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Ability of antibodies specific to the HIV-1 envelope glycoprotein to block the fusion inhibitor T20 in a cell–cell fusion assay

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ABSTRACT

The anti-HIV peptide T20 is able to inhibit the syncytia formation between CHO-WT and HeLa CD4$^+$cells. We found that several sera of HIV-infected patients have the capacity to block the inhibition of fusion by T20. Suggesting that these sera may contain antibody which can block T20 access and prevent membrane fusion, we studied the ability of a panel of antibodies directed to different regions of HIV-1 envelope glycoprotein to block the inhibition of fusion by T20. We found that the C1 and V3 loop regions of gp120 and the heptad repeat 1, the immunodominant C–C region and the Kennedy epitope of gp41 located in the intracytoplasmic tail were the target for antibodies capable to block the inhibition of syncytia formation by T20. We suggest that these antibodies have the capacity to counteract the anti-fusion effect of T20 by preventing its binding to the interaction sites. Further studies are needed to determine if some of them recognize new T20 interaction sites.

Introduction

T20 (Enfuvirtide) is a synthetic peptide used as antiviral to treat HIV-1-infected individuals (Wild et al. 1992; Kilby et al. 1998). T20 represents a fragment of the gp41 heptad repeat 2 (HR2) domain (aa 638–673) located between the disulfide bonded loop region (PID) and the membrane proximal external region (MPER). The disulfide bonded loop region constitutes a hinge between the heptad repeat 1 (HR1) and the HR2 domains. The HR1 domain is located close to the predicted fusion peptide region of the gp41 ectodomain. T20 inhibits HIV-1 mediated cell fusion by preventing the interaction between HR1 and HR2 domains (Chen et al. 1995; Ryu et al. 1999; Kilgore et al. 2003; Trivedi et al. 2003). HIV-1 resistance to T20 is associated with mutations within the HR1 region of gp41 (Heil et al. 2004; Mink et al. 2005).

Several interaction sites of T20 have been located in the inner domain of gp120 (Leavitt et al. 2003; Alam et al. 2004; Yuan et al. 2004; Liu et al. 2005). The sensitivity to T20 varies among HIV-1 strains and is modulated by coreceptor specificity defined by the V3 loop of gp120 (Derdeyn et al. 2000; Xu et al. 2000). In this study, we found that several sera of HIV-infected individuals have the capacity to block completely the inhibition of fusion by T20 using a cell–cell fusion assay for HIV-1-envelope-mediated membrane fusion. To elucidate that finding, we examined the capacity of a panel of antibodies directed against different regions of HIV-1 envelope glycoprotein to counteract the anti-fusion effect of T20.

Materials and methods

Human sera, monoclonal antibodies, peptides

Sera of HIV-1seropositive adults were obtained from the Department of Infectious Diseases, University Hospital of Saint-Etienne.

Monoclonal antibodies (MAbs) III 2,3, I-10,4 and III-4 provided as ascites fluid were obtained from Dr. Pasquali, Hôpital Central, Strasbourg, France. Affinity purified MAbs 5F3, and 3D6 were purchased from Polymun Scientific, Vienna, Austria (Buchacher et al. 1994). MAbs Chessie 6, Chessie 8, 5F7, 4G10, T32, NC-1, 126-7, 50-69, 240-D, 246-D, Z-13, 902, F240, ID6 were obtained through the National Institutes of Health (NIH) AIDS Research and Reference Reagent Program. The monoclonal antibodies 127, 83, 2D7, 33, CRA1, 37.1.1, 60.1.1, 221, 268-D IV, 257-D IV, TH1, 1577 and the rabbit polyclonal directed against the V3 loop (EVA435/group B, EVA436/group C, EVA437/group D), EVA434/group A) were obtained through the Medical Research Council AIDS Reagent Project. Monoclonal antibody D5 was kindly provided by Mike Miller (Merck & Co., NJ, USA) (Miller et al. 2005). CHO-WT cells and HeLa T4 cells were obtained through the NIH AIDS Research and Reference Reagent Program.

The humanized IgA1 monoclonal antibody 1G12 has been generated in the laboratory of Dr. M. Cogné (CNRS, UMR6101, Limoges,

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France) using transgenic mice that express constant regions of heavy and light chains of IgA1 from human origin. The mice have been immunized with the MBP-HR1 construct (see below). Hybridoma obtained after fusion of splenic cells with SP_2O cells were screened by microscopy immunofluorescence on HeLa CD4 cells infected with HIV-1 envelope recombinant vacinia virus as previously described (Malvoisin and Wild 1990).

The following peptides were obtained through the NIH AIDS Research and Reference Reagent Program: C34, N36, T20,

4752 (LGAAGSTMGAASVTLTVQAR; 521–540);
4753 (ASVTLTVQARLLLSGIVQQQ, 531–550);
4754 (LLLSGIVQQQNNLLRAIEAQ, 541–560);
4755 (NNLLRAIEAQQNMLRLTVWG, 553–572);
4756 (QNMLRLTVWGIKQLQARVLA, 561–580);
4757 (IKQLQARVLALERYLRDQQL, 571–590);
4758 (LERYLRDQQLMGIWGCSKL, 581–600);
4759 (MGIWGCSGKLICTTSVPWNV, 591–610);
4760 (ICTTSVPWNSSWSNKSVDDI, 601–620);
4761 (SWSNKSVDDIWNNMTWMEWE, 611–630);
4762 (WNNMTWMEWEREIDNYTDYI, 621–641);
4763 (REIDNYTDYIYDLLEKSQTQ, 631–650);
4764 (YDLLEKSQTQQEKNEKELLE, 641–660);
4765 (QEKNEKELLELDKWASLWNW, 651–670).

The following peptides were obtained through the Medical Research Council AIDS Reagent Project:

ADP 708 (LQARILAVERYLKDQQL, 583–599);
ARP7022 (DQQLLGIWGCSGKLICTTAVPWNC, 593–616;
2027 (DDWINNMTWMQWEREIDNYT, 621–640);
2028 (EREIDNYTSLIYSLLEKS, 633–650);
2029 (SLIYSLLEKSQTQQEKNEQE, 641–660);
2035 (AVLSIVNRVRQGYSPLSLQT, 701–720);
2036 (QGYSPLSLQTRPPVPRGPDR, 711–730);
2037 (RPPVPRGPDRPEGIEEEGGE, 721–740);
2038 (PEGIEEEGGERDRDTSGRLV, 731–750);
2039 (RDRDTSGRLVHGFLAIIWVD, 741–760) and
the following gp120 V3 peptides EVA7012.1 (KSVHIGPGQAFYAT) and EVA7012.2 (KSIHIGPGRAFYTT).

The following recombinant maltose binding protein (MBP) fusion proteins containing fragments of the HIV-1 envelope protein: MBP3 (aa 203–269, GB8.C4, B), MBP-HR1 (aa 536–589, pNL4-3, B), MBP30 (aa 456–683, 92/UG/024.2, D), MBP32 (aa 476–750, LAI, B), MBP18 (aa 274–366, 92/UG/975.10, G), MBP54 (aa 274–362, 92/BR/014.1, B) have been previously described (Vincent et al. 2002, 2004, 2008).

ELISA methods

Peptide ELISA and MPB fusion protein ELISA were done as previously described (Vincent et al. 2002, 2004, 2008).

Analysis of syncytium formation

For measurements of HIV-1 envelope-mediated fusion, we used the CHO-WT cells which stably express HIV-1 HXB2 envelope glycoprotein on their surface (Weiss and White 1993). Purified antibodies or ascites fluid were incubated with CHO-WT cells (2×10^4 cells in a final volume of 100 μl of culture medium) for 2 h at 37 °C in a 96-well cell culture plate. Fifty μl of T20 solution in culture medium was added to the mixture (final concentration 50 nM) for an additional 2 h and then 50 μl of medium containing 2×10^4 HeLa T4 cells (HeLa $CD4^+$cells) were added to each well. The cell mixture was incubated overnight at 37 °C and then the cells were fixed with 4% formaldehyde, stained with Giemsa dye. Three random fields of cells were photographied and the syncytia (>4 nuclei per cell) were enumerated. The number of syncytia was presented as the mean ± SD. Purified antibodies were added at final concentrations ranging from 1 to 10 μg/mL. Five to 20 μL aliquots of culture supernatant, 1–5 μL of ascites fluid, 10 μL of human sera, 10 μL of rabbit antisera (diluted 1:3) were added per well.

Immunization of rabbits

New Zealand White rabbits were immunized four times with 200 μg of purified MBP-protein formulated with Freunds complete adjuvant. Intradermal immunizations were delivered dorsally at 4- to 6-week intervals, and the rabbits were bled 12 days after each immunization.

Results

Ability of sera of HIV-infected patients and human monoclonal antibodies directed against the HIV-1-gp160 to block the fusion inhibitor T20

Fig. 1 showed the syncytia formed when CHO-WT cells were co-cultured with HeLaT4 cells. No syncytia were detectable in the presence of T20 (Fig. 1A, panel a). We studied 22 HIV-patients and found that 3 sera had the ability to block completely the activity

Table 1
Ability of the human monoclonal antibodies directed against the HIV-1 gp160 to block the fusion inhibitor T20.

Designation	Catalog #	Provided as[a]	Mapped to	Syncytia ± SD
				29 ± 2[b]
257-D IV	ARP3023	S	gp120/V3	30 ± 2
268-D IV	ARP3024	S	gp120/V3	<2
126–7	9967	S	HR1/HR2 complex	<2
50–69	531	P	aa 579–613, HR1/HR2 complex	<2
5F3	AB010	P	aa 526–543, HR1/HR2 complex	<2
D5		P	HR1 pocket region	<2
F240	7623	P	gp41/PID	<2
240-D	1242	S	gp41/PID	<2
246-D	1245	P	gp41/PID	<2
T32	11,391	P	gp41/PID	<2
3D6	AB003	P	gp41/PID (GCSGKLICTTAVPW)	31 ± 2
Z13	11,557	P	gp41/MPER (WASLWNWFDITN)	<2

[a] Tissue culture supernatant (S), purified (P).
[b] Control culture.