JOURNAL OF VIROLOGY, Nov. 1993, p. 6815–6819
0022-538X/93/116815-05$02.00/0

Vol. 67, No. 11

Anti-Idiotypic Mimicry of a Neutralizing Epitope on the Glycoprotein B Complex of Human Cytomegalovirus

EILLEEN S. TACKABERRY,[1,2] JOSÉE HAMEL,[1] YOLANDE LAROSE,[1] AND BERNARD R. BRODEUR[1,2]*

National Laboratory for Immunology, Laboratory Centre for Disease Control, Ottawa, Ontario, Canada K1A 0L2,[1] and Department of Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5[2]

Received 21 June 1993/Accepted 11 August 1993

Experiments were carried out to investigate the ability of rabbit anti-idiotype antibodies (Ab2), directed against an anti-human cytomegalovirus monoclonal antibody (Ab1), to induce neutralizing antibodies specific for the immunodominant glycoprotein B viral complex. Mice immunized with Ab2 produced anti-Ab2 (Ab3) that was both antigen and idiotype specific with regard to Ab1. We conclude that the Ab2 antibodies mimicked a neutralizing epitope and acted as a network antigen for inducing a specific anti-human cytomegalovirus antibody response in this experimental system.

Human cytomegalovirus (HCMV) is a widely distributed herpesvirus responsible for severe disease in immunocompromised hosts. Maternal antibody to HCMV correlates with reduced incidence and severity of congenital infection (7, 34), and hyperimmune globulin moderates HCMV-associated disease in transplant patients (32, 34). Immunity was recently provided for bone marrow transplant recipients by adoptive transfer of HCMV-specific T cells (30). Such observations suggest that a vaccine inducing an immune response equivalent to that following natural infection may be effective. Neutralizing antibodies to several envelope glycoproteins in infected individuals have been recognized (19, 29). Of these, glycoprotein B (gB), a complex of 58- and 116 kDa proteins (4, 5), appears to be especially immunodominant (23, 28) and is widely regarded to be a major candidate for vaccine development (22, 34).

Interactions of idiotypic (id) determinants and anti-id antibodies (Ab2) are involved in regulating the immune response. Anti-id antibodies may enhance or suppress specific immunity (9, 15), and id network relationships have been manipulated experimentally to stimulate humoral and/or cell-mediated immune responses to microbial infections (6, 8, 26). Use of anti-id vaccines may be advantageous when the protective epitope(s) is poorly defined, complex (6, 26), or conformational (38). In the present study, we sought to generate polyclonal anti-id antibodies that would mimic a neutralizing epitope on the gB complex of HCMV sufficiently to induce a specific antibody response in naive hosts. The murine monoclonal antibody (MAb) CMVB1 {immunoglobulin G subclass 2a(κ) [IgG2a(κ)]} was selected as the initiating Ab1 for the study. Previous characterization of MAb CMVB1 showed that it recognized a late viral antigen in cells infected with HCMV prototype strains AD169, Towne, and Davis, and all clinical isolates (n = 24) tested thus far (31). In this study, further analysis of purified CMVB1 and HCMV strain AD169 by plaque reduction assay (20) indicated that it was highly neutralizing in the presence of complement, with activity still apparent at less than 28 ng of purified MAb per ml. No neutralization occurred in the absence of complement. Radioimmunoprecipitation assays were carried out to identify the target proteins of MAb CMVB1, following the method described elsewhere (20, 38). As seen in Fig. 1, MAb CMVB1 precipitated viral proteins of 58, 93 to 116, and 130 kDa (Fig. 1, lane 2), a pattern consistent with that defined by other MAbs for the envelope glycoprotein complex gB (23, 27, 33). Similar bands were precipitated by a neutralizing HCMV-seropositive human serum sample obtained from a kidney transplant patient (Fig. 1, lane 4). Neutralizing antibodies to the gB complex routinely develop after natural infection (23, 28), and 48 to 88% of the total neutralizing activity in serum is directed toward the gB complex (23).

Rabbit Ab2 antibodies induce Ab3 with specificity for the gB complex of HCMV. Rabbits immunized with MAb CMVB1 produced specific anti-id antibodies which were detectable in trial bleed samples after a single injection. New Zealand White rabbits were immunized subcutaneously with 200 μg of purified MAb CMVB1 in complete Freund's adjuvant. Ab2 antibodies were detected by an enzyme-linked immunosorbent assay (ELISA) in which microtiter wells were coated with $F(ab')_2$ fragments of MAb CMVB1 (0.15 μg per well) or an isotype-matched control MAb. Dilutions of rabbit Ab2 were added, followed by enzyme-conjugated anti-rabbit IgG (H+L) (Jackson ImmunoResearch Laboratories, West Grove, Pa.). Ab2 antibodies were purified from pooled rabbit antisera collected on days 10 and 17 after immunization. Purification was accomplished by using a series of four affinity columns, eluted with 0.1 M glycine-HCl buffer, pH 3.0, as follows: (i) protein-A Sepharose CL-4B (Pharmacia, Ltd., Montreal, Quebec, Canada), (ii) an isotype affinity column of CNBr-activated Sepharose 4B (Pharmacia, Ltd.) to which two purified irrelevant murine MAbs [both IgG2a(κ)] were bound, (iii) a similar column with purified MAb CMVB1 (Ab1) as a ligand, (iv) protein-A Sepharose to concentrate the purified Ab2. This protocol yielded a preparation containing 1.3 mg of purified rabbit Ab2. The material (at 665 μg/ml) had an ELISA titer of 32,000 against MAb CMVB1 and no reactivity against the control MAb.

Mice were immunized with 30 μg of purified Ab2 (mice 1 to 3) or 30 μg of purified normal rabbit IgG (mice 4 to 6) in conjunction with 25 μg of Quil-A. Identical booster injections were given on days 15 and 46. The presence of mouse Ab3 in immune serum was detected by inhibition ELISA: equal volumes of purified rabbit Ab2 in 5% normal rabbit serum and mouse anti-Ab2 serum in phosphate-buffered saline (PBS) were incubated for 30 min, whereupon residual binding of Ab2

* Corresponding author.

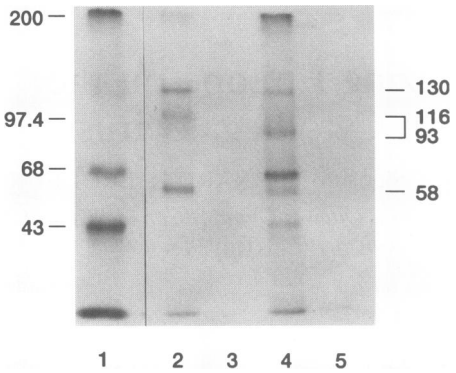

FIG. 1. Viral antigens recognized by MAb CMVB1. Radiolabelling of late viral proteins with [^{35}S]methionine (Tran^{35}S-label; ICN Biomedicals, Inc., Irvine, Calif.) and immunoprecipitation of labelled cell lysates were done by methods described previously (20, 38). Cell lysates from [^{35}S]methionine-labelled HCMV-infected (lanes 2 and 4) or [^{35}S]methionine-labelled control mock-infected (lanes 3 and 5) human foreskin fibroblasts were precipitated with MAb CMVB1 or a neutralizing seropositive human serum sample and then analyzed by electrophoresis under reducing conditions with sodium dodecyl sulfate–10% polyacrylamide (17). Lanes: 1, ^{14}C-molecular mass markers, in kilodaltons; 2 and 3, precipitation by CMVB1 ascitic fluid diluted 1:1,000; 4 and 5, precipitation by HCMV-seropositive human serum diluted 1:1,200.

to Ab1 was measured, as described above. The Ab2 concentration was established beforehand to yield an A_{410} control value (Ab2 plus PBS) of 1.0. The data in Fig. 2 show that all mice immunized with rabbit Ab2 produced Ab3, with reactivity decreasing in a dose-dependent manner. The strongest response was seen in the serum of mouse 1, which exhibited a titer of more than 40. Conversely, there was little response in the sera of mice immunized with equal amounts of normal rabbit IgG.

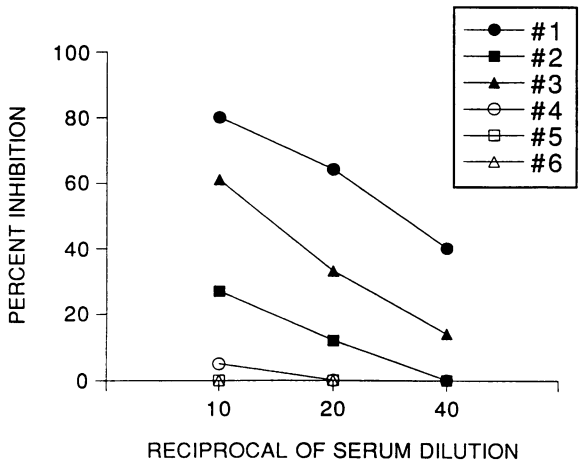

FIG. 2. Detection of Ab3 in mouse sera by inhibition ELISA. Equal volumes of dilutions of immune mouse sera were incubated with purified rabbit Ab2, and residual binding activity of Ab2 for the F(ab')$_2$ fragments of Ab1 was measured. Percent inhibition was determined by comparison with wells in which PBS was substituted for mouse serum. Mice 1 to 3 had been immunized with purified rabbit Ab2, and mice 4 to 6 had been immunized with the same amount of purified normal rabbit IgG.

The immune mouse sera were then evaluated to see whether they contained antibodies directed against HCMV, using assays developed for characterizing the Ab1 MAb CMVB1. First, by indirect immunofluorescence assay (20), Ab3 sera from mice 1 and 2 bound HCMV-infected cells, with titers of 640 and 160, respectively. The pattern of fluorescence was typical of antibodies to many late HCMV proteins, with a prominent perinuclear inclusion body (Fig. 3a), and was similar to that produced by MAb CMVB1 (Fig. 3d). No reactivity was detected with serum from mouse 3 or with sera from mice 4 to 6 (Fig. 3b). To verify the specificity of the fluorescence in Fig. 3a, mouse serum was incubated with either purified rabbit Ab2, an equal amount of purified rabbit IgG, or diluent, after which residual indirect immunofluorescence assay reactivity of the mouse Ab3 serum was tested. Under these conditions, 5 μg of rabbit Ab2 completely inhibited subsequent reactivity of Ab3 (Fig. 3c), whereas normal rabbit IgG at 5 to 20 μg resulted in no inhibition. Further characterization of the Ab3 target protein(s) was achieved by radioimmunoprecipitation assay. As illustrated in Fig. 4, mouse 1 serum (Fig. 4A, lane 3) precipitated viral proteins with the same apparent molecular masses as MAb CMVB1 (Fig. 4A, lane 7), at 58, 93 to 116, and 130 kDa. No viral proteins were precipitated by serum of mouse 4, immunized with normal rabbit IgG (Fig. 4A, lane 5), nor from control cells (Fig. 4A, lanes 4, 6, and 8). An additional experiment confirmed these results and compared proteins precipitated by mouse Ab3 and a human anti-HCMV serum. Figure 4B reveals that HCMV proteins recognized by the Ab3 serum (Fig. 4B, lane 4) were similarly precipitated by the human serum (Fig. 4B, lane 2), which is not an unexpected result given the immunodominance of the gB complex in natural infection (19, 23). Collectively, these experiments demonstrated that Ab1-like antibodies were generated in the Ab2-immunized mice.

Since the Ab1 MAb neutralized viral infectivity in vitro, we investigated whether the mouse Ab3 possessed similar activity. Plaque reduction assays were carried out in at least triplicate, and data were evaluated for levels of significance by using Student's t test. Representative data in Table 1 show that serum from mouse 1 neutralized HCMV at dilutions from 1:5 to 1:40. There was also an indication of neutralizing activity in serum from mouse 3 at a dilution of 1:5, although this was not statistically significant ($P < 0.05$). Neutralization occurred only in the presence of complement, echoing a similar requirement by MAb CMVB1. No neutralization was observed with serum from mouse 2 or with sera from control mice 4 to 6. These results provided further evidence that the combining site of certain subsets of the rabbit Ab2 population bore a degree of structural complementarity to gB, the reference antigen.

Mouse Ab3 antibodies share id specificity with MAb CMVB1 (Ab1). The id specificities of the mouse Ab3 antibodies were examined by utilizing MAb2-3C5 [IgG1(κ)] and MAb2-5C12 [IgG2a(λ)], two of a series of monoclonal anti-id antibodies previously generated against MAb CMVB1 (35). These MAb2 antibodies have distinct target idiotope specificities. However, both appear to be directed to paratope-associated idiotopes of MAb CMVB1, blocking the ability of CMVB1 to bind HCMV-infected cells and inhibiting the viral neutralizing activity of CMVB1. ELISAs were set up to see whether the mouse Ab3 antibodies raised against rabbit Ab2 would bind to immobilized F(ab')$_2$ fragments of MAb2-3C5 or MAb2-5C12. Results demonstrated that all sera from mice immunized with rabbit Ab2 bound to MAb2-5C12, with serum from mouse 1 expressing the highest titer (Fig. 5A). No significant binding to either MAb2-3C5 or the control MAb was seen (Fig. 5A to C), and serum from a mouse immunized with normal rabbit IgG (Fig.

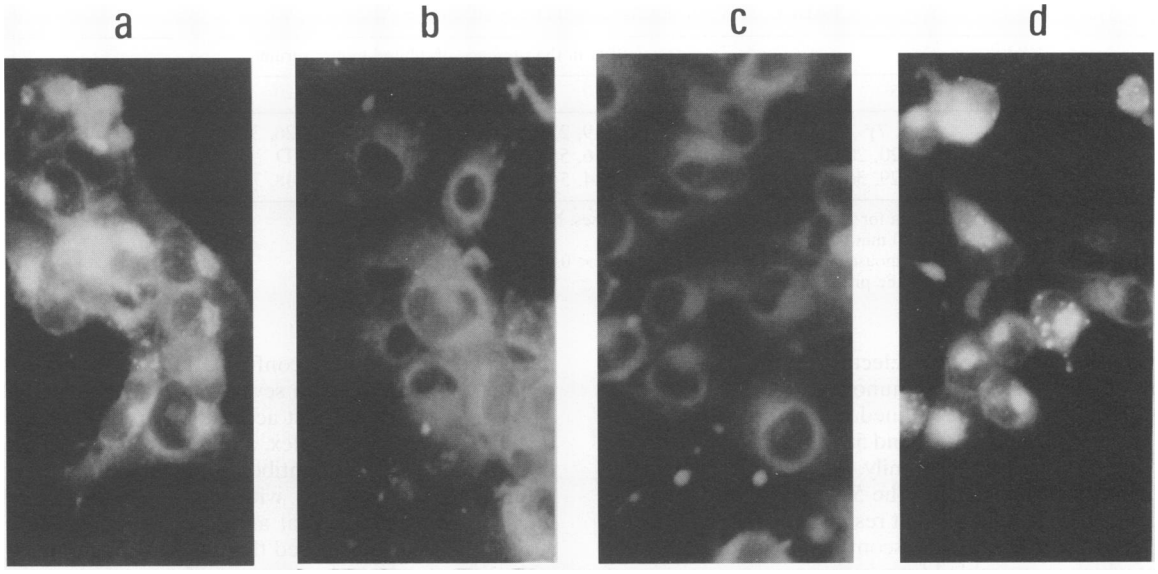

FIG. 3. Immunofluorescence studies of Ab3 mouse serum. Indirect immunofluorescence assay was carried out with fixed HCMV-infected human foreskin fibroblasts (20) visualized with fluorescein isothiocyanate-conjugated anti-mouse Ig. (a) Serum from mouse 1 (1:80 dilution) immunized with purified rabbit Ab2; (b) serum from mouse 4 (1:80 dilution) immunized with normal rabbit IgG; (c) inhibition of the Ab3 immunofluorescent reactivity seen in panel a; (d) immunofluorescence of purified MAb CMVB1.

5D) and normal mouse serum were negative. These data indicate that the mouse Ab3 antibodies expressed an id in common with the initiating Ab1 and that the Ab1 id defined by MAb2-5C12 was recurrent rather than unique (24). Conversely, the Ab1 id defined by MAb2-3C5 was not detectable in the Ab3 sera. This difference in id expression by Ab1 and Ab3 suggested that although antigen-specific antibodies were generated in Ab2-immunized mice, Ab2 stimulated subsets of mouse B cells different from those activated by the nominal antigen. Other publications have reported similar fine variability between Ab1 and the antigen-reactive Ab3. For example, Mariani et al. (21) described the diversity of the anti-HLA-DR Ab3 response induced by MAb2 directed to different ids coexpressed on the same Ab1. Similarly distinctive Ab3 subpopulations have been noted in studies with influenza virus (1) and human respiratory syncytial virus (25). Furthermore, Keay et al. (14) generated syngeneic MAb2 to the 86-kDa glycoprotein of HCMV. The MAb2 antibodies appeared to mimic the reference epitope but also bound a MAb specific for HCMV glycoprotein p130/55, suggesting shared ids on these distinct anti-HCMV MAb (13). These investigations with complex antigen systems, including the present study, illustrate the contribution of id network mechanisms to the generation of diversity in the immune response. Moreover, they suggest that subtle differences in the properties of anti-id antibodies stimulate distinct B-cell populations in responding hosts, consider-

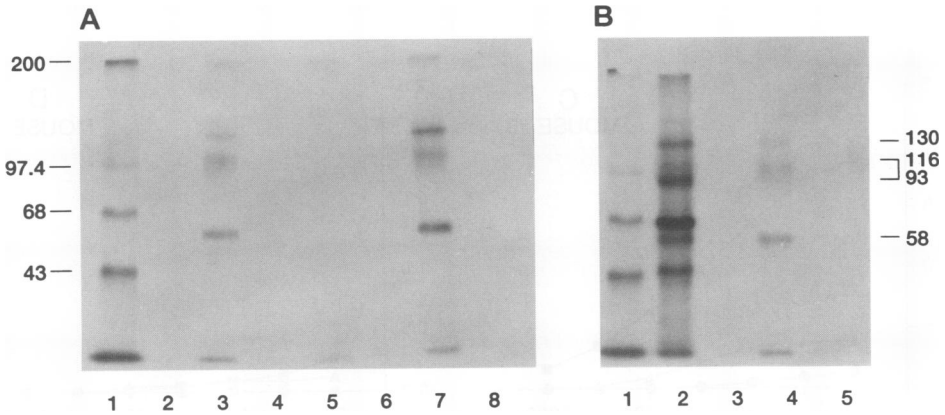

FIG. 4. Specificity of mouse Ab3 for HCMV proteins. Radioimmunoprecipitation assays were performed as described for Fig. 1. Cell lysates from [^{35}S]methionine-labelled HCMV-infected cells (panel A, lanes 3, 5, and 7; panel B, lanes 2 and 4) or [^{35}S]methionine-labelled control mock-infected cells (panel A, lanes 4, 6, and 8; panel B, lanes 3 and 5) were precipitated with sera derived from mice immunized with purified rabbit Ab2 or normal rabbit IgG and then were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Lanes: 1 in panels A and B, ^{14}C-molecular mass markers, in kilodaltons; 3 and 4 in panel A and 4 and 5 in panel B, precipitation by mouse 1 serum (immunized with purified rabbit Ab2) diluted 1:40; 5 and 6 in panel A, precipitation by mouse 4 serum (immunized with normal rabbit IgG) diluted 1:40; 7 and 8 in panel A, precipitation by MAb CMVB1 ascitic fluid diluted 1:1,000; 2 and 3 in panel B, precipitation by HCMV-seropositive human serum diluted 1:400.

TABLE 1. Viral neutralizing activity of mouse AB3 sera

Mouse serum[b]	PFU in the presence of diluted mouse serum[a]			
	1:5	1:10	1:20	1:40
1	4 (2, 3, 7)[c]	19 (14, 19, 23)[c]	26 (22, 26, 31)[c]	28 (28, 27, 30)[c]
3	19 (18, 20, 20)	45 (33, 46, 55)	ND	ND
Preimmune[d]	30 (24, 29, 36)	47 (43, 44, 54)	54 (44, 48, 71)	50 (57, 47, 46)

[a] Average number of PFU per well. Data for individual wells are in parentheses. ND, not determined.
[b] All sera were inactivated at 56°C for 30 min before being tested.
[c] Statistically different from preimmune mouse serum at the same dilution ($P < 0.05$, Student's t test).
[d] Pool of sera from mice 1 to 6 used in the present study.

ations which are relevant to the selection of anti-id antibodies for use as effective surrogate immunogens.

Recent investigations have defined numerous antigenic domains on the 130-kDa precursor and 58- and 116-kDa cleavage products that comprise the gB family. Of these, a major target for neutralizing antibodies is on the 58-kDa glycoprotein (27, 36), adjacent to the cleavage site at residue 460 (5). This region contains both continuous and discontinuous epitopes (2, 12, 27), one of which is assembled from a consecutive sequence of 70 amino acids (37). Though the gB epitope defined by MAb CMVB1 remains uncharacterized, preliminary data suggest that it may reside on the 58-kDa moiety (unpublished observations). Anti-id antibodies are particularly well suited to mimic complex and/or conformational determinants, as recently demonstrated with several viral systems (11, 18, 38).

Our results are the first account of cascades in the immune response to the gB complex, a major focus for HCMV vaccine development. Anti-id antibodies have been categorized as Ab2α, Ab2β, or Ab2γ, with only Ab2β representing the internal image of external antigen (3, 9, 10). More recently, Köhler et al. (16) proposed that these distinctions be replaced with the more inclusive concept of a network antigen. From our study, we conclude that the polyclonal rabbit anti-id antibodies mimic the gB complex sufficiently to function as a network antigen for inducing a specific and neutralizing anti-gB response in this model system. As such, they may be

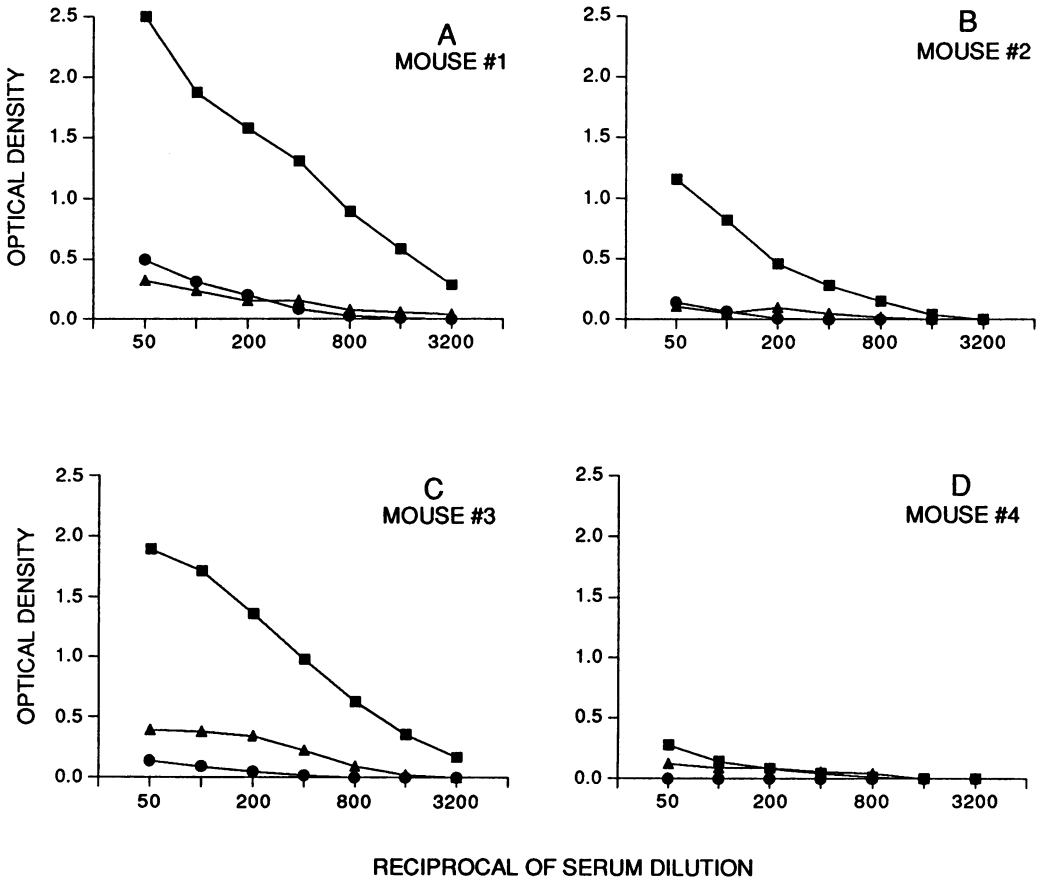

FIG. 5. Detection of shared ids between mouse Ab3 and MAb CMVB1. The F(ab')$_2$ fragments of MAb2-3C5 (●), MAb2-5C12 (■), and an irrelevant control MAb (▲) were immobilized in microtiter wells. Binding of mouse Ab3 sera was detected with enzyme-conjugated anti-mouse Fc-specific IgG. Sera were derived from mice immunized with purified rabbit Ab2 (A, B, and C) or an equivalent amount of normal rabbit IgG (D).

useful probes for further analysis of how id networks influence the immune response to HCMV.

We thank E. Rossier, University of Ottawa, for providing seropositive human serum.

This work was supported by a Studentship Award from the Medical Research Council of Canada to E.S.T.

REFERENCES

1. **Anders, E. M., G. P. Kapaklis-Deliyannis, and D. O. White.** 1989. Induction of immune response to influenza virus with anti-idiotypic antibodies. J. Virol. **63**:2758–2767.
2. **Banks, T., B. Huo, K. Kousoulas, R. Spaete, C. Pachl, and L. Pereira.** 1989. A major neutralizing domain maps within the carboxyl-terminal half of the cleaved cytomegalovirus B glycoprotein. J. Gen. Virol. **70**:979–985.
3. **Bona, C. A., and H. Köhler.** 1984. Anti-idiotypic antibodies and internal images, p. 141–149. *In* J. Venter, C. Fraser, and J. Londstrom (ed.), Receptor biochemistry and methodology, vol. 4. Monoclonal and anti-idiotypic antibodies: probes for receptor structure and function. Alan R. Liss, Inc., New York.
4. **Britt, W. J., and L. G. Vugler.** 1989. Processing of the gp55-116 envelope glycoprotein complex (gB) of human cytomegalovirus. J. Virol. **63**:403–410.
5. **Cranage, M. P., T. Kouzarides, A. T. Bankier, S. Satchwell, K. Weston, P. Tomlinson, B. Barrell, H. Hart, S. E. Bell, A. C. Minson, and G. L. Smith.** 1986. Identification of the human cytomegalovirus glycoprotein B gene and induction of neutralizing antibodies via its expression in recombinant vaccinia virus. EMBO J. **5**:3057–3063.
6. **Finberg, R. W., and H. Ertl.** 1987. The use of antiidiotypic antibodies as vaccines against infectious agents. Crit. Rev. Immunol. **7**:269–284.
7. **Fowler, K. B., D. P. H. Stagno, R. F. Pass, W. J. Britt, T. J. Boll, and C. A. Alford.** 1992. The outcome of congenital cytomegalovirus infection in relation to maternal antibody status. N. Engl. J. Med. **326**:663–667.
8. **Hiernaux, J. R.** 1988. Idiotypic vaccines and infectious diseases. Infect. Immun. **56**:1407–1413.
9. **Jerne, N. K.** 1974. Towards a network theory of the immune system. Ann. Immunol. (Inst. Pasteur) **125C**:373–389.
10. **Jerne, N. K., J. Roland, and P.-A. Cazanave.** 1982. Recurrent idiotopes and internal images. EMBO J. **1**:243–247.
11. **Kang, C.-Y., P. Nara, S. Chamat, V. Caralli, A. Chen, M.-L. Nguyen, H. Yoshiyama, W. J. W. Morrow, D. D. Ho, and H. Köhler.** 1992. Anti-idiotype monoclonal antibody elicits broadly neutralizing anti-gp120 antibodies in monkeys. Proc. Natl. Acad. Sci. USA **89**:2546–2550.
12. **Kari, B., and R. Gehrz.** 1991. Biochemical and immunological analysis of discontinuous epitopes in the family of human cytomegalovirus glycoprotein complexes designated gC-I. J. Gen. Virol. **72**:1975–1983.
13. **Keay, S., T. C. Merigan, and L. Rasmussen.** 1989. Identification of cell surface receptors for the 86-kilodalton glycoprotein of human cytomegalovirus. Proc. Natl. Acad. Sci. USA **86**:10100–10103.
14. **Keay, S., L. Rasmussen, and T. C. Merigan.** 1988. Syngeneic monoclonal anti-idiotype antibodies that bear the internal image of a human cytomegalovirus neutralization epitope. J. Immunol. **140**:944–948.
15. **Köhler, H., S. Kaveri, T. Kieber-Emmons, W. J. W. Morrow, S. Muller, and S. Raychaudhuri.** 1989. Idiotypic networks and nature of molecular mimicry: an overview. Methods Enzymol. **178**:1–35.
16. **Köhler, H., T. Kieber-Emmons, S. Srinivasan, S. Kaveri, W. J. W. Morrow, S. Muller, C.-Y. Kang, and S. Raychaudhuri.** 1989. Revised immune network concepts. Clin. Immunol. Immunopathol. **52**:104–116.
17. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
18. **Lamarre, A., J. Lecomte, and P. Talbot.** 1991. Antiidiotypic vaccination against murine coronavirus infection. J. Immunol. **147**:4256–4262.
19. **Landini, M. P.** 1992. Antibody responses to human cytomegalovirus proteins. Rev. Med. Virol. **2**:63–72.
20. **Larose, Y., E. S. Tackaberry, and B. R. Brodeur.** 1991. Human monoclonal antibodies to cytomegalovirus recognize viral epitopes on the surface of viral-infected cells. Hum. Antibod. Hybridomas **2**:65–73.
21. **Mariani, S. M., E. A. Armandola, and S. Ferrone.** 1992. Diversity of anti-HLA-DR antibodies elicited by distinct anti-idiotypic antibodies recognizing idiotopes co-expressed by the immunizing monoclonal antibody. Immunology **77**:597–603.
22. **Marshall, G. S., and S. A. Plotkin.** 1990. Progress toward developing a cytomegalovirus vaccine. Infect. Dis. Clin. N. Am. **4**:283–298.
23. **Marshall, G. S., G. P. Rabalais, G. G. Stout, and S. L. Waldeyer.** 1992. Antibodies to recombinant-derived glycoprotein B after natural human cytomegalovirus infection correlate with neutralizing activity. J. Infect. Dis. **165**:381–384.
24. **Nisonoff, A.** 1991. Idiotypes: concepts and applications. J. Immunol. **147**:2429–2438.
25. **Palomo, C., J. Albar, B. Garcia-Barreno, and J. Melero.** 1990. Induction of a neutralizing immune response to human respiratory syncytial virus with anti-idiotypic antibodies. J. Virol. **64**:4199–4206.
26. **Poskitt, D., M. Jean-Francois, S. Turnbull, L. Macdonald, and D. Yasmeen.** 1991. Internal image (Ab2β) anti-idiotype vaccines. Theoretical and practical aspects. Vaccine **9**:792–796.
27. **Qadri, I., D. Navarro, P. Paz, and L. Pereira.** 1992. Assembly of conformation-dependent neutralizing domains on glycoprotein B of human cytomegalovirus. J. Gen. Virol. **73**:2913–2921.
28. **Rasmussen, L., C. Matkin, R. Spaete, C. Pachl, and T. C. Merigan.** 1991. Antibody response to human cytomegalovirus glycoproteins gB and gH after natural infection in humans. J. Infect. Dis. **164**:835–842.
29. **Rasmussen, L. E.** 1991. Gene products of cytomegalovirus and their immunologic significance, p. 37–56. *In* M. Ho (ed.), Cytomegalovirus: biology and infection, 2nd ed. Plenum Medical Book Company, New York.
30. **Riddell, S. R., K. S. Watanabe, J. M. Goodrich, C. R. Li, M. E. Agha, and P. D. Greenberg.** 1992. Restoration of viral immunity in immunodeficient humans by the adoptive transfer of T cell clones. Science **257**:238–241.
31. **Rossier, E., K. Dimock, D. Taylor, Y. Larose, P. H. Phipps, and B. R. Brodeur.** 1987. Sensitivity and specificity of enzyme immunofiltration and DNA hybridization for the detection of HCMV-infected cells. J. Virol. Methods **15**:109–120.
32. **Siber, G. R., and D. R. Snydman.** 1992. Use of immune globulins in the prevention and treatment of infections. Curr. Clin. Top. Infect. Dis. **12**:208–256.
33. **Spaete, R. R., R. M. Thayer, W. S. Probert, F. R. Masiarz, S. H. Chamberlain, L. Rasmussen, T. C. Merigan, and C. Pachl.** 1988. Human cytomegalovirus strain Towne glycoprotein B is processed by proteolytic cleavage. Virology **167**:207–225.
34. **Starr, S. E.** 1992. Cytomegalovirus vaccines: current status. Infect. Agents Dis. **1**:146–148.
35. **Tackaberry, E. S., J. Hamel, Y. Larose, R. Kuhl, and B. R. Brodeur.** 1992. Monoclonal anti-idiotypes for the rapid detection of human cytomegalovirus. J. Virol. Methods **40**:175–182.
36. **Utz, U., W. Britt, L. Vugler, and M. Mach.** 1989. Identification of a neutralizing epitope on glycoprotein gp58 of human cytomegalovirus. J. Virol. **63**:1995–2001.
37. **Wagner, B., B. Kropff, H. Kalbacher, W. Britt, V.-A. Sundqvist, L. Östberg, and M. Mach.** 1992. A continuous sequence of more than 70 amino acids is essential for antibody binding to the dominant antigenic site of glycoprotein gp58 of human cytomegalovirus. J. Virol. **66**:5290–5297.
38. **Wiley, J. A., J. Hamel, and B. R. Brodeur.** 1992. Monoclonal anti-idiotypes induce neutralizing antibodies to enterovirus 70 conformational epitopes. J. Virol. **66**:5744–5751.