JOURNAL OF VIROLOGY, Sept. 1990, p. 4123–4129
0022-538X/90/094123-07$02.00/0
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Vol. 64, No. 9

Analysis of a Highly Immunodominant Epitope in the Human Immunodeficiency Virus Type 1 Transmembrane Glycoprotein, gp41, Defined by a Human Monoclonal Antibody†

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Received 27 March 1990/Accepted 1 June 1990

A human monoclonal antibody, 41-7 [immunoglobulin G1(κ)], directed against the transmembrane glycoprotein gp41 of the human immunodeficiency virus type 1 (HIV-1) has been produced by direct fusion of lymph node cells from an HIV-1-infected individual with a human B-lymphoblastoid cell line. The minimal essential epitope for 41-7 was mapped to a conserved seven-amino acid sequence, N-CSGKLIC-C, located within the N-terminal part of gp41. Antibodies blocking the binding of 41-7 could be detected in the serum of all HIV-1-infected individuals tested, irrespective of the stage of the infection. The epitope is located externally to the plasma membrane, and it is accessible to antibody in the native conformation of the glycoprotein. Despite this, no neutralizing activity of 41-7 could be demonstrated in vitro. These data indicate, directly and indirectly, that this immunodominant epitope on gp41, although exposed on the viral surface, elicits antibodies lacking antiviral activity and, hence, should be avoided in future vaccine candidates.

The humoral immune response to the human immunodeficiency virus type 1 (HIV-1) is being extensively studied, with the primary aim of developing immunoprophylactic strategies. Antibodies capable of neutralizing HIV-1 in vitro are present in the serum of many HIV-1-infected individuals, but their protective effect is unknown (17, 48–50). Several human B-cell epitopes have been identified on the external viral surface glycoprotein gp120 (15, 16, 38, 42) and on the transmembrane glycoprotein gp41 (13, 35, 47). Despite an extensive search, only one neutralization epitope consistently recognized by human sera has been identified. This epitope is located in a hypervariable region of gp120 and elicits type-specific neutralizing antibodies with little or no cross-neutralizing potential (15, 16, 24, 37, 42). However, there is indirect evidence for the existence of group-specific human neutralization epitopes on the envelope glycoproteins (31, 44, 49), but such epitopes still need to be identified.

Attempts to induce protective immunity against HIV-1 in chimpanzees by immunization with envelope glycoprotein or *env*-expressing vaccinia viruses have failed, despite a detectable humoral immune response (4, 23). Tools for a more thorough dissection of the function and dynamics of the human humoral immune response to HIV-1 are therefore necessary and needed. One such tool is human monoclonal antibodies.

Several human monoclonal antibodies against *gag*- and *env*-encoded proteins have been produced (3, 9, 11, 14, 18). However, apart from the first study, no detailed characterization of the specificity of these monoclonal antibodies has been provided.

In the present study we describe the development of a new human monoclonal antibody, designated 41-7, defining and allowing a detailed characterization of a human B-cell epitope, N-CSGKLIC-C, located within a highly conserved region of the transmembrane glycoprotein gp41.

MATERIALS AND METHODS

Media and cell lines. Human fusion partners and human hybridomas were grown in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 2 mM glutamine, 50 μg of streptomycin per ml, 50 U of penicillin per ml, 3.5×10^{-5} M 2-mercaptoethanol (Sigma Chemical Co., St. Louis, Mo.), and 10 to 15% heat-inactivated fetal calf serum (all from GIBCO), in the following designated RPMI 1640s. HAT medium consisted of complete RPMI 1640 supplemented with 13.6 μg of hypoxanthine per ml, 0.18 μg of aminopterin per ml, and 3.9 μg of thymidine per ml. HT medium was complete RPMI 1640 supplemented with hypoxanthine and thymidine only. The human fusion partner UC729HF2 (1) (kindly provided by T. Plesner, State University Hospital, Copenhagen, Denmark) is a human B-lymphoblastoid cell line resistant to 6-thioguanine ($HPRT^-$) and is an immunoglobulin nonsecreting variant of the UC729-6 cell line (12).

Production and characterization of the human monoclonal antibody. Lymph node biopsies were obtained from a 39-year-old HIV-1-seropositive individual with persistent generalized lymphadenopathy. Fat was removed from the biopsy material, and a single cell suspension was prepared by homogenization with a ground glass homogenizer. The suspended lymph node cells were washed three times in RPMI 1640. Lymph node cells and UC729HF2 cells harvested in the exponential phase of growth were mixed in a ratio of 2:1 and fused with polyethylene glycol (M_W, 4,000) (50% [wt/vol] in RPMI 1640) (E. Merck AG, Darmstadt, Federal Republic of Germany) as described previously (25). Fusion products were seeded in HAT medium in five 96-well microtiter plates at a density of 10 cells per well. Actively growing hybridomas appeared in 6% of the wells and were screened for production of antibodies reactive with HIV-1 antigens in

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† Dedicated to the memory of Kay Ulrich, mentor and friend, who died 27 September 1989.

enzyme-linked immunosorbent assay (30). A selected positive hybridoma culture was expanded and cloned under standard conditions by limiting dilution. Cloned hybridoma cultures were selected on the basis of positive reaction in ELISA and Western immunoblot and grown up as mass cultures in RPMI 1640. Antibodies were purified from pooled culture supernatants on a protein A-Sepharose column (Pharmacia, Uppsala, Sweden) as described previously (28). The immunoglobulin G subclass and light chain of the monoclonal antibodies were determined in indirect ELISA with peroxidase-conjugated subclass-specific monoclonal antibodies (The Binding Site, Birmingham, United Kingdom) and light-chain-specific antibodies (TAGO, Burlingame, Calif.) according to the instructions of the manufacturers. Biotinylation of 41-7 was performed as described previously (19), except that the molar ratio of *N*-hydroxysuccinimidobiotin to antibody used was four times lower in this study. The isoelectric points (pI) of the human monoclonal antibodies were determined on isoelectric focusing pH 3 to 9 Phast gels (Pharmacia). Running conditions and silver staining of the gels were according to the instructions of the manufacturers.

Western blot of infected cells. HIV-1_{IIIB}-infected H9 cells (39) and uninfected H9 cells were lysed for 1 h at 37°C in 200 mM Tris, pH 8.0–10 mM EDTA–4% (vol/vol) Triton X-100–0.1% (wt/vol) sodium dodecyl sulfate, with 5 mM phenylmethylsulfonyl fluoride, 2 μg of pepstatin A per ml, 10 mM benzamidine, and 1 mM dithiothreitol as protease inhibitors (100 μl of lysis buffer per 2.5×10^7 cells), and the lysates were clarified by centrifugation for 1 h at 20,000 × *g* and 4°C. The lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 5 to 20% gradient gels and electrophoretically transferred to 0.45-μm polyvinylidene difluoride membranes (Millipore Corp., Bedford, Mass.). Unspecific binding sites were blocked with 2% (vol/vol) Tween 20 in phosphate-buffered saline (PBS), and the membrane was cut into strips. The strips were incubated for 2 h at room temperature with primary antibody diluted in 50 mM Tris, pH 10.2–150 mM NaCl–0.1% (vol/vol) Tween 20–10% (wt/vol) nonfat dry milk (Milko, Copenhagen, Denmark). Following four washes, antibodies bound to proteins on the membrane were detected by incubating the strips for 1 h with species-specific peroxidase-conjugated rabbit antibodies to the primary antibody (Dakopatts, Glostrup, Denmark) and staining with DONS/TMB as described previously (27).

Recombinant proteins and synthetic peptides. All nucleotide and amino acid numbering is according to the system described in reference 40. pEX/pUEX designates the expression plasmids and p-purified fusion protein or synthetic peptide. The first number indicates the first amino acid (codon) of HIV-1 *env* present in a fusion protein, peptide, or expression plasmid, and the last number indicates the last amino acid.

The proviral clone BH10-2 (40) was used as source of HIV-1 DNA. All fragments of the BH10-2 *env* gene were expressed as cro-beta-galactosidase fusion proteins by using the pUEX or pEX expression plasmids (6, 45). All transformations of expression plasmids were into the *lacZ lambda* cI857 repressor containing *Escherichia coli* pop2136 (5). To generate the *env* expression plasmids, the desired *env* gene fragment was excised and isolated and either inserted directly into the polylinker of the expression plasmid or first cloned into pUC or pBluescript plasmids to generate compatible restriction overhangs. The expression plasmids used in this study contain the following fragments of BH10-2 (the restriction site is given in parentheses): pEX474-649, *Bgl*II (nucleotide [nt] 7199)-*Hin*dIII (nt 7718); pEX474-759, *Bgl*II (nt 7199)-*Bam*HI (nt 8052); pEX647-759, *Hin*dIII (nt 7719)-*Bam*HI (nt 8052); pEX758-863, *Bam*HI (nt 8053)-*Xho*I (nt 8474); and pUEX350-518, *Dra*I (nt 6188)-(nt 7334). pUEX350-518 was generated by polymerase chain reaction amplification (43) of the gp120-encoding part of the *env* gene by using primers specific for the 5′ and 3′ ends of the region, followed by digestion with *Dra*I and isolation and cloning of the desired fragment in pUEX2.

Small-scale bacterial cultures containing the desired expression plasmids were induced as described previously (10), except that NY medium (0.8% [wt/vol] NZ-amine, 0.5% [wt/vol] yeast extract, 0.5% [wt/vol] NaCl) containing 17 mM KH_2PO_4 and 72 mM K_2HPO_4 was used instead of L broth and induction was performed for 1 h and 40 min. Portions (1.5 ml) of induced culture were cooled on ice, and the bacteria were harvested by centrifugation, suspended in 100 μl of 1× Laemmli sample buffer containing 5% (wt/vol) sodium dodecyl sulfate, and boiled for 7 min. The lysate was clarified by centrifugation for 5 min at 10,000 × *g*. Ten microliters of the lysates was applied for each lane on 7.5% polyacrylamide gels, and the Western blot was performed as described above.

Recombinant fusion proteins were purified from large-scale induced cultures by a modification of the procedure described previously (10). The purification procedure has been described in detail (T. H. Bugge, P. Kusk, B. Ø. Lindhardt, K. Holmbäck, L. L. Hansen, E. Hulgaard, and K. Ulrich, unpublished data).

The peptides p586-606, N-RILAVERYLKDQQLLGIWGCS-C; p592-610, N-RYLKDQQLLGIWGCSGKLI-C; p604-625, N-GCSGKLICTTAVPWNASWSNKS-C, p606-624, N-SGKLICTTAVPWNASWSNK-C; and p611-622, N-CTTAVPWNASWS-C, were a kind gift from Rüdiger Pipkorn, Novabiochem, Sandhausen, Federal Republic of Germany. The synthesis and purification of the peptides have been described previously (26). p600-611, N-LGIWGCSGKLIC-C, and p605-611, N-CSGKLIC-C, and GRF, N-HADAIFTSSYRRILGQLSARKLHEIINR-C, were synthesized on a peptide synthesizer (model 4175; LKB/Biochrom Biolynx, Bromma, Sweden) and purified by reverse-phase high-pressure liquid chromatography. The sequence of the peptides was verified by amino acid composition analysis. The peptides were dissolved in PBS at a concentration of 0.25 to 1.0 mg/ml before use.

Peptide competition ELISA. Synthetic peptides were two-fold serially diluted in PBS, and 55 μl of each dilution was mixed with 55 μl of biotinylated 41-7 (1 μg/ml) in PBS containing 0.1% (vol/vol) Tween 20 and 2% normal rabbit serum. The peptide antibody mixture was incubated for 30 min at room temperature, and 100 μl of the mixture was added to a microtiter plate (Nunc II; Nunc, Roskilde, Denmark) coated with 25 ng of p474-759. The plate was incubated for 2 h at room temperature and extensively washed, and bound antibody was detected with peroxidase-coupled streptavidin (Medac, Hamburg, Federal Republic of Germany) and *ortho*-phenylenediamine.

Surface immunofluorescence analysis. HIV-1_{IIIB}-infected H9 cells or uninfected H9 cells were collected by centrifugation, washed twice in PBS, and resuspended in PBS at a concentration of 5×10^7 cells per ml. A 50-μl portion of the cell suspension was mixed with 50 μl of human monoclonal antibody or serum diluted in PBS containing 1% heat-inactivated normal rabbit serum and incubated for 1 h at 4°C. The cells were washed three times at 4°C in PBS and suspended in 100 μl of fluorescein isothiocyanate-conjugated

rabbit anti-human immunoglobulin G (Dakopatts) diluted 10 times in PBS containing 1% heat-inactivated normal rabbit serum and incubated for 1 h at 4°C. After three washes the cells were mounted in PBS-glycerol (1:1) and inspected in a fluorescence microscope (Leitz Orthoplan).

Immunofluorescence analysis of fixed cells. HIV-I_{IIIB}-infected H9 cells or uninfected H9 cells were collected by centrifugation, washed twice in PBS, and resuspended in PBS at a concentration of 3×10^7 cells per ml. A 10-μl cell suspension was spotted on poly-L-lysine-coated 15-well multitest slides (Flow Laboratories, Inc., McLean, Va.) and immediately removed. The slides were incubated 2 times for 5 min each time in ice-cold acetone and air dried. Monoclonal antibody or serum was diluted in PBS containing 1% normal rabbit serum and 1% (wt/vol) bovine serum albumin, and 10 μl was added per well. The slides were incubated for 1 h at 37°C in a humidified chamber and washed three times in PBS. Ten microliters of fluorescein isothiocyanate-conjugated rabbit anti-human immunoglobulin G (Dakopatts) diluted 20 times in PBS containing 1% normal rabbit serum and 1% (wt/vol) bovine serum albumin was added to each well. The slides were incubated for 1 h, washed three times in PBS, counterstained in Evans blue, and mounted in glycerol-PBS (1:1). The slides were inspected in a fluorescence microscope (Leitz Orthoplan).

Serum competition ELISA. Human serum samples were twofold serially diluted in NT buffer (PBS, 0.5 M NaCl, 1% [vol/vol] Triton X-100) with a starting dilution of 1:20. A 55-μl portion of each serum dilution was mixed with 55 μl of biotinylated 41-7 (5 μg/ml) in NT buffer containing 2% normal rabbit serum. A 100-μl portion of this mixture was added to a microtiter plate (Nunc II) coated with 500 ng of fusion protein, and the plate was incubated for 2 h at room temperature. The plate was extensively washed, and bound 41-7 was detected with peroxidase-conjugated streptavidin (Medac) and *ortho*-phenylenediamine.

Neutralization assays. Peripheral blood mononuclear cells were isolated from normal blood donors and stimulated with phytohemagglutinin as described previously (2). 41-7 was serially diluted in RPMI 1640, and 25 μl of each dilution was mixed in 96-well flat-bottomed microtiter plates (Nunc) with 25 50% tissue culture infective dose units of virus (32) in 25 μl of RPMI 1640–10% fetal calf serum, and the antibody virus mixture was incubated for 1 h at 37°C. Peripheral blood mononuclear cells (10^5) in 50 μl of RPMI 1640 containing 15% fetal calf serum, 10% interleukin-2 (Electro-Nucleonics, Silver Spring, Md.), and antibiotics were added to each well, and the plates were incubated at 37°C and 95% relative humidity in a 5% CO_2 incubator. At day 3, 100 μl of fresh medium was added to the wells. At day 6, 100 μl of medium was removed and replaced with 100 μl of fresh medium. At day 9 the medium was harvested and assayed for reverse transcriptase activity was described previously (21).

RESULTS

Preparation of 41-7. To obtain human monoclonal antibodies directed against HIV-1 antigens, lymph node cells from an HIV-1-antibody positive-individual were fused with the $UC729HF_2$ B-lymphoblastoid cell line, and hybridomas were selected in HAT medium. A single hybridoma produced antibodies reacting with HIV-1 antigens in ELISA and Western blotting. This hybridoma was successfully recloned, and 13 clones were further selected on the basis of HIV-1 ELISA reactivity. One of the cloned hybridomas, 41-7, was selected for further characterization. In Western blot, this antibody showed reactivity with proteins with approximate molecular weights of 160,000 and 41,000, consistent with the expected reactivity of an antibody directed against the transmembrane glycoprotein gp41 (Fig. 1). The subclass of 41-7 was determined to be immunoglobulin G1, and the light chain was determined to be of the kappa type. The isoelectric point pI was found to be 8.3. The subclass, light-chain type, and pI of the remaining 12 hybridomas were identical to 41-7, and all 13 recloned hybridomas therefore likely were descendants of the same B-cell clone. The hybridoma 41-7 has stably produced monoclonal antibody at levels of approximately 1 mg/liter of culture supernatant for more than 3 years.

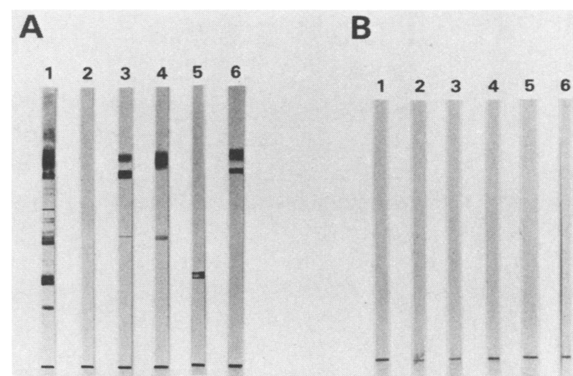

FIG. 1. Reactivity of 41-7 with HIV-1 antigens in Western blot. Strips containing a lysate of HIV-1-infected H9 cells (A) and uninfected H9 cells (B) were stained. Lanes: 1, HIV-1-antibody-positive serum pool, 500 times diluted; 2, HIV-1-antibody-negative serum pool, 500 times diluted; 3, gp120 antiserum (Biochron, Hamburg, Federal Republic of Germany), 500 times diluted; 4, 41-7 (10 μg/ml); 5, p24 monoclonal antibody F5-2 (28); and 6, gp120 monoclonal antibody (E. Hulgaard, unpublished data).

Delineation of the epitope recognized by 41-7. To delineate the epitope recognized by 41-7, the reactivity of the antibody with a series of *E. coli*-produced fusion proteins containing fragments encoded by the HIV-1_{IIIB} *env* gene was initially tested. In a Western blot (Fig. 2), 41-7 showed a strong reactivity with two fusion proteins expressing amino acids 474 to 649 and 474 to 759, respectively. In contrast, no reactivity was observed with fusion proteins expressing amino acids 350 to 518, 647 to 759, or 758 to 863, or with the cro-beta-galactosidase fusion protein itself. The identical pattern of reactivity was observed with purified fusion protein in ELISA (data not shown). This experiment localizes the epitope recognized by 41-7 between amino acids 519 and 649 in the N-terminal presumed extracellular part of gp41, and it further demonstrates that recognition of the epitope is not dependent upon glycosylation of gp41.

The epitope was more precisely mapped by the use of synthetic peptides homologous to the N-terminal part of gp41. The ability of a peptide in solution to mimic the epitope was tested in a competition ELISA by preincubating 41-7 with the peptide before adding the antibody to ELISA wells coated with a purified fusion protein containing the epitope recognized by 41-7. The peptides tested and the results are shown in Fig. 3 and Fig. 4. Three peptides, comprising amino acids 600 to 611, 605 to 611, and 604 to 625, efficiently blocked the binding of 41-7. The sequence of mutual overlap between these peptides is the seven-amino-acid sequence located between cysteines 605 and 611. The peptide p592-610 lacking cysteine 611 showed no competition even at concen-

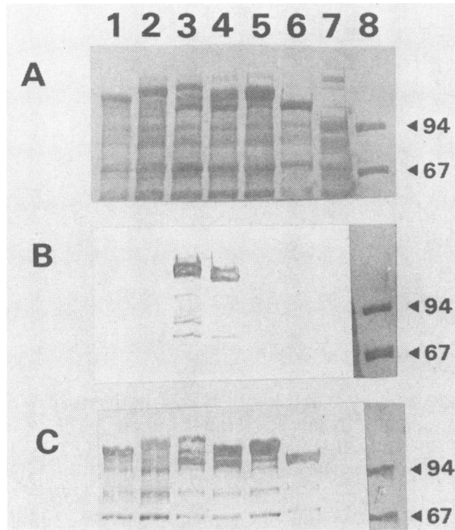

FIG. 2. Reactivity of 41-7 with HIV-1 *env* gene fragments expressed as cro-beta-galactosidase fusion proteins in *E. coli*. Lanes 1 through 5, Lysates of induced cultures containing the following: lane 1, pEX758-863; lane 2, pEX647-759; lane 3, pEX474-759; lane 4, pEX474-649; and lane 5, pUEX350-518. Lane 6, pEX plasmid without insert; lane 7, no plasmid; lane 8, molecular weight markers (in thousands). (A) Stained with Coomassie brilliant blue; (B) 41-7 (10 μg/ml); (C) monoclonal antibody against beta-galactosidase.

trations above 10 μM. Similarly, the affinity of 41-7 for the p606-624 lacking cysteine 605 is, as judged by the amount of peptide needed to block 50% of the binding of the antibody, more than 10,000-fold lower than for the peptide p605-611. The specificity of the reactivity of the peptides p600-611, p604-625, p605-611, and p606-624 with 41-7 was verified by the inability of these peptides to compete with the binding of any of seven different monoclonal antibodies against beta-galactosidase to the fusion protein (data not shown). These results define the minimal essential epitope for 41-7 as the seven-amino-acid sequence N-CSGKLIC-C (Fig. 3). We are currently not able to explain why the affinity of the antibody for the peptide p600-611, which includes the entire postulated minimal essential epitope, appears to be much lower

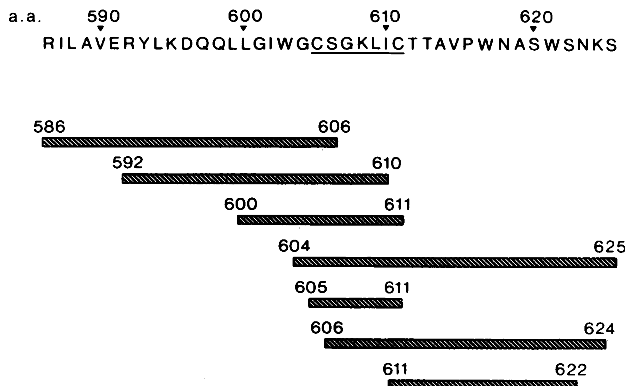

FIG. 3. Synthetic peptides tested for reactivity with 41-7. The amino acid sequence of the binding region for 41-7 on gp41 is shown with the minimal essential epitope underlined. The synthetic peptides tested are shown below.

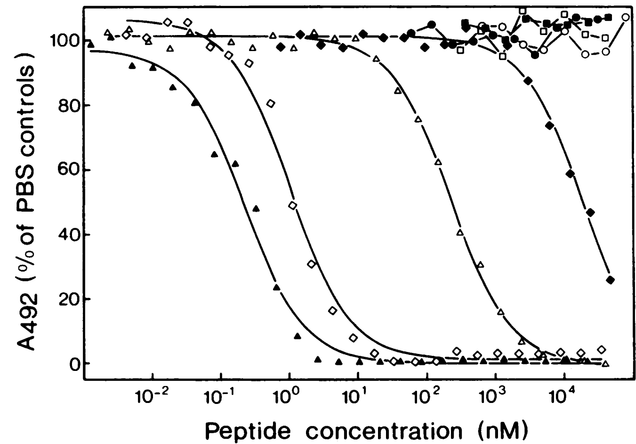

FIG. 4. Results of peptide competition ELISA. The binding of 41-7 to recombinant gp41 was competed with serially diluted peptides as described in Materials and Methods. The absorbance value for each peptide is expressed as a percentage of the mean of six noncompeting controls. □, p586-606; ■, p592-610; △, p600-611; ▲, p604-625; ◇, p605-611; ◆, p606-624; ○, 611-622; ●, irrelevant peptide (GRF).

than for the peptide containing the minimal essential epitope 605-611 itself.

Cell surface localization of the epitope. To investigate the topological localization of the epitope recognized by 41-7, a parallel indirect immunofluorescence analysis of acetone-fixed and unfixed HIV-1_{IIIB}-infected H9 cells was performed. A bright membrane-cytoplasma-associated staining of acetone-fixed infected H9 cells was observed with 10 μg of 41-7 per ml, whereas no staining of uninfected H9 cells was seen even at 50 μg/ml. Further, 41-7 also specifically stained the cell surface of live infected H9 cells, although a five-times-higher concentration of 41-7 was necessary to give the same relative fluorescence intensity (data not shown). This result indicates that the epitope defined by 41-7 is located in an extracellularly-extravirally positioned part of gp41 and, further, that the epitope in the native protein is accessible to the antibody.

In vivo immunogenicity of the epitope defined by 41-7. To examine the in vivo immunogenicity of the epitope defined by 41-7, a competition ELISA was used. Serially diluted serum samples from 30 different HIV-1-infected individuals representing three different stages of the infection were used to compete with the binding of biotinylated 41-7 to p474-759 (Fig 5). Serum samples from 20 healthy donors served as negative controls. All HIV-1-antibody-positive sera tested contained competing antibodies, often in high titers, while no competing activity was found in the control sera (Fig. 5). Further, no evident correlation was found between the ability of the sera to compete with the binding of 41-7 and the stage of the infection. The competition assay shows that the epitope recognized by 41-7 is highly immunodominant or, as blocking of 41-7 binding by steric hindrance of serum antibodies binding proximal epitopes cannot be ruled out, localized in a highly immunodominant region of gp41. Further, antibodies directed against this region seem to persist in the serum of HIV-1-infected individuals throughout the infection, despite the progressive immune dysfunction.

Neutralizing potential of 41-7. The ability of 41-7 to neutralize HIV-1 infectivity in vitro was assessed in an infection inhibition assay by using phytohemagglutinin-stimulated peripheral blood mononuclear cells and the IIIB strain of

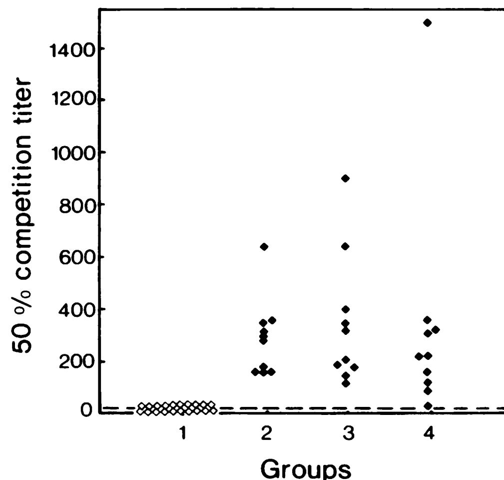

FIG. 5. Results of serum competition assay. Serially diluted serum samples were used to compete with the binding of 41-7 to recombinant gp41. The 50% competition titer is defined as the inverse of the dilution of a serum giving a 50% reduction of the A_{492}. Open diamonds indicate that the 50% competition titer of the serum is less than 20. Groups: 1, uninfected healthy donors; 2, immunologically normal HIV-1-infected individuals; 3, immunologically impaired HIV-1-infected individuals; group 4, AIDS patients. The serum samples were divided into the three groups according to number of CD4-positive T lymphocytes, response to pokeweed mitogen, and clinical status of the infected individuals. For details see references 22 and 29.

HIV-1 as indicator cells and virus, respectively (Fig. 6). No inhibition of virus infectivity was observed even when 25 50% tissue culture infective dose units of virus (the lowest virus concentration giving a consistent infection of peripheral blood mononuclear cells in our laboratory) and an antibody concentration of up to 100 μg/ml were used, indicating that the human monoclonal antibody has no neutralizing ability in vitro.

DISCUSSION

The transmembrane glycoprotein gp41 is the antigen most consistently recognized by human sera (8), and the titers of

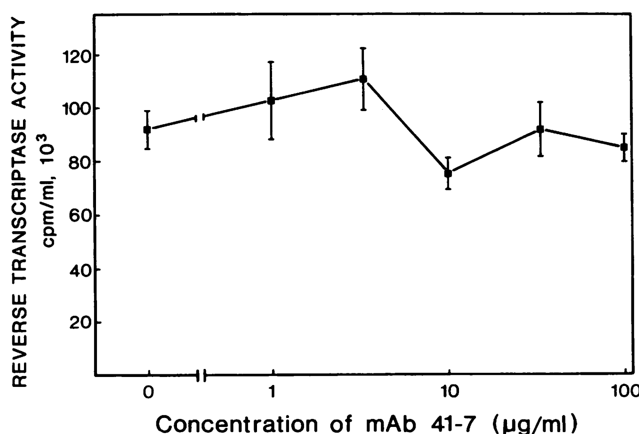

FIG. 6. Neutralizing potential of 41-7. The infectivity of antibody-challenged virus is measured as the amount of particle associated reverse transcriptase in the supernatant 9 days after infection. Data are shown as the means of four experiments. Vertical bars represent the 95% confidence intervals.

detectable antibodies to epitopes on gp41 appear to be much higher than to gp120 epitopes (T. H. Bugge, P. Kusk, B. Ø. Lindhardt, K. Holmbäck, L. L. Hansen, E. Hulgaard, and K. Ulrich, unpublished data). If the number of circulating antibodies against particular epitopes reflect the number of plasma cells producing them, then the method for producing human monoclonal antibodies we describe here contains an intrinsic risk of obtaining antibodies only against a limited set of immunodominant epitopes. Therefore, some kind of selection procedure for desired antibody-producing cells prior to fusion might be preferable.

By using fragments of the HIV-1 *env* gene expressed as fusion proteins in *E. coli* combined with synthetic peptides, the minimal essential epitope for 41-7 was mapped to the seven-amino-acid sequence N-CSGKLIC-C. Comparison of the deduced amino acid sequences of several cloned HIV-1 isolates shows that the sequence appears to be conserved in HIV-1, with the exception of leucine 609, which is changed to isoleucine or histidine in some African isolates (34). Interestingly, a similar sequence motif, N-CAFRQVC-C, is present in the homologous region of the transmembrane glycoprotein of HIV-2 (34). Despite this, most HIV-2-antibody-positive sera fail to compete with the binding of 41-7 (T. H. Bugge, L. L. Hansen, B. Ø. Lindhardt, B. Kvinesdal, P. Kusk, K. Holmbäck, E. Hulgaard, and K. Ulrich, unpublished data), indicating a low serological cross-reactivity in the region, as also reported with HIV-1- and HIV-2-specific peptides derived from the region (36).

The dependence of both cysteines for reactivity with 41-7 as well as a potential beta-turn located between the cysteines (33) suggests that the polypeptide chain may form a disulfide-linked loop. If this is the case and if formation of such a loop is necessary for the efficient recognition by the antibody, then the low affinity of the antibody for p600-611 compared with p605-611 may be explained by the inability or lesser ability of p600-611 to form such a loop. This hypothesis could be tested by the determination of the conformation of the immunoreactive peptides in solution. Such studies are currently in progress, using two-dimensional nuclear magnetic resonance analysis.

Three overlapping synthetic peptides, consisting of amino acids 586 to 606, 599 to 611, and 606 to 620, respectively, have been reported to be immunoreactive with most if not all HIV-1-antibody-positive sera (13, 35, 47). The dependence of both cysteines for the reactivity of 41-7 demonstrates that at least three human B-cell epitopes are present in the region, since the peptides containing the amino acids 586 to 606 and 606 to 620 contain only one cysteine residue each.

Cell surface immunofluorescence analysis shows that the epitope recognized by 41-7 is located externally to the plasma membrane. This indicates that the whole N-terminal part of gp41 is located externally to the membrane, since only one potential transmembrane segment positioned C terminal to the epitope can be identified (1a). This model of the orientation is also supported experimentally by protease digestion protection experiments (20).

In an in vitro infection inhibition assay, 41-7 failed to demonstrate any neutralizing activity even when a low virus inoculum and high antibody concentrations were used. A similar lack of neutralization has been reported for another human monoclonal antibody directed against a proximate or overlapping epitope on gp41 (3). Also, Matthews and coworkers did not observe neutralizing activity of affinity-purified human antibodies directed against the N-terminal half of gp41 (31). Taken together, the data suggest that the immunodominant N-terminal region of gp41 (amino acids

586 to 620) does not elicit neutralizing antibodies in HIV-1-infected individuals. The fact that high titers of neutralizing antibodies are only infrequently found in sera from HIV-1-infected individuals (7, 48, 49), whereas high titers of antibodies against the immunodominant region of gp41 are present in almost all sera (13, 35, 47), indirectly supports this. Reitz and co-workers (41) reported that an HIV-1 variant selected for the resistance to neutralization by one neutralizing human serum contained a single amino acid substitution within the immunodominant region of gp41 (Ala-589 to Thr-589). However, as also pointed out by the authors, "neutralization escape mutations" need not always take place within the epitope of the neutralizing antibody, as thoroughly demonstrated in the picornavirus system (51).

Although not neutralizing, antibodies elicited by the epitope defined by 41-7 may still have other important antiviral activities, such as mediating antibody-dependent cellular cytotoxicity. However, the fact that disease progression seems to occur in virtually all infected individuals despite the presence of persistent high titers of antibodies to the epitope does not indicate a substantial beneficial value of these antibodies. Thus, further work is needed to clarify whether this epitope should eventually be included in future vaccine candidates.

Toxin- or radioisotope-conjugated human monoclonal antibodies against cell surface-exposed epitopes on HIV-1 antigens have potential therapeutic applications, as they should theoretically be able to selectively kill infected cells and thereby reduce or prevent viral replication. Indeed, specific killing in vitro of HIV-1-infected monocytoid and T-cell lines by ricin A-coupled human gp41 monoclonal antibodies has been demonstrated (46). However, attempts to successfully use conjugated human monoclonal antibodies directed against the immunodominant region of gp41 (like the one we present in this paper) therapeutically are likely to be seriously hampered by the persistent high titers of antibodies against this region in HIV-1-infected individuals, efficiently competing with the binding of the conjugated antibody.

ACKNOWLEDGMENTS

We thank Court Pedersen, Department of Infectious Diseases, Hvidovre Hospital, for lymph node biopsies and Torben Særmark, The Protein Laboratory, University of Copenhagen, Copenhagen, Denmark, and Rüdiger Pipkorn, Novabiochem, Sandhausen, Federal Republic of Germany, for synthetic peptides. We are grateful to Helle Johansen and Inge Skibshøj for excellent technical assistance and to Laura Beguinot for critically reading the manuscript.

This work was supported by The Danish Cancer Society, The Neye Foundation, The Meyer Foundation, The Hartmann Foundation, and the Danish Medical Research Council.

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