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Neutralizing Monoclonal Antibodies against Human Immunodeficiency Virus Type 2 gp120

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Monoclonal antibodies (MAbs) were obtained by immunizing mice with synthetic peptides corresponding to the third variable (V3) or the third conserved (C3) domain of the external envelope protein (gp120) of human immunodeficiency virus type 2 (HIV-2_{ROD}). One MAb, designated B2C, which was raised against V3 peptide NKI 26, bound to the surface of HIV-2-infected cells but not to their uninfected counterparts. B2C was capable of neutralizing cell-free and cell-associated virus infection in an isolate-specific fashion. The antibody-binding epitope was mapped to a 6-amino-acid peptide in the V3 variable domain which had the core sequence His-Tyr-Gln. Two MAbs, 2H1B and 2F19C, which were raised against the C3 peptide TND27 reacted with gp120 of HIV-2_{ROD} in a Western immunoblot assay. The C3 epitopes recognized by these two MAbs appeared inaccessible because of their poor reactivity in a surface immunofluorescence assay. Although partial inhibition of syncytium formation was observed in the presence of the anti-C3 MAbs, their neutralizing activity appeared weak. Finally, the effects of these MAbs against CD4-gp120 binding were assessed. Partial inhibition of CD4-gp120 binding was observed in the presence of high concentrations of B2C. On the other hand, no inhibition of CD4-gp120 binding was observed in the presence of anti-C3 MAbs. Since complete neutralization could be achieved at a concentration corresponding to that of partial binding inhibition by B2C, some different mechanisms may be involved in the B2C-mediated neutralization. These results, taken together, indicated that analogous to the function of the V3 region of HIV-1, the V3 region of HIV-2_{ROD} contained at least a type-specific fusion-inhibiting neutralizing epitope. In this respect, the V3 sequence of HIV-2 may be a useful target in an animal model for HIV vaccine development.

The human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) are linked to the current worldwide spread of AIDS (7). While HIV-1 and HIV-2 share a similar genetic organization, they are considerably divergent at the amino acid sequence level. The HIV-1 and HIV-2 envelope glycoproteins, for example, share only approximately 40% amino acid sequence identity (17). HIV-2 is, however, more closely related both immunologically and genetically to simian immunodeficiency virus (SIV) than to HIV-1. The envelope glycoproteins of HIV-2 and the SIV of the rhesus macaque (SIV_{mac}) share approximately 75% amino acid sequence identity (5, 13, 18). Moreover, unlike HIV-1, HIV-2 can infect macaques and baboons, and because these animals do not develop clinical immunodeficiency (11, 12, 33), they can be used to test the protective effect of vaccines against viral infection. However, the usefulness of HIV-2/SIV in monkeys as a model for HIV-1 infection of humans has been questioned because of the apparent biological-pathological difference between these virus groups. In particular, the failure or low efficiency of the HIV-2/SIV linear V3-region peptides to induce neutralizing antibodies compared with that of HIV-1 suggests that an additional region(s) of the HIV-2/SIV envelope protein participates in neutralization (34).

The principal neutralizing determinant in HIV-1 has been localized in the V3 region, which lies between two disulfide bonds thought to confer a loop structure (V3 loop [V3L]) (26) and is subject to a high degree of genetic variation (25). Polyclonal antibodies and monoclonal antibodies (MAbs) raised against HIV-1 V3L had an isolate-specific neutralizing activity in both cell-to-cell infection and cell-free-infection with HIV-1 (28, 32, 35). A recent study suggested that the broadly reactive neutralizing antibody could also be induced against the conserved tip sequence of V3L (19). In contrast, for SIV, low variability within the V3 region has been reported (4, 14, 22, 31). Furthermore, polyclonal antibodies and MAbs against the V3 region of SIV did not have neutralizing activity (21, 23). Instead, antibodies against the V2 region of SIV gp120 have been associated with viral neutralization (23). Recently, Javaherian et al. reported that the V3 region of SIV gp120 did not form a linear neutralizing epitope but was involved in formation of a conformation-dependent epitope (20). For HIV-2, on the other hand, Boeri et al. analyzed the sequence variability of the V3 region and reported that the degree of variation among field isolates of HIV-2 was comparable to that observed in the analogous region of HIV-1 (3). However, neutralization of HIV-2 infection by anti-V3 antibody has not been well studied. Björling et al. reported that neutralizing antibodies were induced by hyperimmunization with peptides representing three different sites, including V3 and C3, in the HIV-2 gp120 (1). In this report, we describe the production and characterization of MAbs against the V3 and C3 regions of the gp120 of HIV-2_{ROD}. Our observations suggest that the V3 region of HIV-2 has a similar function to that of HIV-1 in the infection process.

MATERIALS AND METHODS

Cells and viruses. Uninfected MOLT4 clone 8 (MOLT4C8) cells, CEM cells, and their HIV- and SIV-infected counterparts have been described previously (7, 21, 28, 34). HIV-2 isolates ROD, ISY, and GH1 were kindly provided by L. Montagnier, Pasteur Institute, Paris France, M. Robert-Guroff, National Cancer Institute, Bethesda, Md., and M. Hayami, Kyoto University, Kyoto Japan, re-

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PGN24;PGNKIVKQIMLMSGHVFHSHYQPC
NKI26;NKIVKQIMLMSGHVFHSHYQPINKRC
TND27;TNDTRNISFAAPGKGSDPEVAYMWTNC

FIG. 1. Amino acid sequences of synthetic peptides used for immunization. Peptides PGN24 and NKI26 represent the V3 region of HIV-2_{ROD} gp120. TND27 was derived from a sequence of the C3 region of gp120.

spectively. The SIV_{mac251} isolate was kindly provided by R. Desrosiers, Harvard Medical School, Cambridge, Mass. Cells were maintained in RPMI 1640 medium (GIBCO) supplemented with 15% fetal calf serum and antibiotics (complete medium).

Peptide synthesis and MAb production. Synthetic peptides (Fig. 1) were prepared as described previously (27). Peptides conjugated with keyhole limpet hemocyanin were used to immunize mice by standard protocols. BALB/c mice were immunized on day 0 with 100 μg of keyhole limpet hemocyanin-conjugated peptide in Freund's complete adjuvant, and three subsequent immunizations of 100 μg in Freund's incomplete adjuvant were given at 14-day intervals. Three days after the final immunization, splenic cells were fused with cells of the ×63 mouse myeloma line. The cell fusion, hypoxanthine-aminopterin-thymidine selection, and cloning of hybridoma lines were performed essentially as described previously (28).

Immunological assays. For fluorescent staining of cell surfaces, MOLT4 cells chronically infected with HIV-2_{ROD} (MOLT4/HIV-2_{ROD} cells) or their uninfected counterparts (5×10^5 cells) were incubated for 30 min on ice with 10 μg of the MAb or control immunoglobulin G1 (IgG1; MOPC 21) per ml. Following extensive washing, cells were incubated on ice with fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (Sigma) diluted 1:40 for 30 min, washed, and analyzed by laser flow cytometry (FACStar; Becton Dickinson, Mountain View, Calif.). We used phosphate-buffered saline containing 2% bovine serum albumin and 0.2% sodium azide for washing the cells and diluting the reagents.

We analyzed the expression of the CD4 molecule on the surface of MOLT4/HIV-2_{ROD} cells or its uninfected counterparts by flow-cytometric analysis with two MAbs against the gp120-binding site of CD4 (Leu3a [Becton Dickinson] and Nu-$T_{H/I}$ [Nichilei, Tokyo, Japan]) and one MAb (OKT4 [Ortho Diagnostic Systems, Raritan, N.J.]) not against the gp120-binding site of CD4.

Western immunoblot analysis of the antibody reactivity was performed with lysates MOLT4/HIV-2_{ROD} cells. The cell lysates were fractionated by electrophoresis on sodium dodecyl sulfate–12% polyacrylamide gels and transferred onto nitrocellulose membranes. Individual nitrocellulose strips were reacted with culture supernatants containing mouse MAbs or control human sera. Bound antibodies were visualized by enzyme immunoassay with biotinylated anti-mouse or anti-human antibody and horseradish peroxidase-conjugated avidin.

Syncytial inhibition assay. MOLT4/HIV-2_{ROD} cells (2.5×10^4 cells) were preincubated in 24-well culture plates with MAbs in 0.5 ml of complete medium. After 30 min of incubation at 37°C, 5×10^4 target MOLT4C8 cells were added. MOLT4C8 cells alone and MOLT4/HIV-2_{ROD} cells alone were also included as controls. The plates were cultured at 37°C in a humidified atmosphere of 5% CO_2 in air. The cells were examined under an inverted microscope after 18 h of culture. For the inhibition assay of recombinant soluble (rsCD4)-mediated enhancement of HIV-2 infection, individual culture wells were supplemented with rsCD4 at the indicated concentrations before preincubation was begun.

Neutralization of cell-free HIV infection. Neutralization of cell-free virus infection of MOLT4C8 cells by MAbs was assayed as described previously (28). Briefly, diluted tissue culture supernatant of MOLT4/HIV-2_{ROD} cells (200 50% tissue culture infective doses in 100 μl) was incubated for 1 h at 37°C with MAbs at final concentrations of 10 or 50 μg/ml. Then 5×10^4 MOLT4C8 cells were added to the virus-antibody mixture and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. After 7 days of culture, neutralization of infection was determined by measuring HIV p24 expression by an indirect immunofluorescence assay. The assay was done in duplicate.

Epitope mapping. The epitope-scanning kit was purchased from Chiron Mimotopes Pty Ltd., Clayton, Australia. A series of overlapping peptides were synthesized on each polyethylene rod as specified by the manufacturer. The binding activities of the MAbs to the peptides were assessed by enzyme-linked immunosorbent assay (ELISA). For the epitope mapping of 2H1B and 2F19C, three overlapping peptides were synthesized and used to detect the binding activity by ELISA.

CD4-gp120 binding inhibition assay. rsCD4 was kindly provided by Genentech Inc. (South San Francisco, Calif.). The rsCD4 was conjugated with FITC by the conventional method. The binding activity of FITC-CD4 against HIV-2_{ROD}-infected cells was determined by cell sorter analysis. The concentration that gave 90% of maximum reactivity was used to detect the inhibiting activity of MAbs for the CD4-gp120 binding. As an inhibition-positive control, a MAb to CD4 (Nu-$T_{H/I}$; kindly provided by K. Sagawa, Kurume University, Kurume, Japan) which bound to the similar epitope of Leu3a was used. As a negative control, anti-CD8 MAb (Nu-$T_{S/C}$; kindly provided by K. Sagawa) and anti-HIV-1 MAb (0.5β [28]) were used.

RESULTS

Development and characterization of HIV-2 gp120-specific MAbs. We immunized mice with three different peptides listed in Fig. 1. Two of these peptides (PGN24 and NKI26) were derived from the sequence of HIV-2_{ROD} corresponding to the V3 region of HIV-1 gp120. Peptide TND27, corresponding to the C3 region of HIV-1 gp120, was initially selected because of the similarity in amino acid sequence of the central conserved APGK(R) sequence with the GPGR(K) sequence in the V3 region of HIV-1 gp120. In Western blot assays, antisera against NKI26 and TND27 reacted with HIV-2 gp120 at higher titers than did antisera against PGN24 (data not shown). Neutralizing activity against HIV-2 infection was observed in the presence of these two antisera by fusion inhibition assay (data not shown). On the basis of this observation, we used the two peptides NKI26 and TND27 to immunize mice to obtain the hybridoma lines. Immunization and fusion protocols were as described in Materials and Methods. Culture fluids from growing hybridomas were screened by ELISA for binding activity against the peptides. Supernatants that scored positive were further assayed by immunofluorescent surface staining of virus-producing cells.

Of the several hybridomas tested, we selected three. MAb B2C was raised against peptide NKI26, and MAbs 2F19C and 2H1B were specific for TND27. Figure 2 shows representative sorter profiles obtained with MOLT4/HIV-2_{ROD} cells and uninfected control cells. B2C bound to the surface of HIV-2-infected but not uninfected cells. This reactivity was observed with 71.6% of infected cells. Surface reactivities of the other two MAbs were marginal. However, repeated analysis of cell surface staining showed that 6.0% of infected cells were positive for 2F19C and 3.2% were positive for 2H1B, compared with their uninfected counterparts. Because it was possible that the CD4-binding site of gp120 was masked by complexing with the CD4 molecule on the surface of infected cells, we tested the CD4 expression of these cells. However, the CD4 expression was negative for MOLT4/HIV-2_{ROD} cells (the percent positivities were 0.3% for Leu3a, 0.3% for Nu-$T_{H/I}$, 0.4% for OKT4, and 0.2% for a negative control MAb) compared with the uninfected MOLT4 cells (the percent positivities were 99% for Leu3a, 84.0% for Nu-$T_{H/I}$, 93.1% for OKT4, and 1.5% for a negative control MAb). These findings indicated that the epitope recognized by B2C was exposed on the surface of HIV-2-infected cells whereas those recognized by the other two MAbs were relatively inaccessible from the cell surface.

Recognition of gp120 by the MAb was further shown by the Western blot assay. As shown in Fig. 3, B2C, as well as 2H1B and 2F19C, bound to gp120 of HIV-2_{ROD}. Two nonspecific bands of approximately 55 and 105 kDa were also found on all the Western blots. The nonspecific reactivity of the second reagent (biotin-conjugated anti-mouse or anti-human antibody) or the third reagent (peroxidase-conjugated avidin) with some cellular components may be responsible for these bands.

HIV-2 isolate-specific neutralization by B2C. Antibodies against gp120 have been reported previously to show neutralizing activity against HIV infectivity. Thus, the ability of the MAbs to neutralize virus infection in vitro was assessed. One of the two assays used measured the ability of antibody to block fusion of HIV-2-infected and uninfected cells. MOLT4/HIV-2_{ROD} cells were preincubated with or without MAbs for 30 min, and target MOLT4C8 cells were added. Uninfected MOLT4C8 cells alone and MOLT4/HIV-2_{ROD} alone were also cultured as controls (Fig. 4a and b, respectively). Following the mixing of target MOLT4C8 cells with MOLT4/HIV-2_{ROD} cells in the absence of antibody, numerous syncytia were observed

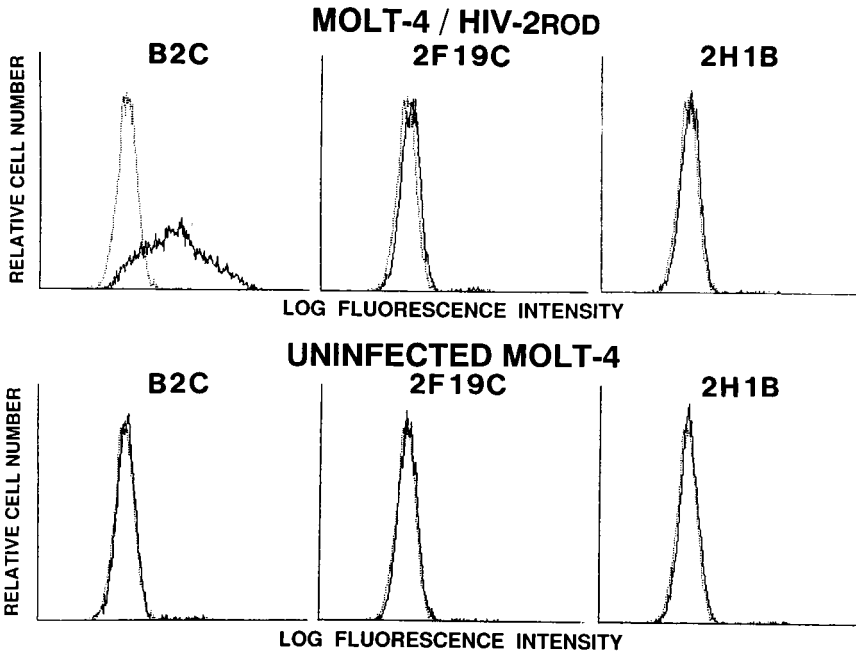

FIG. 2. Representative cell sorter profiles of cells labeled with the MAbs. HIV-2_{ROD}-infected cells and uninfected controls were used to detect the specific surface reactivities of the MAbs. Profiles representing cell populations stained with the indicated MAbs are shown by solid lines, while control populations labelled with control mouse IgG are shown by dotted lines.

after 18 h in culture (Fig. 4c). In contrast, in the presence of B2C, syncytium formation was completely inhibited at an antibody concentration of 50 μg/ml (Fig. 4d). In the presence of 2H1B or 2F19C (Fig. 4e and f, respectively), syncytium formation was partially inhibited. We attempted to inhibit syncytium formation at higher concentrations of these antibodies. However, complete inhibition of syncytium formation was not observed even in the presence of high concentrations (500 μg/ml)

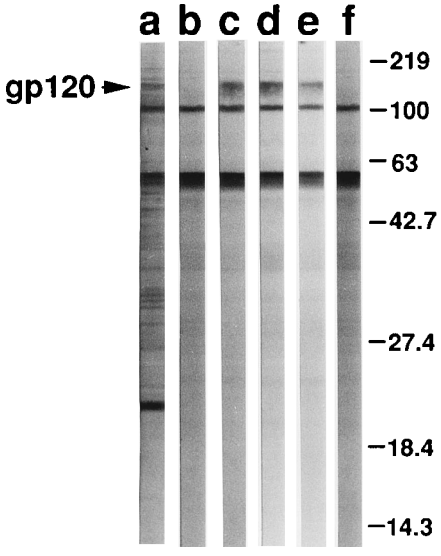

FIG. 3. Western blot analysis for reactivity with HIV-2_{ROD} antigens. Individual strips were reacted with serum from an asymptomatic carrier of HIV-2 (lane a), serum from a normal seronegative donor (lane b), and MAbs B2C (lane c), 2F19C (lane d), 2H1B (lane e), and 0.5β (lane f, as a negative control). MAbs were used at a 1:500 dilution of ascites fluid, and human serum was used at 1:100 dilution. Bound antibodies were visualized by enzyme immunoassay.

of 2H1B or 2F19C (data not shown). Neither control mouse IgG antibody nor MAb 0.5β, which was used as an additional control antibody, had any effect on syncytium formation (Fig. 4g and h, respectively).

The second assay measured neutralization of cell-free virus infection. Diluted culture supernatant of MOLT4/HIV-2_{ROD} cells was incubated with the MAb for 1 h and used to infect MOLT4C8 cells. After 7 days in culture, neutralization of HIV-2 infectivity was determined by measuring virus expression by an indirect immunofluorescence assay with MAb to p24. As shown in Fig. 5, complete neutralization of HIV-2 infection by B2C was observed at a B2C concentration of 50 μg/ml. At a concentration of 10 μg of B2C per ml, partial inhibition of infection was observed. In the presence of 2F19C or 2H1B, on the other hand, partial inhibition of HIV-2 infection was observed at the higher MAb concentration. Percent inhibition of p24-positive cells over the control culture was 41% for 2F19C and 30% for 2H1B. We repeated this experiment with a higher concentration of these MAbs (500 μg/ml). However, complete inhibition of HIV-2 infection was not achieved by 2H1B or 2F19C. This partial inhibition of HIV-2 infection by 2F19C and 2H1B was consistent with that observed in the syncytium induction inhibition assay.

We tested cross-neutralization by B2C against different HIV-2 strains (GH1 and ISY), an SIV isolate (SIV_{mac251}), and HIV-1 isolates (HTLV-IIIB and MN). However, no inhibition of viral infection was observed for the virus tested in the presence of B2C (data not shown). These findings suggest that B2C, which was raised against the V3 domain of HIV-2_{ROD} gp120, has a type-specific neutralizing activity against HIV-2_{ROD}. On the other hand, 2F19C and 2H1B, which were raised against the C3 region of the envelope (TND27), appeared to have weak activity that suppressed the infection of HIV-2 in vitro.

Mapping the epitope. To identify the linear determinant recognized by B2C, Pepscan analysis was performed with 19

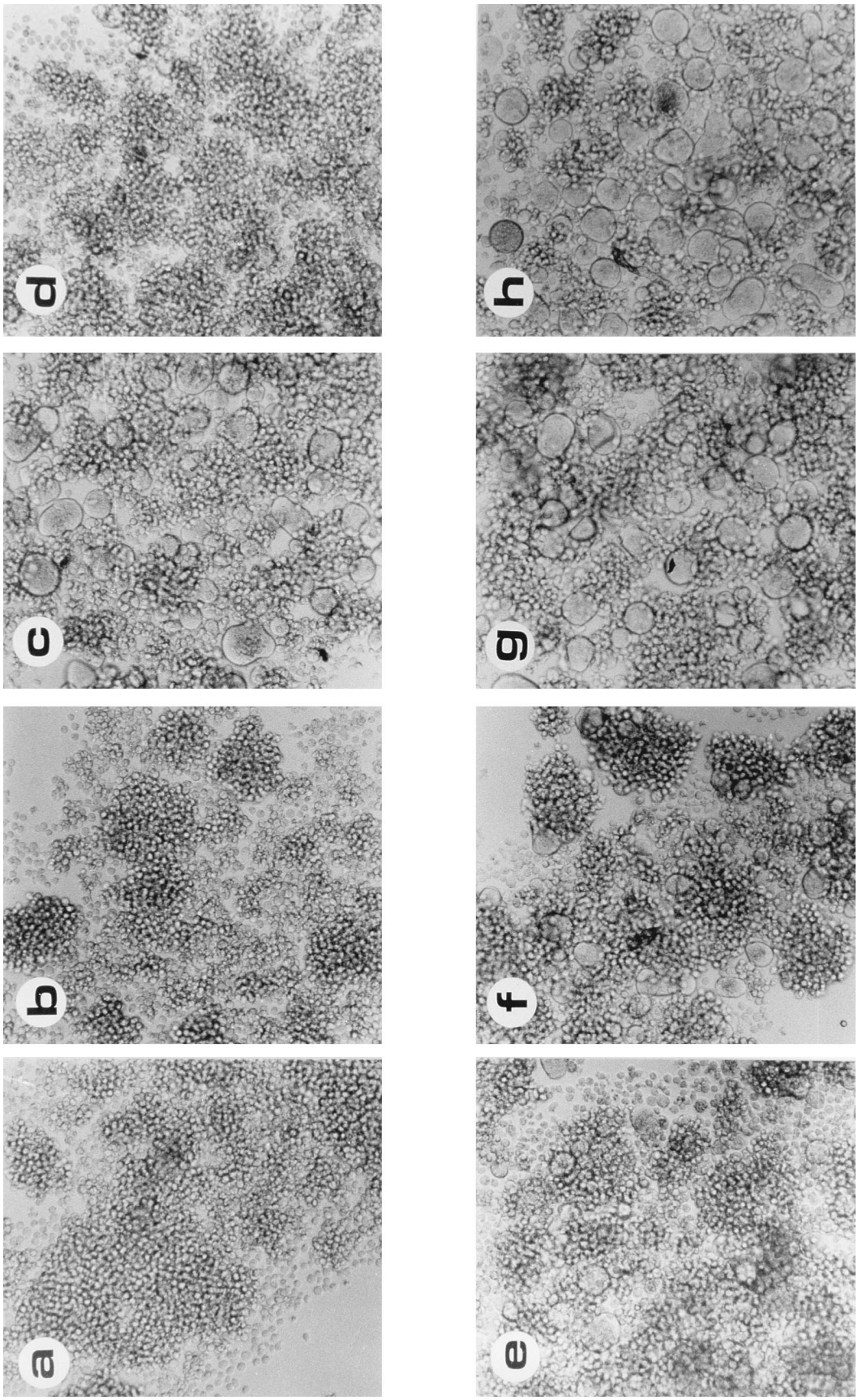

FIG. 4. Inhibition of HIV-2_{ROD}-induced syncytium formation by B2C antibody. Syncytia resulting from preincubation with 50 μg of B2C per ml (d), 50 μg of 2F19C per ml (e), 50 μg of 2H1B per ml (f), 50 μg of control mouse IgG MAb per ml (g), and 50 μg of 0.5β per ml (h) are shown. Control cultures, including MOLT4C8 cells alone (a), MOLT4/HIV-2_{ROD} cells alone (b), and coculture of the MOLT4C8 with MOLT4/HIV-2_{ROD} in the absence of antibody (c) are also shown. The cultures were photographed after 18 h in culture. Magnification, ×372.

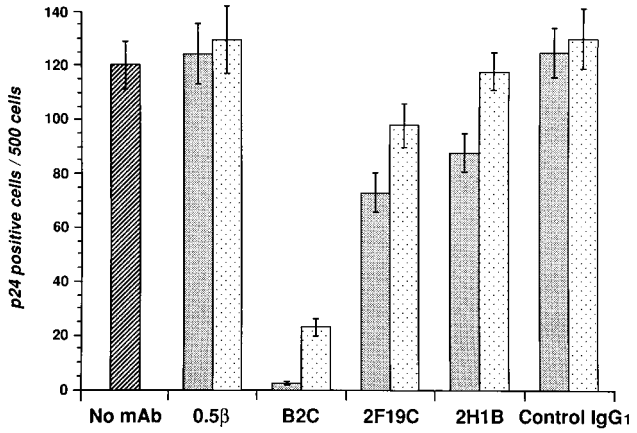

FIG. 5. Neutralization of cell-free virus infection with HIV-2_{ROD} by MAbs. Inhibition of HIV-2 infection by the MAbs was determined by the indirect immunofluorescence assay after 7 days of culture of target MOLT4C8 cells with cell-free HIV-2_{ROD} in the presence of 50 ( ) or 10 ( ) μg of antibodies per ml as indicated in the figure. The target cells were also infected in the absence of MAb ( ). Results are expressed as mean number of HIV-2 p24-positive cells per 500 cells from duplicate determinations. Error bars indicate standard error. More than 500 cells were examined per individual culture.

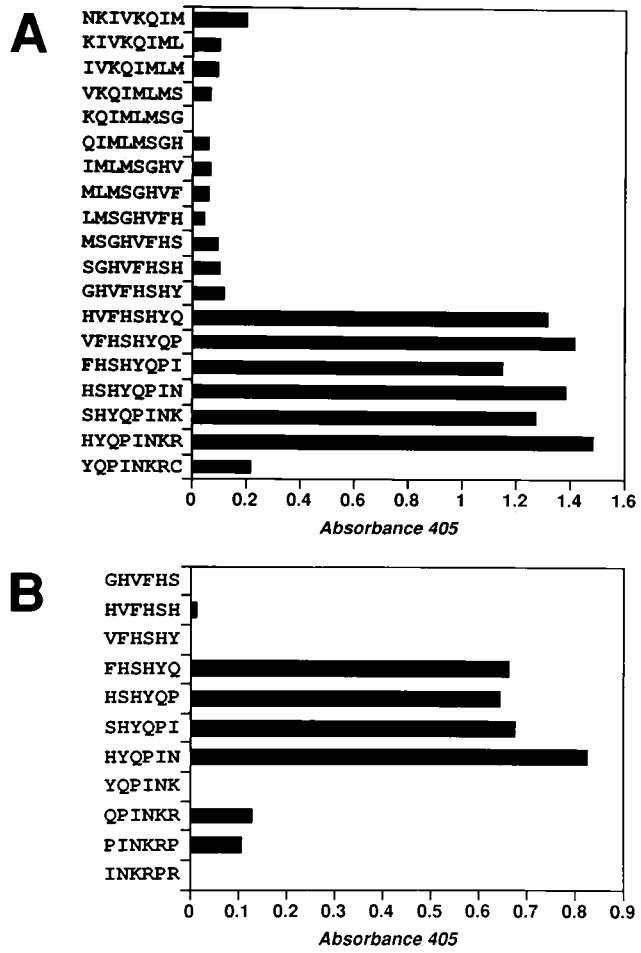

FIG. 6. Mapping of an epitope recognized by B2C by Pepscan analysis. Two sets of synthesized pin peptides were reacted with 1 μg of B2C or a control antibody (0.5β) per ml. Results are shown by subtracting the background absorbance of control antibody binding to each pin peptide from the absorbance of B2C binding. (A) A series of octamer peptides spanning the NKI26 peptide were used to detect the B2C epitope. (B) A series of hexamer peptides spanning the C-terminal part of NKI26 were used to detect the B2C epitope.

octamer peptides, each staggered by one amino acid over the length of NKI25. Reactivity of the B2C MAb with each peptide was determined by ELISA (Fig. 6A). B2C bound to all octamer peptides that contained the His-Tyr-Gln sequence. We next tried to determine the reactivity of the MAb in hexamer peptides (Fig. 6B), and again, B2C bound to peptides which contained the His-Tyr-Gln sequence. These results confirmed that the core of the neutralizing epitope recognized by B2C mAb was His-Tyr-Gln.

The binding sites of 2H1B and 2F19C were determined with three overlapping synthetic peptides. Binding of the antibodies was determined by ELISA. As shown in Fig. 7, 2H1B bound to TND12 and RNI15 peptides, suggesting that the epitope recognized by 2H1B was located at the overlapping region of the two peptides (amino acid sequence RNISFKA). On the other hand, 2F19C bound to RNI15 but not to TND12 or GEG11, suggesting that the epitope was located at around the amino acid sequence APGK.

Effects of the MAbs against CD4 binding to gp120 and rsCD4-mediated enhancement of HIV-2 infection. Although an exception was reported for one strain, the CD4 molecule acts as the primary cell attachment receptor for most HIV2 strains (10, 24, 36), and in HIV-1 gp120, certain amino acids in the C3 region are considered important for the CD4-gp120 interaction (30). Furthermore, a close spatial relationship between the V3 and C4 CD4-binding domain of HIV-1 gp120 was reported (29, 38). To elucidate the effects of these MAbs against CD4-gp120 binding, we incubated FITC-labeled rsCD4 (FITC-CD4) with HIV-2_{ROD}-infected cells in the presence or absence of the MAbs. As shown in Fig. 8, in the absence of antibody, FITC-CD4 bound to the surface of MOLT4/HIV-2_{ROD} cells at a frequency of 39%. In the presence of anti-CD4 MAb (Nu-$T_{H/I}$; positive control), however, complete inhibition of the binding of FITC-CD4 was observed. No inhibition of CD4-gp120 binding was observed in the presence of anti-C3 MAbs (2HIB and 2F19C) or control MAbs (anti-CD8 MAb and 0.5β). In contrast, in the presence of anti-V3 MAb (B2C) partial inhibition of the binding was observed (29.4%). Although the inhibition was marginal ($P = 0.059$), we repeatedly observed this partial inhibition of CD4-gp120 interaction by B2C. The mechanism of this partial inhibition was not clear. However, because of the close spatial relationship between V3 and C4 of HIV-2 gp120, it is possible that binding of B2C to V3 induces a conformational change in the CD4-binding domain of HIV-2.

Enhancement of HIV-2 and SIV infection by rsCD4 has been reported previously for CD4-positive (37) and for CD4-negative (6) cells. We examined the neutralizing activity of B2C under conditions of rsCD4-mediated enhancement of HIV-2 infection. Mixtures of HIV-2-infected and uninfected cells were cultured in the presence or absence of rsCD4. As

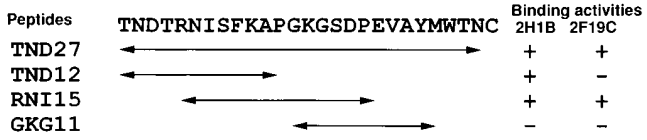

FIG. 7. Epitope mapping of 2F19C and 2H1B with three overlapping synthetic peptides. Binding activities of the antibodies to each peptide were determined by an ELISA as described previously (27).

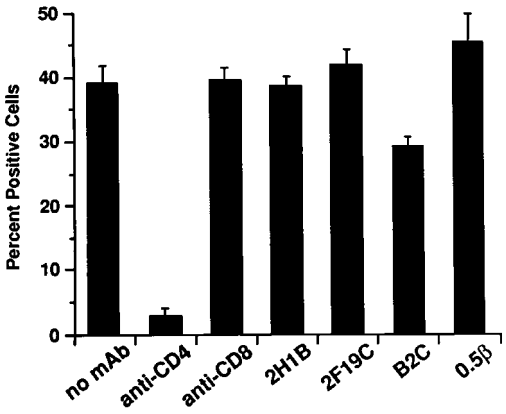

FIG. 8. Inhibition of rsCD-gp120 binding by MAbs. The assay was performed with FITC-CD4. Briefly, MOLT4/HIV-2_{ROD} cells were incubated with the optimum (1:25) concentration of FITC-CD4 in the presence or absence of various competing MAbs (at 50 μg/ml). Bound FITC-CD4 was detected by fluorescence-activated cell sorter analysis. Anti-CD4 MAb (Nu-$T_{H/I}$) was used as an inhibition-positive control. Anti-CD8 MAb (Nu-$T_{S/C}$) and 0.5β (anti-HIV-1 MAb) were used as negative controls. Results are expressed as the mean number of FITC-CD4-positive cells from duplicate determinations; error bars denote standard error.

shown in Fig. 9, following the mixing of MOLT4C8 cells with MOLT4/HIV-2_{ROD} cells in the presence of control MAb (0.5β), numerous syncytia were observed (Fig. 9a). On the other hand, cell mixtures containing rsCD4 at 1 and 10 μg/ml showed an increase in syncytium formation with respect to both the size and the number of giant cells (Fig. 9b and c). In contrast, in the presence of B2C antibody, syncytium formation was significantly inhibited with or without rsCD4-mediated enhancement of HIV-2 infection (Fig. 9d to f).

DISCUSSION

We report here the production and characterization of the MAbs against the V3 or C3 region of HIV-2_{ROD}. The immunological and biological properties of these MAbs are summarized in Table 1.

An analysis of evolutionary relationships suggests that with regard to the *env* gene, the HIV-2 group of viruses are more closely related to the SIV group than to the HIV-1 group (16). Also, in contrast to the infrequent cross-neutralization of HIV-1 by HIV-2- or SIV-positive sera, extensive cross-neutralization of HIV-2 or SIV_{MAC} and SIV_{AGM} has been reported (34). Thus, HIV-2 is considered to be more closely related both immunologically and genetically to SIV than to HIV-1.

The V3 region of the external envelope protein gp120 of HIV-1 has been associated with viral neutralization. On the other hand, antisera against V3 peptides, as well as several MAbs to the SIV V3 region, failed to neutralize SIV infection

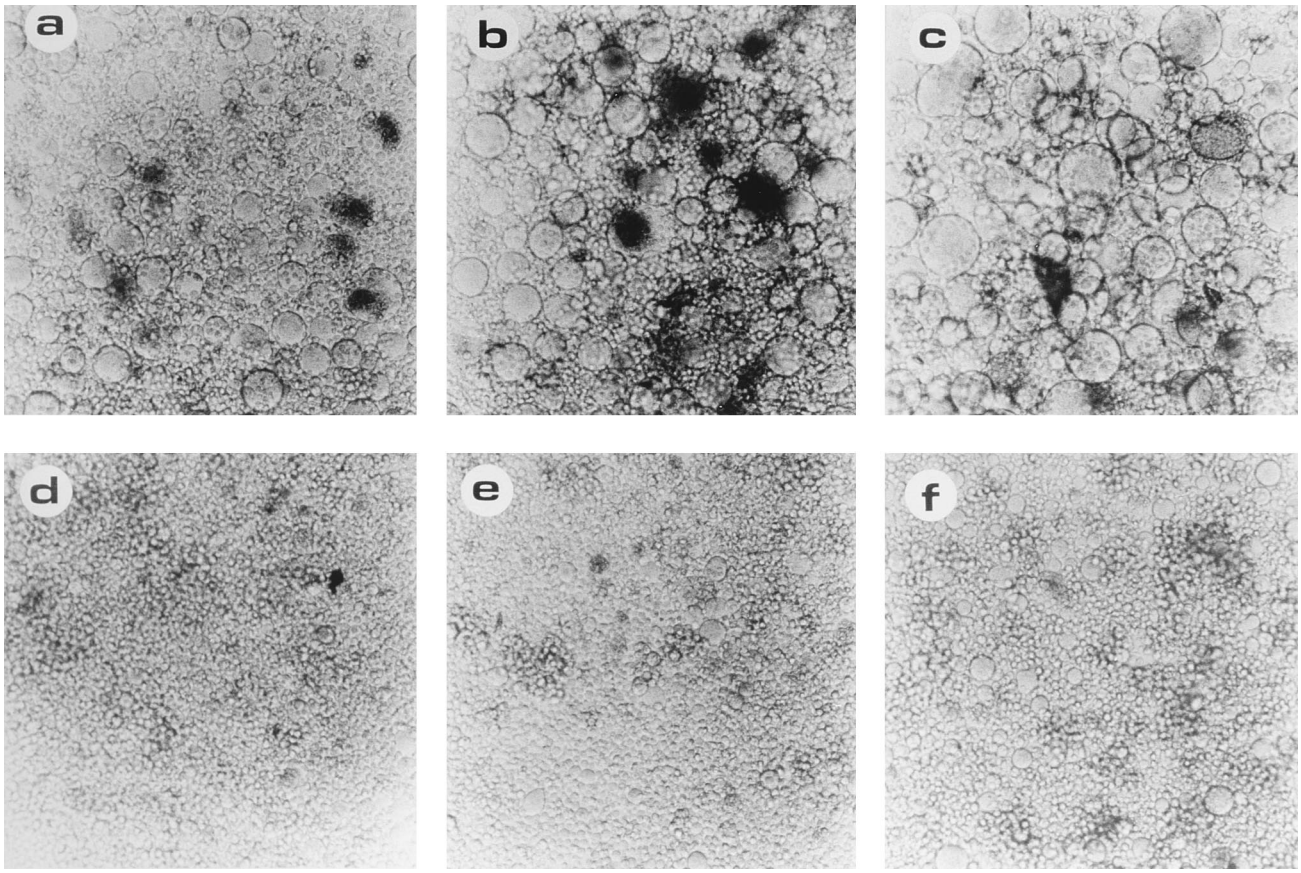

FIG. 9. Inhibition of rsCD4-mediated enhancement of syncytium formation by B2C. MOLT4/HIV-2_{ROD} cells (2.5×10^4) were preincubated with 50 μg of B2C (d to f) or control MAb (0.5β) (a to c) per ml in the presence (1 μg/ml [b and e] or 10 μg/ml [c and f]) or absence (a and d) of rsCD4. After 30 min of incubation, MOLT4C8 cells (5×10^4) were added to each culture well. Cultures were photographed after 18 h. Magnification, ×340.

TABLE 1. Properties of MAbs to the gp120 of HIV-2_{ROD}

MAb	Immunogen	Core epitope	% Surface staining of cells infected with HIV-2_{ROD}	Western blot reactivity	Neutralization of:		CD4-gp120 binding inhibition
					Cell-free virus infection[a]	Syncytium formation[b]	
B2C	NKI26 (V3)	-HYQ-	71.6	gp120	50 μg/ml	+	±[c]
2F19C	TND27 (C3)	-APGK-	6.0	gp120	NA	−	−
2H1B	TND27 (C3)	-RNISFKA-	3.2	gp120	NA	−	−

[a] Antibody concentration that gave more than 90% inhibition of cell-free HIV-2_{ROD} infection. NA, 90% neutralization was not achieved at an antibody concentration of 500 μg/ml.
[b] Complete inhibition of syncytium formation at an antibody concentration of 50 μg/ml.
[c] Although the statistical significance was marginal, partial inhibition of CD4-gp120 binding was observed.

(21, 23). Recently, Javaherian et al. reported that the V3 region of SIV did not form a linear neutralizing epitope but consisted of part of a conformation-dependent neutralizing epitope (20). For HIV-2, however, Björling et al., using several synthetic peptides, reported the existence of a linear neutralizing epitope in the V3 region of gp120 (gp125) (1). Very recently, they developed MAbs against the SBL6669 isolate of HIV-2 and determined two sites which are important for the binding of neutralizing antibodies (2).

In this study, we developed a neutralizing MAb, designated B2C, against the V3 region of HIV-2_{ROD}. This confirms the observation by Björling et al. that the V3 region of HIV-2 contains a linear neutralizing epitope (1). Furthermore, functional characterization showed that the MAb could inhibit HIV-2-induced cell fusion as well as cell-free virus infection in an isolate-specific fashion. This syncytium-inhibiting activity and type specificity of the neutralization of B2C is analogous to that of anti-V3 MAb(s) of HIV-1 (28). The V3 region of HIV-1 has been postulated to interact with cellular protease(s) or galactosylceramide, which are involved in viral entry (8, 9). The analogous characteristics of the neutralizing MAb against the V3 region of HIV-1 and HIV-2 suggest that the HIV-2 V3 may have a similar function in the infection process. This is consistent with the observations by Freed and Myers, using chimeric proteins, that the V3 region of HIV-2 played an important role in a syncytium formation (15). The epitope recognized by B2C was mapped to a 6-amino-acid segment by Pepscan analysis and had the core sequence His-Tyr-Gln. This epitope partially overlaps with the corresponding neutralizing epitope of SBL6669 reported by Björling et al. (2).

Among the neutralizing epitopes recognized by anti-peptide antibodies described by Björling et al., a peptide designated A15-80 contained a sequence corresponding to residues 489 to 509 of the SBL6669 isolate of HIV-2 (2). This peptide contained the corresponding epitope of a ROD isolate recognized by 2H1B and 2F19C. The immunogenicity and the weak neutralizing activity of the MAb against this region were consistent with the observation of Björling et al. (2). Since the amino acid residues in C3, especially 368D (Asp) and 370E (Glu), were considered to be important for the binding of HIV-1 gp120 to CD4 (30), we analyzed the CD4-gp120 interaction in the presence of these MAbs. Unexpectedly, anti-V3 (B2C) but not anti-C3 (2H1B and 2F19C) MAbs partially inhibited the CD4-gp120 interaction. This does not exclude the importance of the C3 region in CD4-gp120 binding. However, as reported for HIV-1 (29), it is possible that inhibition of CD4-gp120 binding was not observed because the C3 region of HIV-2 gp120 was poorly accessible from the surface of infected cells (as shown in Fig. 2).

For HIV-1, conformational interaction of the V3 region and C4 domain was reported (29, 38). Binding of B2C to the V3 region of HIV-2_{ROD} may alter the conformation of the CD4-binding site(s), resulting in reduction of the binding of CD4-gp120. This hypothesis was supported the observation of partial inhibition of the CD4-gp120 interaction at the same antibody concentration which showed complete suppression of HIV-2 infection. This also suggests the involvement of an alternative mechanism(s) other than gp120-CD4-binding inhibition for the neutralization of HIV-2 by B2C.

Enhancement of HIV-2 infection by rsCD4 has been reported previously (6, 37). In this study, we demonstrated that the anti-V3 MAb B2C could inhibit HIV-2-induced cell fusion under conditions of rsCD4-mediated enhancement. The mechanism(s) of this enhancement still remains to be elucidated. However, according to the hypothesis that the HIV-2 strain binds to an alternative cell surface receptor but proceeds to fuse with the cell membrane only after treatment with rsCD4 (6), this result implies that the B2C antibody may inhibit interaction of the V3 region of HIV-2 with the target molecule(s) on the target cell surface.

The present data, which were obtained with a limited number of the HIV-2 strains, show that an isolate-specific neutralizing activity was observed for the anti-V3 MAb (B2C). However, unlike antibodies raised against a peptide, anti-V3 MAb made against intact gp120 may have additional conformational aspects, which would give it wider specificity. Further investigation to elucidate this possibility would be important for studying HIV-2 vaccine models.

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