Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 6439–6443, July 1995
Immunology

Directed selection of recombinant human monoclonal antibodies to herpes simplex virus glycoproteins from phage display libraries

(human antibody repertoires/immune prophylaxis/neurotropic viruses/opportunistic infections/AIDS)

PIETRO PAOLO SANNA*†, R. ANTHONY WILLIAMSON‡, ALESSANDRO DE LOGU*§, FLOYD E. BLOOM*, AND DENNIS R. BURTON‡§

Departments of *Neuropharmacology, §Molecular Biology, and ‡Immunology, The Scripps Research Institute, La Jolla, CA 92037; and §Sezione di Microbiologia e Virologia, Dipartimento di Scienze Chirurgiche e Trapianti d'Organo, Universita' Degli Studi di Cagliari, Cagliari, Italy

Contributed by Floyd E. Bloom, March 23, 1995

ABSTRACT **Human monoclonal antibodies have considerable potential in the prophylaxis and treatment of viral disease. However, only a few such antibodies suitable for clinical use have been produced to date. We have previously shown that large panels of human recombinant monoclonal antibodies against a plethora of infectious agents, including herpes simplex virus types 1 and 2, can be established from phage display libraries. Here we demonstrate that facile cloning of recombinant Fab fragments against specific viral proteins in their native conformation can be accomplished by panning phage display libraries against viral glycoproteins "captured" from infected cell extracts by specific monoclonal antibodies immobilized on ELISA plates. We have tested this strategy by isolating six neutralizing recombinant antibodies specific for herpes simplex glycoprotein gD or gB, some of which are against conformationally sensitive epitopes. By using defined monoclonal antibodies for the antigen-capture step, this method can be used for the isolation of antibodies to specific regions and epitopes within the target viral protein. For instance, monoclonal antibodies to a nonneutralizing epitope can be used in the capture step to clone antibodies to neutralizing epitopes, or antibodies to a neutralizing epitope can be used to clone antibodies to a different neutralizing epitope. Furthermore, by using capturing antibodies to more immunodominant epitopes, one can direct the cloning to less immunogenic ones. This method should be of value in generating antibodies to be used both in the prophylaxis and treatment of viral infections and in the characterization of the mechanisms of antibody protective actions at the molecular level.**

Antibodies play a crucial role in the resistance to viral infections, and the parenteral administration of immunoglobulins can effectively protect man against measles, polio, rabies, hepatitis, and other viral diseases (1–3). Specific high-affinity human antibodies are theoretically more desirable than polyclonal sera for use in immunoprophylaxis and therapy of virus infection, since protective antibodies may be only a minor component in the natural host response (discussed in ref. 4).

Herpes simplex viral disease is a major cause of morbidity and mortality in man (5). Although the development of very effective nucleoside analogs with a large therapeutic index has greatly improved the clinical management of herpetic infections (6), the challenge created by the emergence of mono- and multiple-drug-resistant herpes simplex virus (HSV) strains (7–11) underscores the importance of developing antiviral tools with limited risk of cross-resistance. The effectiveness of monoclonal antibodies (mAbs) in experimental HSV infections in the laboratory animal is well documented (12–15). Evidence of the protective role of humoral immunity to HSV in human disease can also be found in the literature. For instance, the incidence of neonatal herpes is 30–50% in neonates born vaginally to mothers with primary herpetic infections, whereas it is only 1–3% in neonates born vaginally to mothers with recurrent manifestations, presumably due to the passive transfer of protective antibodies transplacentally to the fetus (16). Furthermore, in the older child and in the adult, although herpetic recurrences are often seen despite the presence of neutralizing antibodies in the serum, the incidence and severity of HSV manifestations are inversely correlated with antibody response (17). Moreover, antibodies elicited by natural infection with HSV-1 are believed to provide partial protection against HSV-2-infection and to be behind the lower incidence of HSV-2-infection in people with previous exposure to HSV-1 (5, 18). However, in spite of this evidence, human immune serum has shown little or no capacity to confer protection in passive immunization either in man or in animal models (5, 16, 19). This has been attributed to the fact that protective antibodies against HSV and other viral pathogens are present only as minor components of the natural immune response, and a crucial protective role is restricted to a limited number of epitopes (12, 15, 19). Therefore, it will be critical to isolate and characterize the protective antibodies generated in the course of the natural human immune response to explore the protective mechanisms of humoral immunity in HSV infections and investigate the means for exploiting such antibodies in the treatment of disease due to HSV infection.

The herpes simplex are large enveloped viruses that express 11 glycoproteins on their surface and on the cell membranes of virus-infected cells (5, 20–24). Most of these glycoproteins are believed to be crucial in infection and pathogenesis by mediating attachment, membrane fusion, penetration, and egress from infected cells (25–28). At least 5 glycoproteins are essential for virus infectivity in tissue culture, glycoprotein B (gB), gD, gH, gK, and gL (20–22, 24, 29). Recently, it was shown that the attachment and penetration of HSV involves a cascade of virus cell interactions (26). The initial step in this process seems to require binding of gC to heparan sulfate proteoglycans followed by binding of gB, whereas gD has been proposed to stabilize such binding (26). gH appears instead to be involved in fusion initiation, and still other glycoproteins may be necessary for fusion expansion (26). In good agreement with this model, neutralizing antibodies to gH, gB, and gD prevent membrane fusion but not virus attachment (25–27, 30). Most of the protective responses to HSV both in man and in the murine model are directed to gD and gB (5, 25, 30, 31). Furthermore, an experimental gD vaccine has been shown to be effective in reducing the incidence of recurrent episodes of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HSV, herpes simplex virus; mAb, monoclonal antibody; gB and gD, glycoprotein B and glycoprotein D; BSA, bovine serum albumin.

†To whom reprint requests should be addressed.

reactivation in a guinea pig model and in a recent clinical trial (32–34).

The potential contribution of passive immunization to the prophylaxis and therapy of HSV infections should not, however, be considered redundant because of the availability of an experimental vaccine and of effective antiviral drugs. Passive immunization could provide a valuable alternative to prophylactic and therapeutic vaccination in situations in which active immunization cannot be elicited—e.g., in the immunocompromised, in which the emergence of acyclovir-resistant virus may correlate with treatment failure (8). The development of human mAbs suitable for passive immune therapy could provide therapeutic tools to be used either alternatively to or in combination with chemotherapy with no risk of cross-resistance and virtually free of adverse effects. Furthermore, specific human mAbs could find application in the prevention of vertical and horizontal transmission. The latter may include cases in which a sexual partner is infected with HSV-2, rape cases, and the prevention of HSV transmission among contact sports athletes such as wrestlers (*herpes gladiatorum*).

Based on these considerations, we have devised a rapid and facile approach that allows for the panning of Fab libraries on selected native viral proteins immunoadsorbed ("captured") from virus-infected cell extracts with specific antibodies immobilized on plates for ELISA. This technique represents a rapid way to isolate recombinant antibodies to conformationally intact epitopes and is particularly suited to the cloning of antibodies to complex viruses, like the Herpesviridae, in which multiple glycoproteins can elicit protective responses.

MATERIALS AND METHODS

Viruses and Cells. Vero cells were grown in RPMI 1640 medium supplemented with 5% (vol/vol) fetal calf serum. HSV-1 (strain F) and HSV-2 (strain G) were obtained from American Type Culture Collection.

Capture Panning. Two common specific mouse mAbs against herpes simplex gD (mAbs 1103 and 1010) and one against gB (mAb 1105) were obtained from Goodwin Institute for Cancer Research (Plantation, FL) and were used as antigen-capturing antibodies. Ascites fluid preparations of the capturing antibodies were diluted 1:300–1:1000 in 0.1 M sodium phosphate-buffered saline (PBS) at pH 7.5 and used to coat 96-well ELISA plates (Costar 3690) using 25 μl per well. Cellular extracts were obtained by homogenizing HSV-1- or HSV-2-infected cells in 1% (vol/vol) Nonidet P-40/1% (wt/vol) sodium deoxycholate in PBS (RIPA buffer): 10^7 cells infected at a multiplicity of infection of 5 were homogenized in 5 ml of RIPA buffer by vortexing. The cell extracts were then sonicated and centrifuged at 3000 $\times$ g for 5 min to remove cellular debris. Cell extracts were then aliquoted and frozen at -80°C until needed. Two libraries, designated A and C, were utilized in the study. The former was used to validate the technique by reisolating a previously described Fab specific for gD (35); the latter was used for the isolation of the six additional antibodies described in the present study. Antibody-coated plates were repeatedly washed with water and blocked with 3% (wt/vol) bovine serum albumin (BSA) in PBS for 2 hr at 37°C. The BSA solution was then discarded and replaced with 20 μl of the HSV-infected cell extracts and incubated at room temperature for 20 min. The plates were then washed 10 times with PBS containing 0.05% Tween 20. At this point about 10^{11} colony-forming units per well of one of the two antibody libraries was added and incubated for 1 hr at 37°C as described (36). The library suspension was then removed, and plates were washed for 1 hr with PBS/0.05% Tween 20. Bound phage was eluted with 50 μl of 0.1 M HCl adjusted to pH 2.2 with solid glycine. The eluted phage suspension was immediately neutralized with 3 μl of 2 M Tris base and used to inoculate 2 ml of XL1-Blue *Escherichia coli* cells (OD_{600} = 0.5). After 15 min at room temperature, 10 ml of super broth [3% (wt/vol) tryptone/2% (wt/vol) yeast extract/1% Mops, pH 7.0] containing 20 μg of carbenicillin per ml and 10 μg of tetracycline per ml was added, and the cultures were shaken at 37°C for 1 hr. One hundred milliliters of super broth containing 50 μg of carbenicillin per ml and 10 μg of tetracycline per ml was then added, and the cultures were shaken for an additional 1 hr, when 10^{12} plaque-forming units of helper phage, VCS-M13, were added. After 2 hr of shaking, kanamycin was added at a final concentration of 70 μg/ml. The cultures were then shaken overnight at 30°C. The next day, phage was prepared from the cultures and panning was repeated. Soluble Fabs were prepared after four or five rounds of panning by a *Nhe* I–*Spe* I restriction enzyme digestion followed by self-ligation of the vector, as described (36). In some experiments four or five more rounds of panning were carried out on total infected cell lysates after the original four or five rounds of capture pannings, to eliminate phage particles coding for multispecific antibodies capable of binding to the capturing antibody.

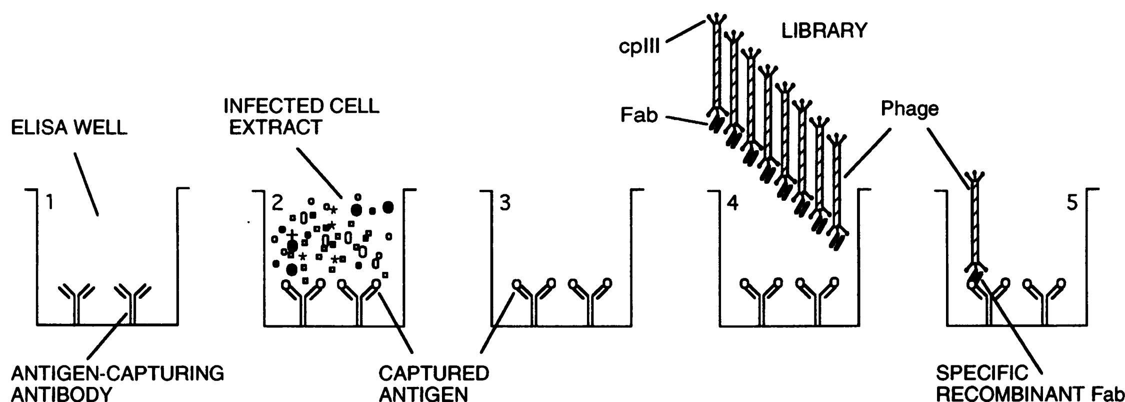

FIG. 1. Schematic representation of the cloning strategy. A mAb against a viral glycoprotein is bound to the bottom of an ELISA well (1); after blocking with BSA, a total protein extract from virus-infected cells is added to the well so that the protein recognized by the first antibody is allowed to be captured (2); the unbound proteins in the extract are removed by washing (3); a phage display combinatorial Fab library is then added to the well (4). Combinatorial Fab libraries express functional Fabs on the surface as a fusion protein with the anchoring domain of the phage coat protein III (cpIII); unmodified coat protein III, which is necessary for *E. coli* infection, is also present on the phage surface. After phage particles expressing specific recombinant Fabs are allowed to immunoreact with the captured protein, the unbound phage particles are removed by washing (5). The enriched phage can now be eluted and used to infect *E. coli.* The process is repeated three to five times, and it can be followed by as many rounds of panning on total cell extracts to eliminate background phage particles capable of binding to the capturing antibodies. We have found that this method is extremely effective in directing the cloning of recombinant antibodies to specific glycoproteins.

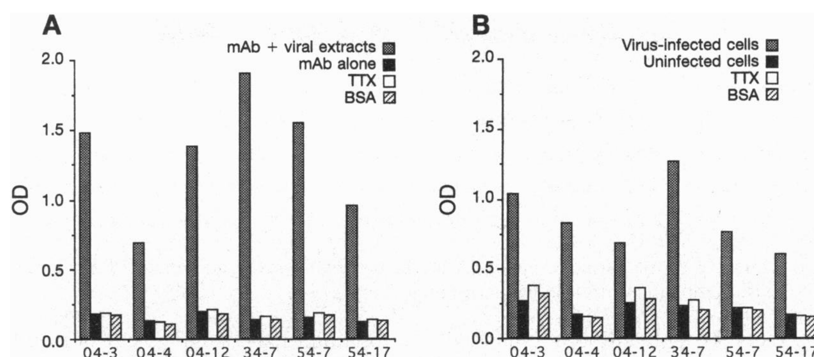

FIG. 2. ELISA assays with the six human antibodies isolated with the method described in the paper. Crude *E. coli* extracts were used in this experiment. (*A*) Antibodies were tested in capture ELISA in the same conditions used for cloning (mAb + viral extracts) or on the capture antibody alone (mAb). (*B*) Antibodies were tested on whole protein extracts from infected or uninfected cells. Tetanus toxoid (TTX) and BSA were also used as controls. OD at 405 nm was measured at 20 min in *A* and at 90 min in *B*.

ELISA. ELISA assays were carried out as described (36). Briefly, crude bacterial pellets from 10-ml cultures of soluble Fab-producing *E. coli* clones were resuspended in PBS and extracted by three cycles of freezing and thawing. They were then centrifuged to eliminate bacterial debris and used directly as a source of antibody in ELISA on either total cell extracts or specific viral glycoproteins captured with the appropriate mouse mAb as described above. A goat anti-human $F(ab')_2$ conjugated to alkaline phosphatase (Pierce) was used as a second antibody with *p*-nitrophenyl phosphate (Sigma) as a substrate. OD was determined at 405 nm.

Immunoprecipitation. Extracts obtained by repeated cycles of freezing and thawing of pelleted Fab-producing *E. coli* (300-ml cultures) were affinity purified on columns of agarose-bound sheep anti-human antibody with standard techniques. Antibodies to gD were tested by immunoprecipitation of non-radiolabeled infected cell extracts and immunoblotting with chemioluminescent immunodetection (Immun-Lite; Bio-Rad). Due to the inability of our murine mAbs to recognize denatured gB, we used HSV proteins radiolabeled with [^{35}S]methionine for detection in the immunoprecipitation with the recombinant antibodies to gB. Vero cells infected with HSV-1 or -2 were homogenized in RIPA buffer as described above and incubated with a purified Fab for 1 hr at 4°C. An agarose-conjugated sheep anti-human Fab was then added (about 20 μl of compacted volume), and the mixture was incubated for 0.5 hr longer at 4°C. The immunoprecipitates were separated by centrifugation and washed repeatedly. Immunoprecipitates were then resolved on SDS/4–12% PAGE gradient gels as described below and immunoblotted. Specific immunoprecipitates obtained with antibodies to gD were revealed with a mixture of the aforementioned mAb 1103 and 1010 (both ascites) each at a dilution of 1:1000; a rabbit polyclonal anti-HSV-1 and -2 serum (Dako) was used as control. Radiolabeled immunoprecipitates obtained with antibodies to gB were subjected to SDS/PAGE and exposed to film directly (Kodak).

Western Blotting. To determine the sensitivity to denaturing conditions of the epitopes recognized by the antibodies described in the present report, we tested them on HSV protein blots obtained from native, denaturing nonreducing, and denaturing reducing PAGE, according to standard techniques (37, 38). Briefly, native sample buffer (1×) contained 20 mM Tris·HCl, pH 8.3/0.00025% bromophenol blue/20% (vol/vol) glycerol; denaturing nonreducing sample buffer (1×) also contained 2% (wt/vol) SDS; and denaturing reducing sample buffer (1×) also contained 2% SDS and 0.5% 2-mercaptoethanol. Native running buffer contained 24 mM Tris (pH 8.3) and 192 mM glycine; denaturing running buffer also contained 1% SDS.

Neutralization. Crude *E. coli* extracts, prepared as described above, were tested for neutralization with both HSV-1 and -2. About 100 plaque-forming units of either strain was incubated with Fab for 1 hr at 37°C and plated on Vero monolayers to determine neutralization activity by plaque reduction assay as described (35). Alternatively, 5 μg of an unlabeled goat anti-human $F(ab')_2$ (Pierce) was added to the Fab/virus mixture and incubated for 30 min longer to simulate divalency. Cell monolayers were stained, and plaques were counted as described (35).

Sequencing. Sequencing was carried out on double-stranded DNA with Sequenase (United States Biochemical) according to the manufacturer's recommendations.

RESULTS AND DISCUSSION

Specific antibodies immobilized on ELISA plates were used to capture HSV surface glycoproteins from infected cells in a similar fashion to a sandwich ELISA (Fig. 1). After elimination of other proteins in the infected cell extract by repeated washings, a phage display Fab library was added to the wells, and specific phage particles coding for Fabs to the captured proteins were selected on the basis of their ability to bind the immunoadsorbed glycoproteins. Initially, we validated this approach by recloning a previously described antibody to gD, Fab AC8 (35). This was accomplished by five rounds of panning of library A on gD immunoadsorbed on ELISA plates

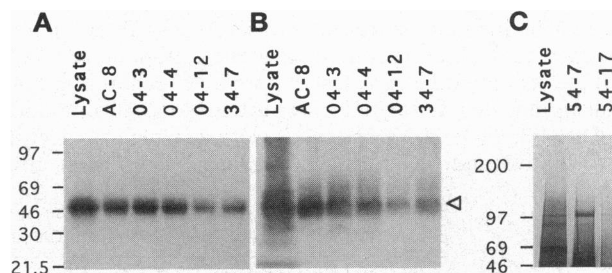

FIG. 3. Immunoprecipitation of HSV glycoproteins with the human antibodies isolated by capture panning. Immunoprecipitation of gD was carried out with nonradiolabeled infected cell extracts and chemioluminescent immunodetection. (*A*) Detection with murine mAbs to gD of specific immunoprecipitates obtained with AC-8 and the four antibodies to gD (04-3, 04-4, 04-12, and 34-7). Immunoprecipitated gD is indicated by the open arrowhead. (*B*) For the purpose of control, the same Western blot displayed in *A* was reimmunoreacted with a rabbit polyclonal antibody to HSV. (*C*) Radiolabeled infected cell extracts were used for the immunoprecipitation of gB since our murine mAbs to gB do not recognize the denatured glycoprotein. The solid arrowhead indicates immunoprecipitated gB. Viral-infected cell lysate is also shown in all sets of immunoprecipitations.

Clone	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
04-3	LESGAELKKPGASVKVSCKASGYPFT	TYYIH	WVRQAPGQGLEWMG	MINPNGGSTRLAQKFQG	RVTMTSDMSAETLYMDLSGLGSEDTAVYYCAR	DRYLGHQYGMDV	WGQGTTVTVSS
04-4	LESGAEVKKPGASVKVSCKSSGNSFS	THYIH	WVREAPGRGLEWLG	WITPTSGRTNYAQKFQG	RVTMTRDTSISTAYMELSRLRSDDTAVYYCAR	GRVLGYFDWLFDY	WGQGALVTVAS
04-12	LEQSGAEVRKPGASVKVSCKASGYTFT	DYYIH	WVRQTPGQGLEWMG	WINPNSADTNIGGTTYAQKFQG	RVTMTRDRSIRTAYMELTKLTSDDTAVYYCAR	EGILGYGDRSPFDI	WGQGTMVTVSS
34-7	LEQSGAEVKKPGASVKVSCKASGYTFT	TYYIH	WVRQAPGQGPEWLG	TINPNGGSTRFSKKFQG	RVTMTIDSSTSTVYMDLSSLKSEDTAVYYCAR	DRLLSHSYGMDV	WGQGTTVIVSS
54-7	LLESGAEVKKPGASVKVSCKASGHTFT	AHFMH	WVRQAPGEGLEWMG	WINPKTGGTHYAQKFQG	RVTMTRDTSTSTAYVELRRLRSDDSAVYYCAR	DRETDLQWNDARVYAMDV	WGQGTTVTVSS
54-17	LLESGAEVKKSGSSVRVACQILGDTFA	SHAFT	WVRQAPGQGLEWVG	TIFPVWRRSDYAQRFQG	RVSMTADDSARTIYMEMRNLLSGDTATYFCTR	DRRMYGGGHGWWFHP	WGPGTQVSVSS

FIG. 4. Deduced amino acid sequences of the variable regions of the heavy chains of the human anti-HSV antibodies described in the present report. FR, framework region; CDR, complementarity determining region.

from HSV-2-infected cell extracts through antibody 1103 (see *Materials and Methods*). The presence of AC8 among enriched clones, positive both by ELISA with total infected cell extracts and by capture ELISA, was confirmed by sequencing (data not shown). Although this antibody was reisolated by a straight series of pannings on captured gD, we noticed that the best strategy to isolate antibodies with this method was to carry out four or five pannings on the captured glycoprotein, followed by three or four pannings on unfractionated total infected-cell extracts to eliminate phage particles coding for multispecific antibodies capable of binding to the capturing antibody.

The two-step approach was then used to isolate six additional recombinant Fabs from library C (Figs. 2–4). These Fabs were designated 04-3, 04-4, and 04-12 (selected by panning on gD captured with murine mAb 1010), 34-7 (selected by panning on gD captured with murine mAb 1103), and 54-7 and 54-17 (selected by panning on gB captured with murine mAb 1105). All the Fab isolates were tested by ELISA (Fig. 2) and by immunoprecipitation (Fig. 3). The sequences of the heavy-chain constant regions of these antibodies are shown in Fig. 4. Two of the anti-gD Fab fragments (04-3 and 34-7) appear to be close clonal variants, although they were obtained from panning with different and noncompeting capturing murine mAbs. This suggests that they may recognize a third epitope independent of those recognized by the two murine mAbs.

Although antibodies to gD were obtained by capture panning using HSV-1-infected cell proteins and antibodies to gB were obtained with HSV-2-infected cell proteins, all Fabs appeared to immunoreact with proteins from both strains in ELISA, probably reflecting the immunological history of the patient. None of the Fabs was capable of neutralizing either strain if used alone. However, the addition of purified goat anti-human $F(ab')_2$ immunoglobulins to mimic divalency revealed that all the Fabs isolated could neutralize HSV-2, and three of them could neutralize both serotypes (Table 1). The sensitivity to denaturing conditions of the epitopes recognized by these six antibodies was tested on native, denaturing nonreducing, and denaturing reducing protein blots. All antibodies to gD (04-3, 04-4, 04-12, and 34-7) were capable of detecting gD in native and denaturing nonreducing blots even after boiling of the samples but not on denaturing reducing blots. Antibodies to gB (54-7 and 54-17) were only capable of immunoreacting on native blots and, if the samples were not boiled, on denaturing nonreducing blots (Table 1).

The establishment of other human mAbs against HSV surface glycoproteins has been achieved by different approaches, including conventional hybridoma technology (39), humanization (40), and affinity selection from recombinant antibody libraries (35). Central problems in the recombinant approach are the availability of conformationally intact antigens and the difficulty of isolating rare ligands to specific epitopes. In the case of Herpesviridae and other complex viruses, the isolation of specific antibodies against potentially protective epitopes of surface glycoproteins is further complicated by the fact that the host humoral immune response is not restricted to determinants of the surface glycoproteins but is directed to all viral polypeptides (41). To target the panning to specific proteins, one can utilize purified glycoproteins produced either by recombinant DNA technology in eukaryotic cell systems or by purification from virus-infected cells. This, however, can be particularly laborious if one aims at the establishment of antibodies to multiple glycoproteins, and the conformational integrity of the purified glycoproteins may not be totally preserved during the necessary manipulations. The method described here is an attractive alternative to the use of purified individual glycoproteins. With this approach, a directed cloning of recombinant mAbs against specific viral proteins in their native conformation is achieved by the capture of individual viral polypeptides with immobilized antibodies. To avoid missing potential recombinant Fabs directed against the same or adjacent epitopes to that recognized by the mAb used to capture the native viral protein of interest, two or more noncompeting mAbs can be used in parallel for each viral protein. Furthermore, by using a capturing antibody directed to an immunodominant epitope, one can isolate antibodies to less immunogenic ones, and the sequential use of different mAbs may allow one to restrict the specificity of the Fabs cloned to one or a few epitopes. In addition, mAbs to a nonneutralizing epitope can be used in the capture step to clone antibodies to neutralizing epitopes, or antibodies to a

Table 1. Cross-reactivity in ELISA of the antibodies isolated, neutralizing activity, and conformational sensitivity of the epitopes recognized

Fab	Glycoprotein recognized	HSV serotype recognized	HSV serotype neutralized	Epitope characteristics
04-3	gD	1, 2	2	Sensitive to reducing conditions
04-4	gD	1, 2	2	Sensitive to reducing conditions
04-12	gD	1, 2	1, 2	Sensitive to reducing conditions
34-7	gD	1, 2	1, 2	Sensitive to reducing conditions
54-7	gB	1, 2	1, 2	Sensitive to SDS + boiling
54-17	gB	1, 2	2	Sensitive to SDS + boiling

All of the antibodies recognized both HSV-1 and -2 proteins, but half of them only neutralized HSV-2. Immunoreactivity on Western blots of all antibodies to gD was preserved by boiling in the presence of SDS but not by exposure to 2-mercaptoethanol. Consistent with the notion that gB elicits conformationally sensitive antibodies (31), the immunoreactivities of 54-7 and 54-17 were eliminated by boiling in the presence of SDS, although they were resistant to exposure to SDS alone.

neutralizing epitope can be used to clone antibodies to a different neutralizing epitope.

In conclusion, we report here a modification of the classical phage display antibody cloning approach that allows for the directed cloning of antibodies to specific proteins captured (immunoadsorbed) on ELISA wells from unfractioned protein extracts. With this strategy we have been able to isolate specific antibodies to HSV gD and gB, two of the surface glycoproteins to which most of the protective humoral and cellular responses are directed (5, 31). This method has the potential to greatly simplify the isolation of human recombinant antibodies against complex viruses like the Herpesviridae. The major benefits of this method are its rapidity and the fact that the antigens are presented to the Fab library in the panning in their native conformation, allowing for the isolation of antibodies directed to conformationally sensitive epitopes.

This work was partially supported by Grants AI36192 (P.P.S.), MH47680 (F.E.B.), AI33292 (D.R.B.), and MO1RR0833 from The National Institutes of Health.

1. Dulbecco, R. & Ginsberg, H. S. (1980) *Virology* (Harper & Row, Philadelphia).
2. White, D. O. & Fenner, J. F. (1986) *Medical Virology* (Academic, Orlando, FL).
3. Chanock, R. M., Crowe, J. J., Murphy, B. R. & Burton, D. R. (1993) *Infect. Agents Dis.* **2,** 118–131.
4. Burton, D. R. (1992) *Hosp. Pract.* **27,** 67–74.
5. Whitley, R. J. (1990) in *Herpes Simplex Viruses*, eds. Fields, B. N. & Knipe, D. M. (Raven, New York), pp. 1843–1888.
6. Gilman, A. G., Goodman, L. S., Rall, T. W. & Murad, F. (1985) *Goodman and Gilman's: The Pharmacological Basis of Therapeutics* (MacMillan, New York).
7. Coen, D. M. (1991) *Antiviral Res.* **15,** 287–300.
8. Nugiër, F., Colin, J. N., Aymard, M. & Langlois, M. (1992) *J. Med. Virol.* **36,** 1–12.
9. Safrin, S., Kemmerly, S., Plotkin, B., Smith, T., Weissbach, N., De, V. D., Phan, L. D. & Cohn, D. (1994) *J. Infect. Dis.* **169,** 193–196.
10. Yoshida, T., Suzutani, T. & Azuma, M. (1988) *Tohoku J. Exp. Med.* **156,** 279–290.
11. Hwang, C. B., Ruffner, K. L. & Coen, D. M. (1992) *J. Virol.* **66,** 1774–1776.
12. Eis Hubinger, A., Schmidt, D. S. & Schneweis, K. E. (1993) *J. Gen. Virol.* **74,** 379–385.
13. Dix, R. D., Pereira, L. & Baringer, J. R. (1981) *Infect. Immun.* **34,** 192–199.
14. Hayashida, I., Nagafuchi, S., Hayashi, Y., Kino, Y., Mori, R., Oda, H., Ohtomo, N. & Tashiro, A. (1982) *Microbiol. Immunol.* **26,** 497–509.
15. Mester, J. C., Glorioso, J. C. & Rouse, B. T. (1991) *J. Infect. Dis.* **163,** 263–269.
16. Kohl, S. (1992) *Curr. Top. Microbiol. Immunol.* **179,** 75–88.
17. Kohl, S. (1989) in *Herpes Virus Infections*, ed. Kelly, W. N. (Lippincott, Philadelphia), pp. 1647–1666.
18. Allen, W. P. & Rapp, F. (1982) *J. Infect. Dis.* **145,** 413–421.
19. Eis Hubinger, A., Mohr, K. & Schneweis, K. E. (1991) *Intervirology* **32,** 351–360.
20. Gompels, U. & Minson, A. (1986) *Virology* **153,** 230–247.
21. Hutchinson, L., Goldsmith, K., Snoddy, D., Ghosh, H., Graham, F. L. & Johnson, D. C. (1992) *J. Virol.* **66,** 5603–5609.
22. Ligas, M. W. & Johnson, D. C. (1988) *J. Virol.* **62,** 1486–1494.
23. Lipton, H. L. & Gonzalez, S. F. (1978) *J. Infect. Dis.* **137,** 145–151.
24. Roop, C., Hutchinson, L. & Johnson, D. C. (1993) *J. Virol.* **67,** 2285–2297.
25. Fuller, A. O. & Spear, P. G. (1985) *J. Virol.* **55,** 475–482.
26. Fuller, A. O. & Lee, W. C. (1992) *J. Virol.* **66,** 5002–5012.
27. Navarro, D., Paz, P. & Pereira, L. (1992) *Virology* **186,** 99–112.
28. Spear, P. G. (1985) in *Glycoproteins Specified by Herpes Simplex Virus*, eds. Roizman, B. & Lopez, C. (Plenum, New York), pp. 315–356.
29. Cai, W. Z., Person, S., Warner, S. C., Zhou, J. H. & DeLuca, N. A. (1987) *J. Virol.* **61,** 714–721.
30. Highlander, S. L., Sutherland, S. L., Gage, P. J., Johnson, D. C., Levine, M. & Glorioso, J. C. (1987) *J. Virol.* **61,** 3356–3364.
31. Eing, B. R., Kuhn, J. E. & Braun, R. W. (1989) *J. Med. Virol.* **27,** 59–65.
32. Burke, R. L. (1991) *Rev. Infect. Dis.* **13,** S906–911.
33. Straus, S. E., Corey, L., Burke, R. L., Savarese, B., Barnum, G., Krause, P. R., Kost, R. G., Meier, J. L., Sekulovich, R., Adair, S. F. & Dekker, C. L. (1994) *Lancet* **343,** 1460–1463.
34. Stanberry, L. R., Harrison, C. J., Bernstein, D. I., Burke, R. L., Shukla, R., Ott, G. & Myers, M. G. (1989) *Antiviral Res.* **11,** 203–214.
35. Burioni, R., Williamson, R. A., Sanna, P. P., Bloom, F. E. & Burton, D. R. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 355–359.
36. Williamson, R. A., Burioni, R., Sanna, P. P., Partridge, L. J., Barbas, C. d. & Burton, D. R. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 4141–4145.
37. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
38. Laemmli, E. K. (1970) *Nature (London)* **227,** 680–685.
39. Fujinaga, S., Sugano, T., Matsumoto, Y., Masuho, Y. & Mori, R. (1987) *J. Infect. Dis.* **155,** 45–53.
40. Co, M. S., Deschamps, M., Whitley, R. J. & Queen, C. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 2869–2873.
41. Kahlon, J. & Whitley, R. J. (1988) *J. Infect. Dis.* **158,** 925–933.