit antisera against antireovirus type 3 Ig.

The Id3 Determinant Is Linked Specifically to Anti-HA 3 Ab. A competition RIA using the rabbit antisera against antireovirus 3 Ig tested for binding to ^{125}I-labeled G5 monoclonal Ab was developed, and served as a selective assay for the Id3 determinant. This assay is highly sensitive and specific for Id3 activity because it measures competitive binding toward a specific monoclonal determinant. Fig. 3 illustrates the HA specificity of the Id3 determinant. In Fig. 3 A the ability of antireovirus Ig to inhibit the binding of ^{125}I G5 Ig by rabbit antisera raised to antireovirus 3 Ig was measured. Absorbed antireovirus 3 Ig (with HA 3 selectivity) inhibited this assay much more than antireovirus 1 Ig. Confirmation that this Id3 determinant shared by antireovirus 3 Ig and the G5 hybridoma product marks the HA type 3 binding specificity is shown in Fig. 3 B. Antireovirus 3 Ig was absorbed on reovirus 1.HA3 to remove the HA-binding globulins and retested for the presence of the Id3 determinant. Absorportion on reovirus 1 served as a control for non-HA binding. The inhibition seen with antireovirus 3 Ig was removed only by the type 3 specific absorption. Id3 appears to identify an idiotypic HA type 3 binding structure present in the antireovirus 3 Ig preparation.

The Id3 Determinant Is Present on Reovirus-binding Cells. The Id3 determinant has been serologically identified on antireovirus Ig and one antireovirus hybridoma Ab. We have used cell surface immunofluoresence in a series of experiments to determine whether the Id3 determinant marks an HA-binding idiotypic structure on cells as well. We have screened a variety of cell populations with known reovirus-binding capacities for ability to bind the Id3-specific rabbit antireovirus 3 antisera. Examples of such data are shown in Fig. 4 and summarized in Table II.

Cell line R1.1 is a T cell-derived culture-adapted thymoma that binds reovirus type 3 and not reovirus type 1 (M. Greene and John Noseworthy, unpublished observations). Fig. 4 A shows the cytofluorographic profile obtained staining R1.1

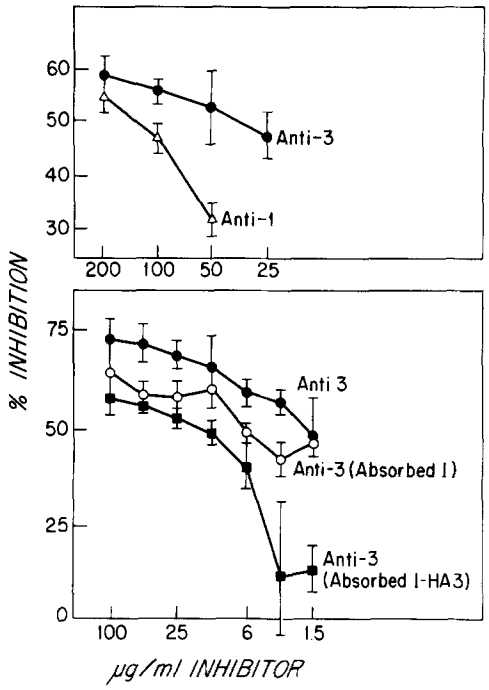

FIG. 3. The Id3 determinant is reovirus 3 HA specific. Competitive binding RIA were performed to assess the specificity of recognition of G5 hybridoma antibody by the rabbit anti-idiotypic antisera. (A), Ig from anti-reovirus 3 antisera inhibits the binding much more than Ig from anti-reovirus 1 antisera, and (B), the Id3 determinant present in the antireovirus 3 antisera is removed only by selective absorption on HA type 3.

with rabbit antisera to antireovirus 3 Ig compared with rabbit antisera to antireovirus type 1 Ig. A marked shift in fluorescent intensity of the cell population is observed with the antisera to type 3 idiotype and not with the control sera. Fig. 4 B demonstrates that the binding is indeed due to Id3 determinants on R1.1 cells; rabbit antisera to the type 3 idiotype were passed over an immunoadsorbent containing G5 hybridoma Ab attached to Sepharose 4B. Both unbound (b) and bound and recovered (a) fractions were tested for immunofluorescence on R1.1 The fraction enriched for antiserum against the Id3 determinant contained the R1.1-binding Ig. This fraction is presumably anti-idiotypic, accounting for the shared recognition of the G5 Ab, the R1.1 cell, and the antireovirus 3 Ig. Rabbit antisera to non-G5 determinants (curve b) was negative for staining on R1.1 cells; thus, the R1.1-binding Ig was removed by adsorption on the G5 Ig.

Confirmation that a single Ab in the anti-idiotypic antisera was responsible for the observed binding is shown in Fig. 4 C. Fluorescence profiles on R1.1 cells are shown using a syngeneic monoclonal Ab raised in BALB/c mice against the G5 Ab and selected for idiotypic specificity (J. Nepom, J. Noseworthy, L. Perry, and M. I. Greene, manuscript in preparation). This Ab, from a cloned B cell hybridoma, recognizes the same G5 idiotype characterized by the rabbit anti-idiotype sera described above. Curve a shows that this monoclonal Ab to the G5 idiotype also bound R1.1 cells; after absorption with G5 Ig, no R1.1 staining was seen (curve b). Because the shared recognition observed using the rabbit antisera was confirmed

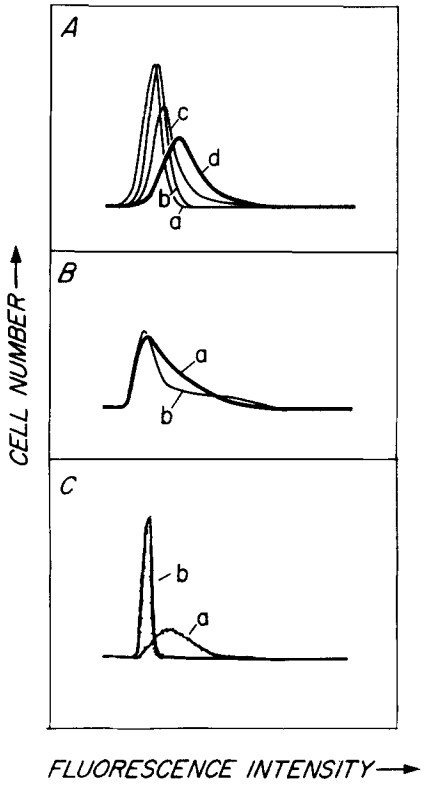

FIG. 4. Cytofluorographic profiles of R1.1 thymoma cells stained with anti-idiotypic antisera. (A), indirect immunofluorescence of 50,000 R1.1 cells with no rabbit serum (a), unimmunized rabbit serum (b), rabbit antisera to antireovirus type 1 HA (c), rabbit antisera to antireovirus type 3 HA (d). Sera were tested at 1:5 dilution followed by FITC-coupled protein A to give the profiles shown. (B) indirect immunofluorescence of 50,000 R1.1 cells with affinity-purified rabbit Ab to the Id3 determinant anti-idiotype enriched for Id3 binding by elution from a G5 containing immunoadsorbent (a), and anti-idiotype depleted of Id3 binding activity, with the unbound fraction from a G5 containing immunoadsorbent (b). 15 μl of each Ab was tested at 200 μg/ml followed by FITC-coupled protein A to give the profiles shown. (C) indirect immunofluorescence of 40,000 R1.1 cells with a syngeneic monoclonal Ab to the Id3 determinant, raised by immunization with the G5 monoclonal Ab. Monoclonal Ab culture supernatant followed by FITC-coupled rabbit anti-mouse Ig (a), and monoclonal Ab culture supernatant after removal of the G5-binding Ig on an immunoadsorbent, followed by FITC-coupled rabbit anti-mouse Ig (b).

using a syngeneic monoclonal Ab that has only a single specificity, we conclude that the anti-idiotypic Ab made against antireovirus 3 Ig does indeed identify a common determinant on reovirus-binding cells and Ab. The YAC lymphoma binds neither reovirus 1 nor 3, and immunofluorescent studies with anti-idiotypic anti-Id3 Ab were also negative for selective binding on YAC cells.

This same anti-idiotypic antiserum was screened on explanted cells from the rat and mouse nervous system and examined by visual immunofluorescence and phase microscopy. Anti-Id3 Ab purified by elution from the G5 immunoadsorbent bind cultured neuronal cells, as shown in Fig. 5 B. Control rabbit antisera with other non-Id3-binding Ab is negative for staining on neurons (Fig. 5 D). Similar lack of staining on neurons is seen both with rabbit antisera to reovirus type 1 Ig and with rabbit

TABLE II

Immunofluorescence Patterns of Reovirus Binding and Anti-Idiotypic Ab Staining When Tested on Lymphoid and Neuronal Tissues

Cell type	Reovirus bound*		Anti-idiotype bound‡	
	Type 3	Type 1	Id3+	Id3−
R1.1 thymoma	+	−	+	−
YAC lymphoma	−	−	−	−
Neuronal cells	+	−	+	−
Ependymal cells	−	+	−	+

* Stained with FITC-rabbit antireovirus Ig.

‡ Anti-idiotype Ab affinity purified by binding to the G5 monoclonal Ab on a sepharose immunoadsorbent. Staining with unbound (Id3−) or bound and eluted (Id3+) rabbit Ig followed by FITC goat anti-rabbit Ig.

antisera to reovirus type 3 Ig that has been absorbed with G5 Ig to remove the anti-idiotypic specificity. Additional evidence for anti-idiotype specificity within the nervous system comes from the observation that these same purified anti-Id3 Ab do not bind to single cell preparations of ciliated ependymal cells. Neurons in these experiments were identified by their characteristic appearance of phase bright somata with multiple dendritic processes. These cells have been definitively identified as neurons in extensive studies in Dichter's laboratory (10) by tetanus toxin immunofluorescence and intracellular microelectrode recordings. Although the exact association of the reovirus receptor to the Id3 determinant remains to be clarified, it appears that the central nervous system tropism of reovirus 3 and the presence of this idiotype coincide; that is, cells that bind reovirus 3 HA also bind the anti-Id3 antisera. Thus, we have recently described the binding of reovirus 3 and not reovirus 1 to similarly cultured rat neuronal cells, and suspensions of murine ependymal cells purified from mouse brain bind neither reovirus 3 nor the anti-Id3 antisera, although they do bind reovirus type 1. These data are summarized in Table II.

Discussion

Antigen recognition by immune B cells operates via receptors with idiotypic properties corresponding to the variable region-encoded domains of Ig. T cells, in some cases, also express idiotypic structures as part of their binding site (13–15). Receptors on nonimmune cells also demonstrate highly specific ligand recognition, an example being the HA-selective binding of neuronal cells for reoviruses. We have probed the relationship between receptors on immune and nonimmune cells towards the mammalian reovirus and have identified a common idiotype associated with HA binding.

We describe here the identification of a distinct serologically recognized determinant that is present on structures to which reovirus type 3 binds via the viral HA. This determinant, which we call Id3, was identified by its ability to inhibit the binding of a rabbit antisera generated against antireovirus 3 Ig to Ab with reovirus 3 HA-binding activity. We were able to characterize a selected HA-binding determinant by the use of monoclonal Ab from hybridomas made to the HA of reovirus 3. The Id3 determinant was shown to be pesent on (*a*) a monoclonal Ab (G5) to reovirus

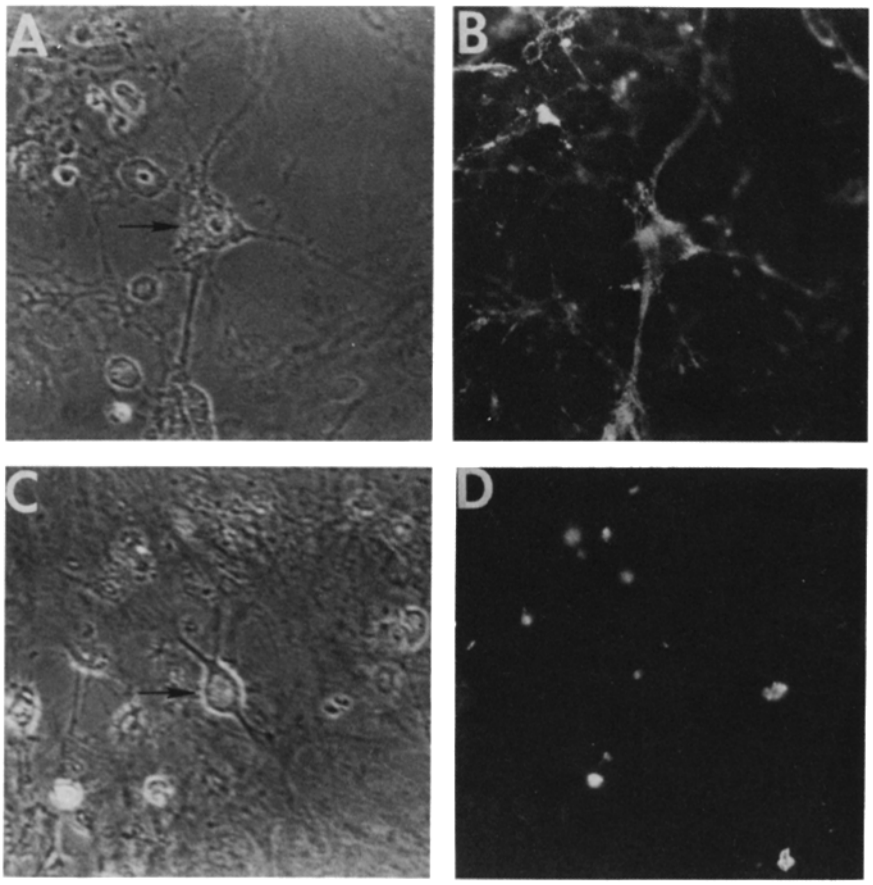

FIG. 5. Mouse spinal cord neurons in cell culture stained with anti-Id3 Ab. (A and B), phase contast photomicrograph and corresponding field in fluorescence of large multipolar neuron (arrow) and other, non-neuronal cells. Intense fluorescence of the neuron cell body and processes is seen as well as some other neuronal processes coursing through the microscopic field. Culture was stained with purified anti-idiotypic Ab eluted from a column containing monoclonal Ab G5. (C and D), Similar phase contrast and fluorescence photomicrographs of one field from a culture stained with Ab not bound to the G5 column. Note the neuron in the center of the field in C (arrow) and the total lack of specific immunofluorescence in D. Panel D shows lack of staining by the same antisera that was purified for use in panel B except that anti-G5 Ab have been removed, rather than recovered, by immunoadsorption. Negative staining was also seen using rabbit antisera to reovirus type 1 Ig rather than to type 3 Ig. Magnification, × 344.

HA type 3, (*b*) highly purified BALB/c anti-HA type 3 Ab, (*c*) R1.1, a T cell-derived cell line with selective reovirus 3 binding activity, and (*d*) neuronal cells in primary cell cultures. The Id3 determinant is not present on Ig from antisera to reovirus 1 with anti-HA type 1 activity, on Ig from numerous other hybridomas producing Ab to either the type 3 or type 1 HA, on YAC cells, a nonreovirus-binding murine tumor line, or on ependymal cells that bind reovirus type 1 but not type 3. This anti-Id3 activity present in the rabbit antisera to antireovirus type 3 Ig appears to be anti-idiotypic because it selectively identifies determinants on HA 3-binding structures that are removed by absorption on the HA type 3. Rabbit antisera to antireovirus type 1 Ig does not have any of the properties that identify this anti-idiotypic antisera, further documenting the specificity of the Id3 determinant.

The idiotypy of cell surface receptors has been extensively studied, primarily in analysis of B cell and T cell recognition mechanisms in the immune system. After immunization, a clonal distribution of idiotype-bearing determinants present on B cell receptors, secreted Ig, and on some T cell receptors and factors arises and serves as a focus of internal immune regulation (15). The nature of the B cell receptor is especially well understood at the nucleic acid (16, 17), primary amino acid (18, 19), and even the tertiary structural level (20). This has been feasible because secreted Ig from B cells have identical ligand-binding structural elements as the membrane-bound Ig (21) that functions as an antigen receptor. Although differences in the membrane and secreted Ig type exist, these differences do not relate to the portion of the Ig that mediates ligand binding.

Anti-idiotypic antisera generated against idiotypic Ig in such clonally distributed situations often has potent immunologic effects and may interact directly wth T cell receptors or mimic the antigen itself in terms of ability to prime a nonimmune animal. The idiotypy of receptors that do not require immunization and may not involve expansion of a rearranged V_H product for expression has been less extensively studied. Precedent for anti-idiotypic interaction with such nonimmune receptors, however, has been reported: antiserum generated against anti-insulin Ab binds and even activates insulin receptors on nonimmune cells (4) as do 'auto-anti-receptor' Ab present in the sera of some individuals that mimic the insulin effect on suitable target cells.

Our observations of a shared idiotypy of antireovirus 3 Ig and the R1.1 T cell line might permit study of the genetic basis for HA-binding structures on nonimmune cells. V_H genes code for T and B cell idiotypic receptors, and it is possible that they also code for the nonimmune receptors described here. However, we have so far been unable to demonstrate a linkage of the reovirus receptor to the Igh locus, so tht if V_H genes do contribute, it is likely that some relatively invariant public specificity is involved. A somatic cell genetic approach might also help determine whether the receptor is encoded on the same or different chromosome than V_H or V_L elements. Alternatively, similar HA-binding structures on disparate tissues might really reflect convergent forms deriving from the presence of an internal image of the HA by the anti-idiotype. That is, the anti-idiotypic structure might truly resemble the HA as seen by an idiotypic receptor.

The presence of the Id3 idiotypic determinant on neuronal cells raises the possibility that this same idiotype is intimately involved in some manner in ligand recognition by neurons. To date, we have found that the selective tropism of reovirus HA-binding to neuronal and ependymal cells corresponds precisely to the selective binding of our anti-idiotypic antisera as detected by immunofluorescence.

If the Id3 determinant indeed identifies the viral receptor, the use of anti-idiotypic antisera provides a useful tool for analysis of virus-cell interactions and possible mechanisms of auto-immune phenomena in the nervous system after viral infection.

Summary

A xenogeneic antiserum raised to antireovirus immunoglobulin was used to define an idiotypic determinant present on antibodies to reovirus type 3 hemagglutinin. The same idiotype was identified on nonimmune lymphoid cells and on neuronal cells that specifically bind the hemagglutinin of type 3 reovirus. This idiotypic determinant, called Id3, is shared by (*a*) a monoclonal antibody to the neutralization site of

hemagglutinin from type 3 reovirus; (*b*) BALB/c serum antibodies to the hemagglutinin of reovirus type 3; (*c*) R1.1, a murine thymoma cell line that binds reovirus type 3; (*d*) primary cultures of murine neuronal cells. The presence of an idiotype shared by antihemagglutinin antibodies and by structures on nonlymphoid cells suggests a general relationship between disparate receptors that recognize a common determinant. Furthermore, this suggests a novel approach for the study of viral receptor interactions and for analysis of mechanisms of autoimmune responses.

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