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Enhancement of Human Immunodeficiency Virus Type 1 Envelope-Mediated Fusion by a CD4-gp120 Complex-Specific Monoclonal Antibody

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The entry of human immunodeficiency virus type 1 (HIV-1) into cells is initiated by binding of the viral glycoprotein gp120-gp41 to its cellular receptor CD4. The gp120-CD4 complex formed at the cell surface undergoes conformational changes that may allow its association with an additional membrane component(s) and the eventual formation of the fusion complex. These conformational rearrangements are accompanied by immunological changes manifested by altered reactivity with monoclonal antibodies specific for the individual components and presentation of new epitopes unique to the postbinding complex. In order to analyze the structure and function of the gp120-CD4 complex, monoclonal antibodies were generated from splenocytes of BALB/c mice immunized with soluble CD4-gp120 (IIIB) molecules (J. M. Gershoni, G. Denisova, D. Raviv, N. I. Smorodinsky, and D. Buyaner, FASEB J. 7:1185–1187 1993). One of those monoclonal antibodies, CG10, was found to be strictly complex specific. Here we demonstrate that this monoclonal antibody can significantly enhance the fusion of $CD4^+$ cells with effector cells expressing multiple HIV-1 envelopes. Both T-cell-line-tropic and macrophage-tropic envelope-mediated cell fusion were enhanced, albeit at different optimal doses. Furthermore, infection of HeLa $CD4^+$ (MAGI) cells by HIV-1 LAI, ELI1, and ELI2 strains was increased two- to fourfold in the presence of CG10 monoclonal antibodies, suggesting an effect on viral entry. These findings indicate the existence of a novel, conserved CD4-gp120 intermediate structure that plays an important role in HIV-1 cell fusion.

The binding of the human immunodeficiency virus type 1 (HIV-1) envelope (gp120-gp41) to its cellular receptor (CD4) induces conformational changes in the viral and cellular proteins. This is followed by a cascade of postbinding events (including recruitment of the newly discovered coreceptors [1, 7, 8, 10, 12, 14, 23]) leading to formation of the fusion complex at the cell membrane (30, 33). Monoclonal antibodies (MAbs) against either the HIV-1 envelope or CD4 were found to be useful tools in the dissection of the HIV-1 fusion process. Some antibodies were found to block the initial binding event, while others inhibit the fusion process by blocking postbinding events. The latter class of MAbs bind to either linear or nonlinear epitopes in gp120-gp41 or in CD4 that map outside of the direct binding regions (16, 20, 25–27, 32, 34, 37–39, 42). In an attempt to generate more effective blocking antibodies, several groups have used the CD4-gp120 complex as the immunogen in mice (6, 9, 17, 21). Some of the MAbs derived from the splenocytes of immunized mice were found to be complex dependent, while others could recognize gp120 or CD4 separately but bound to the complex with higher avidity (9, 17, 21, 38). The biological activities of such MAbs seemed to vary. Furthermore, several groups have identified antibodies with complex-specific or complex-enhanced binding that have neutralizing activities. The breadth and efficiency of neutralization compared with non-complex-specific MAbs have not been rigorously studied. The MAb CG10 was generated from the spleen of a BALB/c mouse immunized with soluble CD4 (sCD4)-gp120 (IIIB) complex. This MAb was found to be strictly complex specific (17).

In the present study, we tested the activity of CG10 in a variety of viral fusion and viral infectivity assays. It was found that CG10 can have a potent enhancing activity on HIV-1-mediated fusion of both T-cell-line-tropic (T-tropic) and macrophage-tropic (M-tropic) envelopes. It also can enhance viral entry into HeLa-CD4 (MAGI) cells. Thus, CG10 may stabilize a postbinding configuration of the gp120-CD4 complex necessary for HIV-1 cell entry.

MATERIALS AND METHODS

MAbs and envelope-expressing recombinant vaccinia viruses. The CG10 and CG4 MAbs used in the study were produced in the laboratory of Jonathan Gershoni as previously described (17). Briefly, a BALB/c mouse was immunized with sCD4-gp120 (IIIB) complex, and its splenocytes were fused with NS-0 myeloma cells. The hybridoma supernatants were screened for their ability to bind to the CD4-gp120 complex or to sCD4 or gp120 alone. CG10 MAb was found to be strictly complex specific (17). CG4 MAb binds directly to gp120, and its binding is not affected by association of gp120 with sCD4 (17). The 48d MAb was produced in the laboratory of James Robinson (University of Connecticut, Farmington). The binding of this MAb to a complex conformation-sensitive epitope on gp120 was shown to increase after its association with CD4 (36, 38). The recombinant vaccinia virus vectors vCB28, vCB39, vCB43, and vPE16 expressing HIV-1 envelope from the M-tropic strains JR-FL, ADA, and Ba-L (5) and the T-tropic strain IIIB (13), respectively, were used. MIP-1α and MIP-1β

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(R@D) were obtained from the NIH AIDS Research and Reference Reagent Program (Rockville, Md.).

Flow cytometry measurements of CG10 binding to CD4-gp120 complexes on cells expressing T-tropic or M-tropic envelopes. HeLa cells were infected overnight with the vaccinia virus recombinants described above at 10 PFU/cell. Infected cells were incubated with sCD4 (10 μg/ml; Intracel, Cambridge, Mass.) for 30 min at 37°C. CG10 MAb was added at four concentrations for 2 h at 4°C followed by the addition of fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse antibodies. Stained cells were analyzed by the Epic Profile (Coulter Counter, Hialeah, Fla.). The mean fluorescence channels were converted to mean fluorescence units by using standard curves generated by fluorescent beads with increasing intensities (Flow Cytometry Standards, Research Triangle Park, N.C.). The experimental data were fitted to the Langmuir adsorption isotherm: relative fluorescence = (experimental fluorescence − background fluorescence)/maximal fluorescence. Relative fluorescence was calculated as $ac/(b+c)$, where a is a proportionality constant, b is the equilibrium dissociation constant, and c is the concentration of CG10 used. The data were fitted by the least squares method by using the Scientist program (MicroMath, Salt Lake City, Utah).

In another experiment, CEM ($CD4^+$) cells were preincubated with (or without) soluble gp120 (10 μg/ml; Intracel, Seattle, Wash.) for 30 min at 37°C followed by incubation with CG10 MAb or CG10 Fab fragments at a concentration of 10 μg/ml for 1 h at 4°C. All treated cells were then washed and incubated for an additional 1 h at 4°C with FITC-conjugated goat anti-mouse (Fc specific) antibodies or FITC-conjugated goat anti-mouse immunoglobulin G (IgG) (Fab) (Sigma, St. Louis, Mo.) in phosphate-buffered saline containing 1% bovine serum albumin and 0.1% sodium azide. Binding constants of the intact CG10 or its Fab fragments were calculated as described above.

Syncytia assays. The syncytia assays were described previously (19). A $CD4^-$ cell line, 12E1, was infected with gp120-gp41-expressing recombinant vaccinia viruses at 10 PFU/cell overnight. Envelope-expressing 12E1 cells were mixed (1:1) with either CEM (expressing full-length CD4), A2.01.CD4.401 (expressing tailless CD4) (3), or PM1 (a derivative of the Hut78 cell line which is susceptible to infection by both T-tropic and M-tropic HIV-1 strains [24]) cells. HIV-1 chronically infected H9 cells (IIIB, MN, or RF) were mixed at a 1:3 ratio with CEM cells. The various MAbs were added at different concentrations to the cell mixtures at the initiation of coculturing. The numbers of syncytia were scored at various times after cocultures were initiated. All groups were plated at two to three replicates, and all experiments were repeated at least three times.

Viruses and plasmid DNA. Infectious molecular clones of HIV-1 LAI (pLAI) and HIV-1 ELI (pELI1 and pELI2) have been described previously (15, 28, 29). Viral stocks were prepared from molecular clones of LAI, ELI1, and ELI2 as previously described (28). 293 cells were transfected with 5 μg of plasmid DNA by the calcium phosphate coprecipitation method, and the amount of virus produced was determined by a reverse transcriptase assay.

MAGI cell single-round infectivity assay. To study the effect of CG10 MAb on the early stages of viral infection, we used the HeLa–CD4–LTR–β-Gal cell line termed MAGI (multinuclear activation of a galactosidase indicator [22]). This cell line requires only a single round of replication to detect infectious HIV-1. It exploits the ability of HIV Tat protein to transactivate the *Escherichia coli lacZ* gene (coding for β-galactosidase) driven by the HIV-1 long terminal repeat promoter (22). The MAGI cells were plated in 24-well plates at 3×10^4 to 4×10^4 per well in duplicate. When 30 to 50% confluence was reached, the cells were infected with different T-tropic viruses (LAI, ELI1, and ELI2 [15, 28]), at different multiplicities of infection (MOI) and in the absence or presence of CG10 (or control CG4) MAb. The number of infected cells (blue-stained nuclei) were scored 48 h after infection as previously described (22).

Preparation of CG10 Fab fragments. Purified CG10 (100 μg/ml) in TBS (25 mM Tris-HCl [pH 7.4], 150 mM NaCl) containing 1 mM EDTA and 10 mM L-cysteine was incubated with papain (50 μg/ml; Sigma) for 8 h at 37°C. The reaction was terminated with 0.1 M iodoacetamide for 30 min at room temperature and dialyzed overnight in phosphate-buffered saline. To remove residual intact CG10 molecules, the reaction mixture was passed through a Centricon concentrator (Amicon Inc., Beverly, Mass.), and the molecules were adsorbed with protein-G-coupled Sepharose beads (Sigma) for 3 h at 37°C. By using flow cytometry, it was determined that the CG10-Fab preparation contained no contaminating whole molecules. Quantitative mathematical analysis of the binding isotherms of the intact antibodies and the Fab fragments demonstrated that the intact molecules bound with only slightly higher (20%) affinity than the Fab fragments.

RESULTS

CG10 MAb binds to multiple gp120-CD4 complexes. The CG10 MAb was derived from splenocytes of a BALB/c mouse immunized with CD4-gp120 (IIIB) complex. This MAb was shown to be strictly complex specific in that it did not bind to either CD4 or gp120 alone but only to the complex (17). The binding specificity of CG10 was also demonstrated by flow cytometry of envelope-expressing cells incubated with sCD4. Importantly, CG10 bound to cells expressing either T-tropic or M-tropic envelopes (after addition of sCD4) with very similar avidities (Fig. 1), even though it was originally derived from a mouse immunized with the IIIB (T-tropic) envelope complex to sCD4.

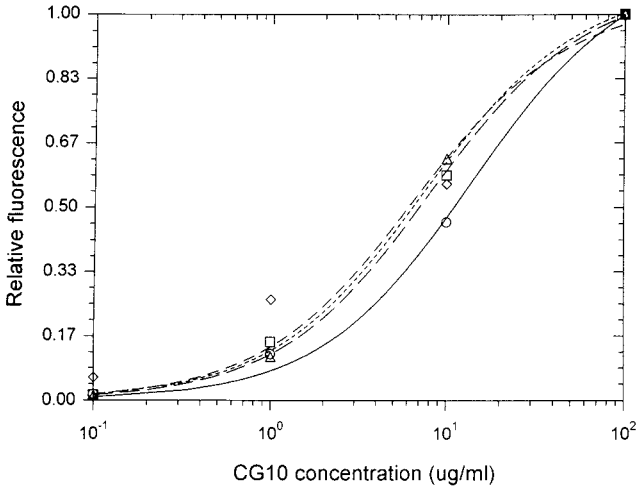

FIG. 1. Binding of CG10 MAb to sCD4-treated cells expressing T-tropic and M-tropic HIV-1 envelopes. HeLa cells were infected overnight with the gp160-expressing recombinant vaccinia viruses vPE16 (IIIB) (□), vCB28 (JR-FL) (○), vCB39 (ADA) (◇), and vCB43 (Ba-L) (△) at 10 PFU/cell. Infected cells were pretreated with sCD4 (10 μg/ml, 30 min, 37°C). Cells were then incubated with the indicated concentrations of CG10 MAb, followed by staining with FITC-conjugated goat anti-mouse IgG antibodies. Control cells were not pretreated with sCD4. No binding of CG10 to these cells was measured (data not shown). The experimental data were fitted to the Langmuir adsorption isotherm (see Materials and Methods).

CG10 MAb enhances HIV-1 envelope-mediated cell fusion. Because HIV-1 envelope-mediated cell fusion is a multistep process that involves conformational changes in both the cell proteins and the viral proteins, antibodies that recognize the postbinding gp120-CD4 complex could potentially have either an inhibitory (6, 38) or an augmenting effect on the fusion process.

The activity of CG10 MAb was tested initially with 12E1-vPE16-infected effector cells in cocultures with CEM cells which express full-length CD4 molecules. Four concentrations of CG10 MAb (0.01, 0.1, 1, and 10 μg/ml) were added at the time of effector/target cell mixing. The numbers of syncytia formed were determined over 6 h. An enhancement of fusion capacity was observed in the presence of CG10. The facilitating effect was most pronounced at the early time points (90 to 120 min) (Fig. 2), suggesting that CG10 can accelerate the kinetics of fusion. Interestingly, the enhancement seen at high concentrations of CG10 (≥10 μg/ml) was less pronounced than that seen with lower concentrations (≤1 μg/ml). In multiple experiments, significant enhancement was seen in the presence of subsaturating concentrations (0.01 and even 0.001 μg/ml) of the antibody (Fig. 2 and data not shown). The fusion-enhancing effect was not seen with CG4, a MAb specific for gp120 (Table 1). Another MAb, 48d, previously shown to prefer gp120-CD4 complex over gp120 alone, did not demonstrate any enhancing activity. In fact, this MAb had an inhibitory activity on syncytium formation between 12E1-vPE16-infected effectors and A2.01.CD4.401 targets (and to a lesser degree CEM targets) (Table 1). These findings are in agreement with previous reports on the potential of 48d to block infection of certain clones of HIV-1 IIIB (36, 38). The fusion-enhancing effect of CG10 was not restricted to CEM and CEM-derived

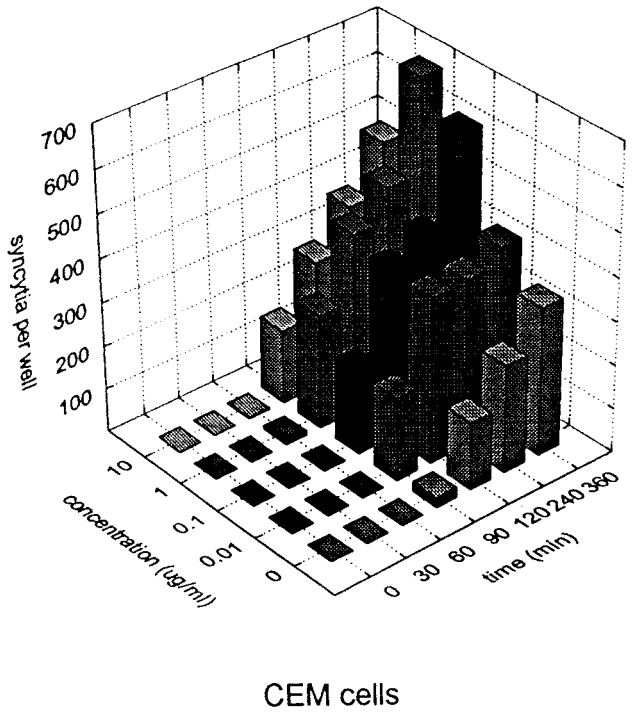

FIG. 2. CG10 MAb enhances syncytium formation by $CD4^+$ cells. 12E1 ($CD4^-$) cells were infected overnight with gp160-expressing recombinant vaccinia virus vPE16 (10 PFU/cell). These effector cells were mixed at 1:1 ratio with CEM ($CD4^+$ cells) in the absence or presence of CG10 MAb at increasing concentrations. All groups were plated in duplicates. Syncytia were scored at 30-min intervals over a total period of 6 h.

cell lines. It was also observed with other $CD4^+$ cell lines, such as H9 and HeLa-CD4 transfectants (data not shown).

CG10 can enhance the fusion of multiple HIV-1 T-tropic envelopes. In order to determine if the enhancing effect of CG10 was restricted to the HIV-1 LAI strain, the MAb was added to cocultures of CEM with H9 cells chronically infected with IIIB, MN, or RF. As can be seen in Table 2, similar levels of enhancement (2.0- to 2.7-fold) were observed for all of the three different chronically infected cells at CG10 concentrations of 0.1 to 1.0 μg/ml. Thus, the enhancing activity of CG10 on the fusion of HIV-1 chronically infected cells is not restricted to the LAI envelope.

CG10 can enhance fusion of M-tropic HIV-1 envelopes. To determine whether the fusion-enhancing activity of CG10 is restricted to T-tropic strains or includes M-tropic envelopes, a panel of vaccinia virus vectors expressing envelopes derived from the M-tropic strains JR-FL (vCB28), ADA (vCB39), and Ba-L (vCB43) (5) was used to infect 12E1 ($CD4^-$) as effector cells. The M-tropic envelope-expressing effectors were mixed with the PM1 cell line, a derivative of HUT78 cells previously shown to support replication of both T-tropic and M-tropic HIV-1 strains (24). It was found that CG10 had a pronounced augmenting activity on syncytium formation with all three M-tropic envelope-expressing cells (Table 3). However, the dose required for maximal enhancement was 10 μg/ml, which is 10- to 100-fold higher than the dose required for optimal enhancement of T-tropic envelope-mediated fusion. This shift in dose response was not a property of the PM1 cells, because in the same experiment, fusion between PM1 and 12E1-vPE16 (IIIB envelope [env]) effectors was enhanced by CG10 at a dose range of 0.1 to 1 μg/ml. Since CG10 was derived from the spleen of a BALB/c mouse immunized with a gp120 (IIIB)-sCD4 complex, it is possible that it associates with M-tropic envelopes bound to CD4 with lower affinities. However, this is an unlikely explanation, since CG10 bound to HeLa cells infected with recombinant vaccinia viruses expressing T-tropic or M-tropic envelopes with very similar binding curves (Fig. 1). The calculated equilibrium dissociation constants were 60, 103, 47, and 53 nM for IIIB, JR-FL, ADA, and Ba-L, respectively, suggesting that CG10 binds with similar affinities to all four surface-expressed envelopes after addition of sCD4 under saturating conditions. We also tested the ability of CG10 to in-

TABLE 1. CG10 but not 48d MAb enhances HIV-1 envelope-cell fusion

Effector	Target	MAb concn (μg/ml)	No. of syncytia [±SD (% control)]
12E1-vPE16	CEM		205 ± 43 (100)
		CG10	
		10	371 ± 60 (180)
		1	512 ± 25 (250)
		0.1	445 ± 30 (220)
		48d	
		10	148 ± 65 (70)
		1	215 ± 4 (100)
		0.1	257 ± 16 (125)
		CG4	
		10	230 ± 10 (112)
		1	249 ± 20 (121)
		0.1	262 ± 26 (127)
12E1-vPE16	A2.01.CD4.401		142 ± 8 (100)
		CG10	
		10	160 ± 5 (110)
		1	368 ± 39 (260)
		0.1	342 ± 12 (240)
		48d	
		10	59 ± 1 (40)
		1	70 ± 16 (50)
		0.1	99 ± 11 (70)
		CG4	
		10	140 ± 8 (100)
		1	134 ± 1 (94)
		0.1	139 ± 9 (98)

TABLE 2. CG10 MAb increases syncytium formation of several HIV-1 laboratory strains[a]

Effector (HIV-1 strain)	Target	CG10 concn (μg/ml)	No. of syncytia [±SD (% control)]
H9 (IIIB)	CEM	0	251 ± 24 (100)
		10	252 ± 33 (100)
		1	495 ± 8 (200)
		0.1	362 ± 23 (150)
		0.01	280 ± 25 (110)
H9 (MN)	CEM	0	176 ± 16 (100)
		10	349 ± 6 (200)
		1	419 ± 37 (240)
		0.1	281 ± 34 (160)
		0.01	229 ± 38 (130)
H9 (RF)	CEM	0	318 ± 35 (100)
		10	497 ± 9 (160)
		1	618 ± 40 (190)
		0.1	863 ± 94 (270)
		0.01	598 ± 31 (180)

[a] Chronically infected H9 cells (2.5×10^4/well) were cultured with CEM ($CD4^+$) cells (7.5×10^4/well) in the absence or presence of CG10 MAb in triplicate wells. Syncytia were scored after 2.5 h.

TABLE 3. CG10 MAb enhances fusion of PM1 target cells and elutriated human monocytes with effector cells expressing M-tropic HIV-1 envelopes[a]

Effector (HIV-1 strain)	Target	No. of syncytia [±SD (% control)] in presence of CG10 MAb concn (μg/ml):			
		None	10	1	0.1
12E1-vPE16 (IIIB)	PM1	303 ± 20 (100)	410 ± 46 (140)	501 ± 47 (170)	685 ± 7 (230)
12E1-vCB28 (JR-FL)	PM1	142 ± 16 (100)	286 ± 30 (200)	151 ± 20 (100)	131 ± 3 (92)
12E1-vCB43 (Ba-L)	PM1	18 ± 2 (100)	86 ± 1 (470)	70 ± 8 (380)	23 ± 3 (130)
12E1-vCB39 (ADA)	PM1	137 ± 38 (100)	445 ± 31 (320)	238 ± 3 (170)	189 ± 20 (140)
12E1-vCB39 (ADA)	Monocytes	42 ± 3 (100)	102 ± 9 (240)	55 ± 10 (130)	45 ± 5 (107)

[a] 12E1 cells were infected with different recombinant vaccinia vectors overnight. They were mixed with either PM1 cells or elutriated monocytes at a 1:1 ratio in the absence or presence of CG10 MAb. Syncytia were scored either at 6 h (PM1 targets) or at 18 h (monocytes).

crease the fusion of elutriated human monocytes with 12E1-vCB39 (ADA env). Again, a significant enhancement in the number of syncytia formed was seen with CG10 at a concentration of 10 μg/ml. Together these experiments suggest that the complex-specific MAb CG10 can promote fusion between a diverse array of HIV-1 envelopes and $CD4^+$ cell lines.

CG10 increases HIV-1 viral infection of HeLa–CD4–LTR–β-Gal cells. The observed increase of envelope-mediated cell fusion in the presence of CG10 MAb predicted that this MAb should increase viral infectivity by augmenting virus cell entry. To test this hypothesis, we used the HeLa–CD4–LTR–β-Gal cell line termed MAGI. This cell line detects a single-round infection with HIV-1 (22). The ability of CG10 to enhance infection was tested with three HIV-1 isolates: LAI, a syncytium-inducing T-tropic virus that is capable of infecting all T-cell lines tested (28), and two variants of the ELI strain, ELI1 and ELI2. ELI1 is a T-tropic virus that has a restricted host range on T-cell lines and has many properties of a primary isolate (15, 28, 40). ELI2 was derived from ELI1 by adaptation to growth in H9 cells and differs from ELI1 at a single residue in gp41 (Met-7 to Val); this change confers an increased replicative capacity and expanded host range on the virus (15). These viruses were used to infect MAGI cells at different MOI in either the absence or presence of CG10 (or CG4 control MAb). The numbers of infected cells were determined after 48 h. As can be seen in Table 4, CG10 significantly increased infection of MAGI cells with LAI, ELI1, and ELI2. The infection of MAGI cells by ELI2 was fivefold lower than that by LAI in the absence of antibodies. However, in the presence of CG10 MAb (1 μg/ml), the level of ELI2 infection was closer to that found with LAI. As with the fusion assay, CG10 at a 1-μg/ml dose was more effective than the higher (5 μg/ml) dose with T-tropic strains (Table 4). Similar results were obtained with ELI1. The gp120-specific MAb CG4, which is not complex specific, did not have an augmenting effect on the infection of MAGI cells (data not shown).

TABLE 4. CG10 enhances infection of MAGI cells by HIV-1 LAI, ELI1, and ELI2 strains

Virus strain	MOI (cpm)[a]	No. of infected cells [±SD (fold[b])] in presence of CG10 MAb concn (μg/ml):		
		None	1	5
LAI	10^5	779 ± 3 (1.0)	TNTC[c]	TNTC
	10^4	140 ± 1 (1.0)	258 ± 15 (1.8)	236 ± 14 (1.7)
ELI1	10^5	178 ± 5 (1.0)	594 ± 67 (3.3)	411 ± 41 (2.3)
	10^4	25 ± 1 (1.0)	79 ± 4 (3.2)	38 ± 8 (1.5)
ELI2	10^5	171 ± 25 (1.0)	747 ± 36 (4.3)	603 ± 54 (3.5)
	10^4	26 ± 1 (1.0)	121 ± 10 (4.6)	76 ± 8 (2.9)

[a] Determined by reverse transcriptase assay.
[b] Numbers in parentheses indicate the fold increase in the number of infected cells (blue-stained nuclei) compared with the no antibody control (set at 1.0).
[c] TNTC, too numerous to count.

CG10 does not increase the binding affinity between CD4 and gp120. The biological activity of CG10 could be explained by an effect on the binding affinity between CD4 and gp120 (due to an increased association rate or to a decreased dissociation rate). To test this possibility, the binding of horseradish peroxidase (HRP)-conjugated gp120 (LAI; Intracel, Seattle, Wash.) to CD4 was measured in the presence of different concentrations of CG10. In a preliminary experiment it was established that CG10 binds to CD4 complexes with gp120 or gp120-HRP equally well (8a). As shown in Fig. 3, CG10 had no effect on the association of CD4 and gp120. This was confirmed by monitoring the dissociation rate of the complex in the presence of various CG10 concentrations. No difference in this rate could be detected in the presence or absence of CG10. Similarly, the association and dissociation of sCD4 to and from gp120-gp41-expressing cells, respectively (oligomeric form), determined by flow cytometry analyses, were not affected by the presence of CG10 MAb (data not shown). Thus, CG10 most likely exerts its effect at a postbinding step of viral entry and not on CD4-gp120 binding.

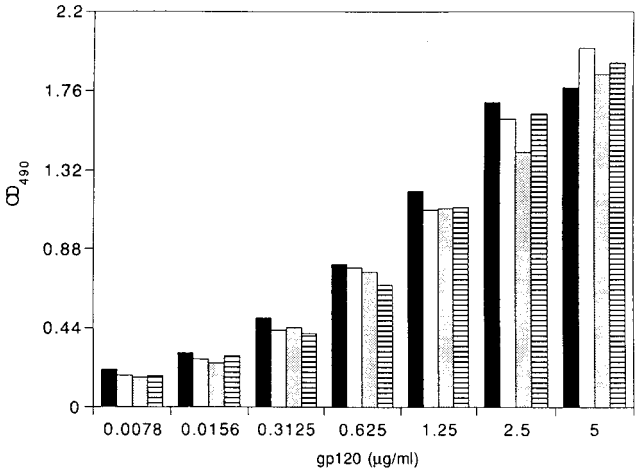

FIG. 3. CG10 MAb does not affect the binding of gp120 to CD4. Enzyme-linked immunosorbent plates were coated with anti-CD4 MAb (17), and after blocking with 3% (wt/vol) bovine serum albumin in TBS, were saturated with sCD4 (SmithKline, Philadelphia, Pa.). Various concentrations (0.0078 to 5 μg/ml) of HRP-labelled gp120 (LAI) (Intracel, Seattle, Wash.) were added to the plate and incubated for 4 h at room temperature in the absence (solid bars) or in the presence of CG10 at the following concentrations: 0.1 μg/ml (open bars), 1 μg/ml (stippled bars), and 10 μg/ml (hatched bars). After the plates were washed, the substrate *o*-phenylenediamine dihydrochloride was added, and the optical densities of the plates were read at 490 nm (OD_{490}).

TABLE 5. CG10 MAb can partially reconstitute the fusion capacity of PMA-treated A2.01.CD4.401 cells

Effector	Target	PMA treatment[a]	No. of syncytia [±SD (fold[b])] in presence of CG10 MAb concn (μg/ml):		
			None	1	0.1
12E1-VPE16	A2.01.CD4.401	−	162 ± 1	262 ± 1 (1.6)	376 ± 7 (2.3)
		+	30 ± 7	128 ± 15 (4.2)	180 ± 17 (6.0)

[a] A2.01.CD4.401 cells were (+) or were not (−) treated with PMA (25 ng/ml, 3 h) and washed three times before being mixed with 12E1-VPE16 effectors.
[b] Numbers in parentheses indicate the fold enhancement of syncytia formed compared with the no antibody control (set at 1.0).

CG10 can partially reconstitute the fusion of PMA-treated A2.01.CD4.401 cells. We have previously shown that the phorbol ester phorbol myristate acetate (PMA) induces down-modulation of a membrane component that is distinct from CD4 yet associates with CD4 after its interaction with gp120. This component was recently proven to be the coreceptor for T-tropic HIV-1 strains, CXCR-4 (fusin) (23). Therefore we investigated the ability of CG10 to reconstitute the capacity of A2.01.CD4.401 cells pretreated with PMA (25 ng/ml, 3 h) to fuse with 12E1-gp120-gp41 effectors. As can be seen in Table 5, syncytium formation by PMA-treated cells was reduced by 81% compared with untreated cells. However, in the presence of CG10 MAb at a 1- or 0.1-μg/ml concentration, a significant increase (four- and sixfold, respectively) in the number of syncytia was observed.

CG10 can partially reverse MIP-1β-mediated blocking of PM1 cell fusion with M-tropic envelopes. It was recently shown that β-chemokines (MIP-1α, MIP-1β, and RANTES), which bind to the chemokine receptors CCR-5 and CCR-3, can block infection of the PM1 cell line by M-tropic strains (8). The activity of CG10 in the same system was tested. As indicated in Table 6, preincubation of PM1 cells with MIP-1β (1 μg/ml) blocked their subsequent fusion with 12E1-JR-FL env effectors by 85%. The addition of CG10 (but not CG4) could significantly reconstitute the fusion ability of the MIP-1β-treated PM1 cells. Similar results were obtained with MIP-1α-treated cells and with effector cells expressing the Ba-L envelope (data not shown). The data in Tables 5 and 6 suggest that CG10 is effective under conditions of limited availability of HIV-1 coreceptors.

TABLE 6. CG10 MAb can partially reverse the fusion-blocking activity of MIP-1β

Target	Effector (HIV-1 strain)	MIP-1β treatment[a]	MAb	MAb concn (μg/ml)	No. of syncytia [±SD (% control[b])]
PM1	12E1-vCB28 (JR-FL)	−			345 ± 12 (100)
		+			51 ± 8 (15)
		+	CG10	1	156 ± 5 (45)
		+	CG10	10	243 ± 46 (70)
		+	CG4	10	50 ± 1 (15)
	12E1-vPE16 (IIIB)	−			280 ± 15 (100)
		+			292 ± 8 (104)
		+	CG10	1	624 ± 68 (220)
		+	CG10	10	431 ± 25 (150)
		+	CG4	1	260 ± 20 (93)

[a] Target cells were (+) or were not (−) treated with MIP-1β (1 μg/ml) for 2 h at 37°C. They were then mixed with effector cells in the absence or presence of MAb as indicated.
[b] Numbers in parentheses indicate percentages of syncytia formed compared to the no chemokine and/or no MAb control groups (set at 100%).

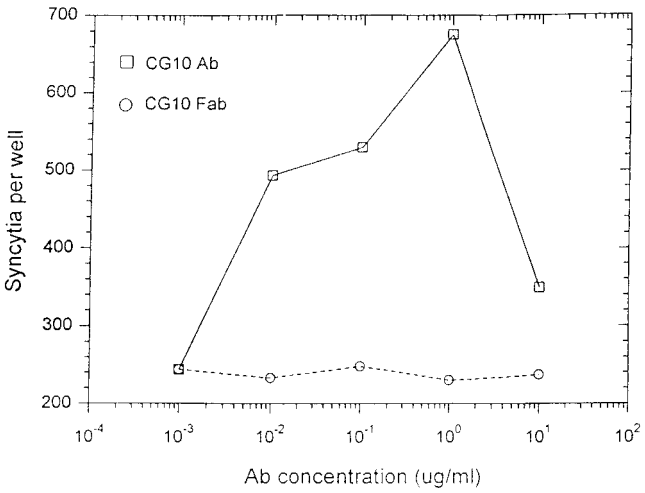

FIG. 4. Intact CG10 molecules but not Fab fragments have syncytium-augmenting activity. CEM cells were cocultured with 12E1 cells, infected overnight with vPE16 recombinant vaccinia virus expressing IIIB envelope, in the absence or presence of intact CG10 or CG10-Fab fragments (0.001, 0.01, 0.1, 1.0, or 10 μg/ml [in triplicate]). Syncytia were scored at 2.5 h. Standard deviations were ≤15% of the means.

Fab fragments of CG10 do not enhance HIV-1 envelope-mediated fusion. As mentioned above, CG10 exhibits an unusual dose response on the fusion of T-tropic viruses. Higher concentrations (≥10 μg/ml) are less fusion augmenting than lower doses (1 and 0.1 μg/ml). This could be explained if CG10 exerts its enhancing effect only when each molecule cross-links two adjacent CD4-gp120 complexes on the viral or cell membrane. To test this hypothesis, we generated Fab fragments of CG10 and compared their augmenting activity with that of the intact molecules. It was found (Fig. 4) that the CG10-Fab fragments were devoid of biological activity in the fusion assay, even though they bound to surface CD4-gp120 complexes with an affinity similar to that of the intact molecules (see Materials and Methods). This suggests that, whereas CG10 MAb and its Fab are capable of binding to preformed membrane CD4-gp120 complexes, augmentation of the fusion process requires cross-linking.

DISCUSSION

HIV-1 cell entry is a multistep process whose details are yet to be fully elucidated. The initial binding of the HIV-1 envelope (gp120-gp41) to membrane-associated CD4 molecules is required but not sufficient for completion of the viral-cell fusion process. MAbs specific for gp120 and CD4 have helped in delineating the regions in both proteins that are involved in the initial binding event. However, multiple MAbs that recognize epitopes outside of the binding regions of gp120 and CD4 nevertheless can have significant neutralizing capacity (13, 20, 25, 26, 30, 37, 42). One explanation for these findings is that additional regions of the gp120 and CD4 molecules are involved in secondary interactions with other membrane components (31). These secondary interactions may be required for the eventual exposure of the gp41 hydrophobic N terminus and its insertion into the target cell membrane. These accessory molecules or coreceptors were identified recently. They differ between M-tropic and T-tropic isolates (1, 7, 10, 14) and were shown to have a restricted tissue and species distribution (1, 2,

4, 11, 31, 35). We have recently demonstrated that, following the binding of gp120 to CD4-expressing human cells, the coreceptor CXCR-4 (fusin) is recruited into a trimolecular complex with CD4-gp120 that can be coimmunoprecipitated with antibodies against either CD4 or gp120 (23).

In an attempt to generate MAbs with biological activities affecting the postbinding steps of the fusion process, we (17) and other researchers have immunized mice with preformed soluble gp120-CD4 complexes. Many of the MAbs generated from the spleens of these mice could be mapped to either the gp120 or CD4 component but demonstrated enhanced binding to the complex (9, 17, 21). Several of these complex-enhanced MAbs were shown to have viral-neutralizing activities. In addition, two human MAbs generated by immortalization of B cells from infected individuals were shown to bind with increased affinity to gp120 complexed with CD4 and to neutralize a number of divergent HIV-1 isolates (36, 38). Notable among these MAbs is the MAb CG10, which was found to be exquisitely complex specific, since no binding to either CD4 or gp120 was seen in multiple assays (17).

In the present study, it was found that low concentrations of CG10 MAb do not inhibit but rather can enhance HIV-1–cell fusion. By using either chronically infected cell lines or recombinant vaccinia viruses expressing T-tropic or M-tropic envelopes, it was shown that CG10-enhancing activity can be observed with a variety of HIV-1 envelopes and a variety of $CD4^+$ cell lines. Thus, the epitope recognized by CG10 must be relatively conserved. The enhancing effect of CG10 was also evident during infection of MAGI cells by several HIV-1 strains. Since this assay measures a single round of replication, it suggests that CG10 can increase the efficiency of virus entry.

In kinetic experiments, it was found that CG10 MAb had the greatest effect during the earlier stages of syncytium formation (90 min), suggesting that this MAb may shift the kinetics of the fusion process as well as increase the number of syncytia formed. Interestingly, the dose response of CG10 in assays involving T-tropic envelopes showed that the optimal concentration for enhancing activity was between 0.01 and 1 μg/ml. One possible explanation for this phenomenon is the requirement for each intact molecule of CG10 IgG (bivalent) to cross-link two adjacent CD4-gp120 complexes in the oligomeric form on the envelope-expressing cells or virions (as was reported for an anti-CD4 domain 2 MAb, 5A8 [27]). At antibody excess, this cross-linking could be diminished. Indeed, it was found that the Fab fragments of CG10 bound to the CD4-gp120 complex but had no fusion-augmenting activity.

In the case of M-tropic envelopes, the dose response shifted. The enhancing effect required higher concentrations of CG10. This shift did not reflect a lower binding affinity of CG10 for these envelopes, since the binding curves of CG10 to cells expressing T-tropic (IIIB) and M-tropic (JR-FL, ADA, and Ba-L) envelopes (after the addition of sCD4) were practically indistinguishable. Thus, the difference in dose response between T-tropic and M-tropic envelopes may reflect CG10's "stabilizing effect" on a postbinding event.

The mechanism underlying the fusion-enhancing effect of CG10 is not clear. Several lines of reasoning suggest, but do not prove, that it may be related to its binding to epitopes which are involved in interactions with the coreceptor molecules: (i) the CG10 MAb was raised against a CD4-gp120 complex and recognizes only a complex-dependent conformational epitope; (ii) in multiple experiments, including those presented in the present study, no evidence was found to support the notion that CG10 increases the avidity of gp120-CD4 interaction by either increasing their association rate or decreasing their dissociation rate; (iii) in two different experimental systems aimed at either reducing the level of surface CXCR-4 expression (by PMA treatment) or blocking CCR-5 (by treating PM1 cells with the β-chemokines MIP-1α or MIP-1β), we found that CG10 can significantly reconstitute cell fusion with effector cells expressing T-tropic or M-tropic envelopes, respectively; and (iv) in a recent publication by Wu et al., it was found that a complex of gp120 (M-tropic) and sCD4 can interact specifically with CCR-5 and inhibit the binding of the natural CCR-5 ligands MIP-1α and MIP-1β (41). In that system, addition of CG10 to the sgp120-sCD4 complex before it was added to cells blocked (but not completely) the interaction of the complex with CCR-5-expressing cells. We believe that our data are not in conflict with the above findings since in our system CG10 is added to $CD4^+$ and envelope-expressing cells at the initiation of coculturing. Thus, in the fusion assay, CG10 binds to the trimolecular CD4-gp120-coreceptor complexes that are formed at the interface between the membranes of effector and target cells. It is very important to realize that a MAb which can block in a coreceptor binding assay may not have a neutralizing activity and, as described here for CG10 MAb, may have an augmenting activity in the fusion and infectivity assays. Further experiments will show exactly how CG10 enhances fusion. It may do so by interacting with fusion complexes containing exposed fusion intermediate structures, thus enhancing the conformational changes required for fusion to progress. It is possible that in the absence of CG10 not all fusion complexes are active due to a variety of reasons including incomplete interactions between the exposed conformational epitopes and coreceptors. In this case, increasing the number of reactive fusion complexes by interaction with CG10 would likely lead to an increase in fusion efficiency. One should note that these interactions probably involve oligomeric complexes, which may explain our finding that CG10 Fab fragments are not active in enhancing fusion.

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