JOURNAL OF VIROLOGY, Oct. 2000, p. 9749–9754 Vol. 74, No. 20
0022-538X/00/$04.00+0
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Identification of gp120 Regions Targeted by a Highly Potent Neutralizing Antiserum Elicited in a Chimpanzee Inoculated with a Primary Human Immunodeficiency Virus Type 1 Isolate

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Received 17 May 2000/Accepted 18 July 2000

We have previously reported that a chimpanzee infected with a primary human immunodeficiency virus type 1 (HIV-1) isolate (HIV-1_{DH12}) developed an extremely potent virus-neutralizing antibody. Immunoglobulin G purified from this animal conferred sterilizing immunity following passive transfer to macaques which were subsequently challenged with simian immunodeficiency virus/HIV-1 chimeric virus strain DH12. In addition to being highly strain specific, the chimpanzee antiserum did not bind to the V3 loop peptide of HIV-1_{DH12}, nor did it block the interaction of gp120 with the CD4 receptor. When neutralization was examined in the context of virus particles carrying chimeric envelope glycoproteins, the presence of all five hypervariable regions (V1 to V5) was required for optimal neutralization. Virions bearing chimeric gp120 containing the V1–V2 and V4 regions of HIV-1_{DH12} could also be neutralized, but larger quantities of the chimpanzee antiserum were needed to block infection. These results indicate that the HIV-1 gp120 epitope(s) targeted by the chimpanzee antiserum is highly conformational, involving surface elements contributed by all of the hypervariable domains of the envelope glycoprotein.

Neutralizing antibodies (NAbs) have been shown to be of critical importance for controlling infections and preventing disease induced by viral pathogens; in many instances they represent the first step of the adaptive immune response. For several reasons, however, the role of NAbs in clearing acute human immunodeficiency virus type 1 (HIV-1), simian immunodeficiency virus, or simian immunodeficiency virus/HIV-1 chimeric virus (SHIV) infections remains unclear. First, the emergence of NAbs during primary lentiviral infections is not coincident with the decline of plasma viremia (28). Second, the potency of NAbs in the sera of HIV-1-infected individuals is generally quite low and usually capable of only partially reducing virus infectivity of primary isolates in in vitro assays (19, 27). In addition, sera containing measurable neutralizing activity frequently fail to confer resistance to a subsequent virus challenge in prophylactic-vaccine experiments conducted with nonhuman primates (2, 15). Other studies indicate that vaccinated animals are able to control a virus inoculum in the absence of detectable NAbs (3, 10, 35, 43). In no case has immunization elicited potent, broadly cross-reactive NAbs against primary isolates (1, 8, 25, 38, 46, 47).

Although these results raise questions about the protective value of NAbs, several recent reports have described resistance to lentivirus challenge following the passive transfer of such antibodies to both hu-PBL-SCID mice (14, 31, 37) and nonhuman primates (12, 23, 24, 26, 34, 41). In one of these studies, the administration of 10 to 100 times the amount of immunoglobulin G1b12 (IgG1b12) human monoclonal antibody needed to neutralize 90% of virus infectivity in vitro completely protected SCID mice from an HIV-1 challenge (14). In other previously reported experiments, the transfer of high-titered IgG derived from a chimpanzee (number 1206) chronically infected with the dualtropic primary HIV-1_{DH12} isolate resulted in sterilizing immunity of macaques that were subsequently challenged with an SHIV bearing the same envelope glycoprotein (41). In the latter case, complete protection was observed when 100 times the amount of polyclonal chimpanzee IgG needed to achieve 100% neutralization of $SHIV_{DH12}$, as measured in in vitro assays, was administered to pig-tailed monkeys. These types of experiments clearly indicate that the presence of preexisting NAbs can control a subsequent virus challenge.

It is worth mentioning that although several mechanisms have been proposed for HIV-1 neutralization, the strong binding of antibody to virus particles or to oligomeric gp120 expressed on the surfaces of infected cells remains the best correlate of robust neutralizing activity (13, 32, 36, 39, 41). This is in contrast to the majority of antibodies detectable in the plasma of HIV-1-infected individuals, which are directed against the gp160 precursor glycoprotein and intact or fragmented monomeric gp120 molecules released from either virus particles or the surfaces of infected cells and not the virion-associated, oligomeric envelope glycoprotein complex (30).

As noted earlier, the titers of NAbs in HIV-1-seropositive individuals are usually quite low (19, 27), even in the case of long-term nonprogressors who maintain low levels of circulating virus (17, 33). The isolation of only three human monoclonal antibodies possessing broad, high-titered virus-neutralizing activities over the nearly two decades of the AIDS epidemic further testifies to the rarity of this type of humoral response during natural infections (4, 5, 29). We were therefore intrigued by the appearance of extremely high-titered NAbs in

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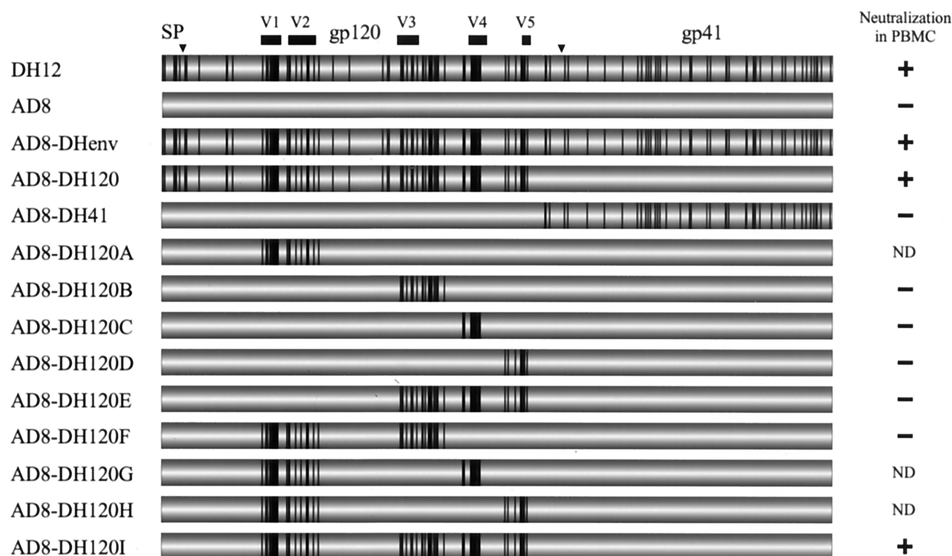

FIG. 1. Schematic diagram of the parental and chimeric HIV-1 envelope glycoproteins and results of neutralization assay with chimpanzee 1206 antiserum in human PBMC. The regions encoding the signal peptide (SP), surface glycoprotein gp120, transmembrane glycoprotein gp41, hypervariable regions (V1 to V5), and cleavage sites (▼) are indicated. The individual vertical lines represent amino acid residues that are different between HIV-1_{DH12} and HIV-1_{AD8}. Neutralization assays were performed using anti-CD3 and -CD28 antibody-activated PBMC, as previously described (16). Neutralization is reported as positive (calculated V_n/V_o = 0.1, serum titers > 1:1,600) or negative (calculated V_n/V_o = 0.1, serum titers < 1:100). ND, neutralization could not be determined using PBMC as target cells. HIV-1_{AD8} was previously reported to be neutralization resistant (50).

chimpanzees following the inoculation of the uncloned primary isolate HIV-1_{DH12} (40, 42, 44). The neutralizing activity in such persistently infected animals was initially measured in an assay in which duplicate, fourfold dilutions of chimpanzee serum were incubated with virus for 1 h prior to the addition of human peripheral blood mononuclear cells (PBMC) previously activated for 2 days with antibodies to CD3 and CD28 in the presence of interleukin-2. The surviving virus fraction (V_n/V_o = virus level in the presence of neutralizing serum/control serum) in the culture supernatants at 7 to 10 days postinfection, measured by a reverse transcriptase (RT) assay, was plotted as a function of serum dilution; the HIV-1 neutralization titer was defined as the reciprocal of the serum dilution causing a 10-fold reduction in virus production (V_n/V_o = 0.1) (16). Neutralizing activity against HIV-1_{DH12} became detectable in serum samples from one of these chimpanzees (number 1206) at 6 weeks postchallenge (titer = 1:270), peaked at week 21 (titer = 1:25,000), and stabilized at a titer of 1:10,000 at 1 year postinfection. When an assay that measures 100% neutralization was used, the titer of NAbs in the serum collected from the same animal at week 40 postinfection was approximately 1:2,800 (50). The virus-neutralizing activity of chimpanzee 1206 antiserum was highly strain specific; no blocking activity (<1:20) against HIV-1_{RF}, HIV-1_{SF2}, HIV-1_{IIIB}, or HIV-1_{MN} was measured.

The availability of high-titered anti-HIV-1_{DH12} chimpanzee antiserum possessing durable neutralizing activity has permitted us to conduct passive-transfer experiments assessing different aspects of primary lentiviral infections. It has been shown that HIV-1 NAbs capable of blocking infections in vitro are also protective in vivo (41) and that the administration of IgG from another chimpanzee chronically infected with HIV-1_{DH12} greatly accelerated the physical and biological clearance of cell-free virus particles from the blood (18). As a first step in characterizing the neutralization epitope(s) recognized by chimpanzee 1206 serum, the entire *env* gene from the HIV-1_{DH12} molecular clone DH125 (40) was inserted into the genetic background of HIV-1_{AD8}, a molecular clone of the HIV-1_{Ada} isolate, which is refractory to neutralization by the antiserum. As previously reported, the 100% neutralization titers directed against both HIV-1_{DH12} and this chimeric virus, designated AD8/DHenv, were nearly equivalent (approximately 1:2,000) (50), indicating that the chimpanzee NAbs are primarily directed against the HIV-1_{DH12} envelope glycoprotein.

The construction and characterization of additional chimeric viruses have also been described in which gp120 or gp41 coding sequences, as well as individual or multiple HIV-1_{DH12} gp120 variable regions, were transferred into HIV-1_{AD8} (7). Stocks of these chimeric viruses were generated by transfecting plasmids encoding full-length molecular clones into HeLa cells (40). When the sensitivities to the chimpanzee antiserum of the chimeric viruses bearing gp120 or gp41 coding sequences from HIV-1_{DH12} were evaluated in anti-CD3 and -CD28 antibody-activated human PBMC as described above, only the AD8-DH120 virus, which carried the HIV-1_{DH12} gp120, was neutralized (Fig. 1, right column). This result clearly indicates that the neutralizing activity of the chimpanzee antiserum is directed against the gp120, not the gp41, of HIV_{DH12}.

In view of the strain-specific nature of the chimpanzee NAbs, an enzyme-linked immunosorbent assay assessing the reactivity of chimpanzee antiserum with a 25-amino-acid peptide encompassing most of the V3 region of HIV-1_{DH12} gp120 was carried out. As has been reported for other primary HIV-1 isolates (45, 48), no binding of serum samples, collected at 10 different times during the course of the chimpanzee infection, to the V3 peptide was detected (data not shown). To determine whether the antiserum from animal 1206 targeted the CD4 binding site, soluble HIV-1_{DH12} gp120 released from recombinant baculovirus-infected insect cells was incubated with chimpanzee serum, and its reactivity with CD4 was monitored. No reduction in CD4 binding was observed with any of the samples of chimpanzee antiserum tested (data not shown).

To further evaluate potential HIV-1_{DH12} gp120 neutralization epitopes, the sensitivities of six of the nine intra-gp120

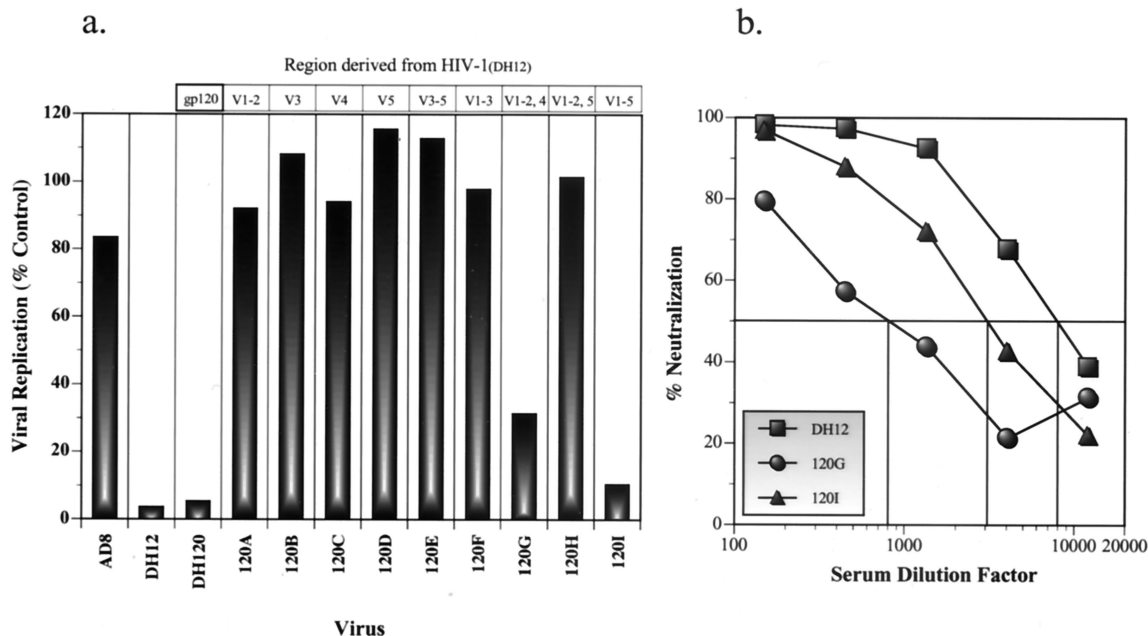

FIG. 2. Neutralization of the parental and chimeric viruses using the HOS-CD4.CCR5 cell line for target cells. (a) Replication of each virus in the presence of chimpanzee 1206 antiserum is shown as a percentage of that for the no-serum control. Each virus was incubated with or without the antiserum (1:300 dilution) prior to infecting cells. Culture medium was replaced at 1 day postinfection and culture supernatant was analyzed for RT activity at 4 days postinfection. (b) The parental HIV-1_{DH12} and two chimeric viruses (AD8-DH120G and -I) were incubated with various dilutions of the antiserum to determine relative sensitivities to the antiserum.

chimeric viruses capable of replicating in human PBMC to chimpanzee 1206 antiserum were assessed. Chimeric viruses AD8-DH120A, -G, and -H do not replicate to detectable levels in PBMC (6). As indicated in the right column of Fig. 1, the only intra-gp120 chimeric virus neutralized by chimpanzee 1206 serum was AD-DH120I, which contains all five variable regions of the HIV-1_{DH12} gp120 in the genetic background of HIV-1_{AD8}. Taken at face value, all of these results indicate that the gp120 epitope targeted by NAbs in the serum from animal 1206 is conformational, involving surfaces contributed by hypervariable domains V1 to V5 of the envelope glycoprotein.

Although, as noted in the preceding paragraph, the chimeric viruses AD8-DH120A, -G, and -H are essentially replication incompetent in human PBMC, it was previously reported that they are able to infect primary monocyte-derived macrophages and a human osteosarcoma (HOS) cell line expressing both CD4 and the CCR5 chemokine receptor (6). Using HOS-CD4.CCR5 (9) target cells, we examined the sensitivities of the two parental HIV-1 strains and the full ensemble of chimeric viruses to neutralization by the chimpanzee antiserum. In these neutralization assays, cell-free virus was incubated with the chimpanzee serum at room temperature for 30 min, and the mixture was then added to the adherent HOS-CD4.CCR5 cells, using the previously described tissue culture conditions (6). As shown in Fig. 2a, serum from chimpanzee 1206 readily neutralized both the parental HIV-1_{DH12} and the chimeric virus containing HIV-1_{DH12} gp120 (DH120), but not the parental HIV-1_{AD8}. Also consistent with the neutralization results with human PBMC (Fig.1), the chimpanzee antiserum neutralized the AD8-DH120I chimeric virus, which carries all five variable regions of HIV-1_{DH12} gp120. Surprisingly, one other intra-gp120 chimeric virus, AD8-DH120G, which contains the V1–V2 and the V4 regions of HIV-1_{DH12} gp120, was partially neutralized. To better assess the neutralization sensitivities of the two intra-gp120 chimeric viruses relative to the parental HIV-1_{DH12}, the three virus preparations were incubated with serially diluted serum and their infectivities were measured by RT production in the HOS-CD4.CCR5 cells (Fig. 2b). In this HOS cell system, the titers of chimpanzee antiserum which neutralized 50% of the infectivity of HIV-1_{DH12} and the chimeric viruses AD8-DH120I and -G were approximately 1:8,000, 1:3,000, and 1:800, respectively. Thus, although AD8-DH120G could be neutralized by the chimpanzee 1206 serum, it was far less sensitive than the parental HIV-1_{DH12}.

Although it is now generally appreciated that neutralization of HIV-1 is correlated with the binding of antibodies to oligomeric gp120 on virus particles, we still wished to identify and characterize epitopes associated with monomeric wild-type and chimeric gp120 molecules that are recognized by the chimpanzee antiserum. To this end, HeLa cells were infected with recombinant vaccinia viruses expressing either the parental or chimeric envelope glycoproteins (21), and the gp120 released into the culture supernatant was immunoprecipitated with chimpanzee 1206 serum and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Western blot analysis using rabbit anti-gp160 antiserum (22, 49). The gp120s analyzed were derived from HIV-1_{IIIB} (vPE-16) (11), the parental strains HIV-1_{AD8} and HIV-1_{DH12} (vvADenv and vvDHenv, respectively), and the chimeric envelope AD8-DH120A to -I (vv120Aenv to -Ienv) (6, 21, 22). As shown in Fig. 3a, the chimpanzee antiserum exhibited high specificity for and readily immunoprecipitated the gp120 from HIV-1_{DH12} but not the gp120 from HIV-1_{AD8} or HIV-1_{IIIB}. In a control experiment for this and other gp120 immunoprecipitations, the direct analysis of the two parental and nine chimeric gp120s produced in HeLa cells following infection with recombinant vaccinia viruses revealed similar reactivities with the polyclonal anti-gp160 rabbit antibody (Fig. 3b). When the chimeric gp120s were subjected to immunoprecipitation using the chimpanzee antiserum, only two (AD8-DH120A [V1–V2] and AD8-DH120I [V1 to V5]) were efficiently immunoprecipi-

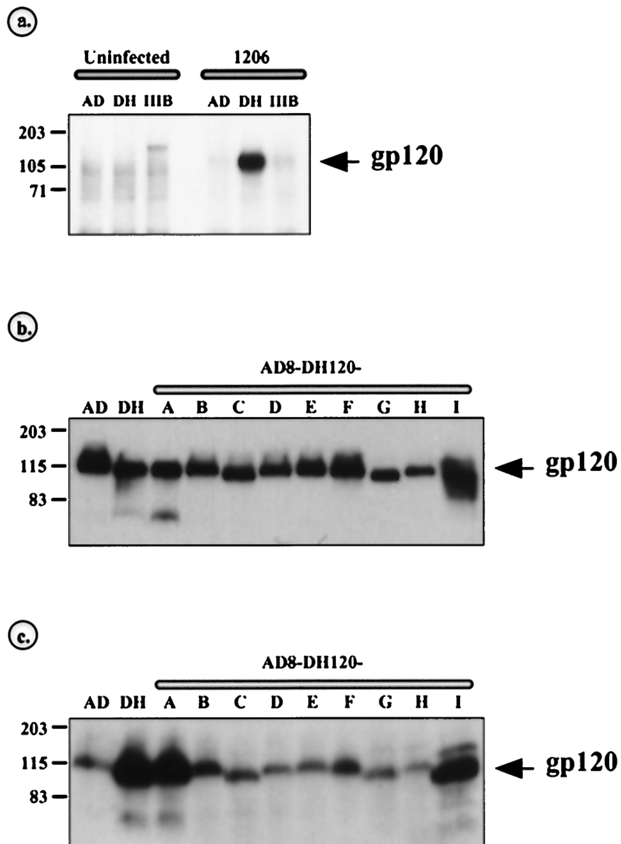

FIG. 3. Immunoprecipitation of parental and chimeric gp120s with chimpanzee 1206 antiserum. (a) gp120 from HIV-1_{AD8} (AD), HIV-1_{DH12} (DH), or HIV-1_{IIIB} (IIIB) was immunoprecipitated with serum from either an uninfected chimpanzee or chimpanzee 1206, which had been previously inoculated with HIV-1_{DH12}. (b) The parental HIV-1_{AD8} (AD) and HIV-1_{DH12} (DH) gp120s or chimeric gp120s were subjected to SDS-PAGE followed by Western blot analysis using rabbit anti-gp160 antiserum. (c) The same parental and chimeric gp120s were first immunoprecipitated (using 10 times the amount of protein that was directly loaded on the gel in panel b) with the antiserum from chimpanzee 1206. The immunoprecipitated proteins were subsequently subjected to SDS-PAGE followed by Western blotting using rabbit anti-gp160 antiserum. Numbers, on the left are molecular masses, in kilodaltons.

tated (Fig. 3c). Like the parental HIV-1_{AD8} gp120, only small amounts of the other chimeric gp120s were immunoprecipitated and recognized by the rabbit anti-gp160 polyclonal antibody in this overexposed Western blot (Fig. 3c). Thus, the HIV-1_{DH12} V1–V2 region, when present alone in a chimeric gp120, was readily immunoprecipitated by the chimpamzee 1206 serum but was nonreactive when it was associated with the HIV-1_{DH12} V3, V4, and V5 regions in other chimeric envelopes.

In this study, we have characterized the immune serum from a chimpanzee previously inoculated with the uncloned primary isolate HIV-1_{DH12}. Both the neutralizing and gp120-immunoprecipitating antibodies in the chimpanzee antiserum exhibited high specificity for HIV-1_{DH12}. No reactivity was observed with a peptide spanning the V3 loop of the HIV-1_{DH12} gp120, and a monomeric chimeric gp120 carrying this variable region was not immunoprecipitated. Unlike some HIV-1 NAbs, the chimpanzee antiserum did not block binding to the CD4 receptor. When analyzed in the context of virions bearing a chimeric envelope glycoprotein, all of the highly variable regions were required for optimal neutralization, implying that the HIV-1_{DH12} neutralization epitopes are conformational. Although neutralization required at minimum the presence of the V1–V2 and V4 regions of the HIV-1_{DH12} gp120, the high sensitivity of AD8-DH120I (V1 to V5) compared to that of AD8-DH120G (V1–V2 and V4) to neutralization by the chimpanzee antiserum suggested that the structure of the AD8-DH120I gp120 is more similar to that of the parental HIV-1_{DH12} gp120 than is the structure of the AD8-DH120G gp120.

The highly conformational nature of the HIV-1_{DH12} epitope(s) recognized by the chimpanzee serum resulted in the paradoxical results obtained in immunoprecipitation and neutralization experiments conducted with chimeric envelope glycoproteins. For example, although the chimeric virus AD8-DH120G, which contains the V1–V2 and V4 regions of HIV-1_{DH12}, was neutralized by the chimpanzee antiserum, the same chimeric gp120 released from recombinant vaccinia virus-infected HeLa cells could not be immunoprecipitated. Conversely, the chimeric gp120 monomer containing the V1–V2 region of HIV-1_{DH12} (AD8-DH120A) was immunoprecipitated as efficiently as the parental HIV-1_{DH12} gp120 expressed in HeLa cells (Fig. 3b), yet virus bearing the same chimeric envelope was neutralization resistant. Perhaps the antibodies that immunoprecipitate this monomeric gp120 are unable to bind this antigen when it is particle associated. Nonetheless, it is highly likely that in the context of the virus particle, the V1–V2 and V4 regions contain binding sites for the NAb and the seemingly discordant results in various assays may reflect vastly different levels of neutralizing and immunoprecipitating antibody activities in the chimpanzee antiserum. It is worth noting that although the precise location of the V1–V2 and V4 regions in the proposed structure of the HIV-1 gp120 have not been determined (because the V1–V2 region was deleted from the crystallized protein and the V4 domain was disordered in the crystal lattice), these two variable domains have been positioned on opposite sides of monomeric gp120 (20). This raises the interesting possibility that the neutralizing epitopes we have identified are formed from V1–V2 and V4 regions of two adjacent gp120 molecules within the trimeric complex rather than from an individual gp120 molecule. Additional experiments will be needed to confirm this hypothesis.

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