Designer antigens for elicitation of broadly neutralizing antibodies against HIV

Tuckweng Kok1,2, Adriana Gaeguta1, John Finnie1,2, Paul R Gorry3, Melissa Churchill3 and Peng Li1

Broadly neutralizing antibodies (bNAbs) are a consistent protective immune correlate in human immunodeficiency virus (HIV) patients as well as in passive immunotherapy studies. The inability to elicit bNAbs is the core reason underlining the repeated failures in traditional HIV vaccine research. Rare monoclonal bNAbs against HIV, however, have been produced. The significance of producing and studying more monoclonal bNAbs against HIV is underlined by its capability of defining critical epitopes for antigen designs aimed at the development of a serum-neutralizing HIV vaccine. In this regard, traditional antigen preparations have failed. There is a need to clearly advocate the concept, and systematic study, of more sophisticated ‘designer antigens’ (DAGs), which carry epitopes that can lead to the elicitation of bNAbs. Using an extremely efficient cell-to-cell HIV infection model for the preparation of HIV prefusion intermediates, we have investigated a novel and systematic approach to produce (not screen for) potential bNAbs against HIV. We have established the concept and the experimental system for producing formaldehyde-fixed HIV DAGs that carry temperature-arrested prefusion intermediates. These prefusion intermediates are structures on the cell surface after viral attachment and receptor engagement but before fully functional viral entry. Using defined HIV prefusion DAGs, we have produced monoclonal antibodies (mAbs) specific to novel epitopes on HIV prefusion intermediates. These mAbs do not react with the static/native surface HIV or cellular antigens, but react with the DAGs. This is a paradigm shift from the current mainstream approach of screening elite patients’ bNAbs.

Clinical & Translational Immunology (2014) 3, e24; doi:10.1038/cti.2014.22; published online 26 September 2014

Elicitation of broadly neutralizing antibodies (bNAbs) against the constantly mutating HIV is at the heart of meeting the global health challenge of human immunodeficiency virus (HIV) vaccine development and treatment. In recent years, we have been repeatedly reminded of the fundamental lesson that a successful prophylactic vaccine and treatment. In recent years, we have been repeatedly reminded of the fundamental lesson that a successful prophylactic

1School of Molecular and Biomedical Science, University of Adelaide, Adelaide, South Australia, Australia; 2SA Pathology, Adelaide, South Australia, Australia and 3Burnet Institute, Melbourne, Victoria, Australia.

Correspondence: Associate Professor T Kok, School of Molecular and Biomedical Science (Level 3), The University of Adelaide, North Terrace, Adelaide, South Australia 5005, Australia. Email: tuckweng.kok@adelaide.edu.au

Received 29 April 2014; revised 31 July 2014; accepted 3 August 2014
recognize them and to produce specific antibodies. The virus–cell interaction process is a continuously changing target and membrane fusion is a temperature-dependent process. There is a practical possibility of making ‘designer antigens’ (DAGs) under prefusion temperature arrest states (TAS), which will carry those potentially conserved, broadly neutralizing B-cell epitopes in fixed forms to mimic the transient state of HIV prefusion intermediates and consequently induce bNAb's against the virus. This report describes the cellular model for the production of novel DAGs at selected TAS using a high multiplicity of infection cell-to-cell transmission model, reviewed by Sattentau and Sattentau selection and characterization of mouse monoclonal DAG-specific antibodies for potentially broadly neutralizing activity against HIV.

**RESULTS**

**Cellular model for temperature-arrested HIV infection—I: efficient viral receptor binding**

Using indicator cells and a dye-based fusion assay, Mkrtchyan and colleagues showed that a ternary HIV prefusion intermediate complex, composed of viral envelope proteins gp120/gp41, CD4 and CCR5/CXCR4, can be arrested at 23 °C. By using T cells and a B clade HIV in a cell-to-cell HIV infection model that has been successfully used in our laboratory for more 20 years, we first show that the viral receptor binding is very efficient.

Figure 1 shows the immunofluorescence (IF) staining of cell-to-cell HIV infection temperature arrested for 3 h at 23 °C (HuT78: H3B=10:1, d–f are higher magnification of panel a–c). The CD4 molecules are identified by red staining (arrows in d). HIV antigens are identified by green staining (stained with a HIV patient’s serum, arrows in e). When both green and red fluorophores were excited, it is apparent that the vast majority of HuT78 cells were dual-stained for both HIV and CD4, indicating HuT78 cells have grabbed the virus from virus donor H3B cells, that is, virus–receptor engagement is accomplished efficiently (arrows in f). Note that under temperature-arrested prefusion intermediate state, all transferred HIV is studded only on the surface of HuT78 cells and there is no cell fusion despite 3 h incubation after receptor engagement (also see Figure 2).

**Cellular model for temperature-arrested HIV infection—II: no functional viral entry**

Using the above cell-to-cell HIV infection model, when H3B virus donor and HuT78 virus acceptor cells were co-incubated at selected TAS, we showed that the fusion-mediated HIV infection was unable to progress further.

H3B virus-donor and HuT78 virus-acceptor cells were co-incubated in a 1:4 mix respectively at 23 °C for 15 min. (cell processing time, essentially no low temperature arrest) or incubated for 3 h (temperature arrested). The co-culture mix was then further incubated at 37 °C for 0–120 min. Functional viral entry and replication was judged by estimation of episomal DNA with analysis for early and late HIV reverse transcription products by real time PCR using primer pairs specific for HIV minus strand strong-stop DNA (ss-DNA) and HIV DNA after first (1ST-DNA) and second (2ST-DNA) strand-transfer. The levels of early ss-DNA (a), 1ST-DNA (b) and late 2ST-DNA (c) were expressed as copy numbers per 2 × 10^6 mixed cells (Figure 2).

The data in Figure 2 show that: (1) HIV does not fully enter virus-acceptor cells after 3 h at low temperature arrest (similar levels of viral DNA in black and grey columns, with or without 3 h co-incubation at 23 °C, before temperature shift to 37 °C); and (2) co-incubation at 23 °C prepares the virus-acceptor cells for HIV entry, as progression of viral reverse transcription was much faster (black columns) than without a prolonged incubation at 23 °C (grey columns), when the temperature was subsequently increased from 23 to 37 °C. The target is a stable cellular state where viral entry events have been initiated but arrested at prefusion intermediate stage.

In summary, the above cellular model that we used delivered efficient viral receptor/co-receptor binding but stops short of viral entry. However, cellular receptor /co-receptor engagement and the formation of viral prefusion intermediates have changed the conformational structure at the cell–virus interface. The TAS has prepared viral acceptor cells for HIV entry. As a result, the onset of viral reverse transcription is expected to be much faster when the co-culture incubation temperature is subsequently shifted to 37 °C than without a prolonged incubation step at a temperature below 25 °C. This and recent studies by Henderson and Hope suggest that, before the fatal exposure of gp41 N-terminal fusion peptide, stable HIV prefusion...
Antisera derived from T cell-tropic HIV fusion DAGs neutralize both T cell-tropic and macrophage-tropic HIV

In a preliminary experiment, DAGs prepared at TAS at 16, 19 or 23 °C (viz. DAG16, DAG19 or DAG23) were used to immunize guinea pigs to produce polyclonal sera. The antisera against DAGs were heat inactivated at 56 °C for 30 min to minimize complement activity. The antisera were absorbed against $3.5 \times 10^6$ freshly prepared viable HuT78 cells once, then with $3.5 \times 10^6$ formaldehyde (final 0.05%) fixed HuT78 cells separately and then absorbed with $3.5 \times 10^6$ formaldehyde fixed (final 0.05%) H3B cells twice. These immune sera were referred to as ‘absorbed sera’. The absorbed sera are expected to contain antibodies against the viral DAG prefusion intermediates, but minimal or no antibodies against static/native HIV antigens and cellular antigens that are not involved in viral fusion. One half volume of each of the absorbed immune sera was then further absorbed against the corresponding DAG16, DAG19 or DAG23 (ca. 2–3 × 10^3 cell equivalent). These were referred to as ‘depleted sera’.

The depleted sera (depleted of those antibodies against the viral prefusion intermediates) and the absorbed sera were compared for their ability to neutralize the virus in a HIV single cycle infectivity assay. The results in Figure 3 showed that after extensive absorption to remove antibodies directed against static HIV and cellular antigens that were not involved in the fusion process, the T-cell tropic HIV-HXB2 fusion DAG-derived antisera contained neutralizing antibodies which reduced T-cell tropic HIV-HXB2 infectivity by 65–68% and, importantly, reduced macrophage-tropic HIV-Bal infectivity by 39–46%.

HIV prefusion DAG-specific mAbs

We were encouraged by the guinea pig antisera results described above. To further study the properties of DAG-specific antibodies, we have produced mouse mAbs using DAGs that were prepared at TAS of 19 °C (DAG19).

Figures 4a and b show the IF staining of different cells by a HIV-specific mAb (8B3), which detects a static/native antigen of HIV and that of a HIV prefusion DAG-specific mAb (5E1), which detects an antigen only present on the viral prefusion intermediates. The results clearly show that mAb 5E1 does not react to mature budding HIV particles on H3B cells—rather, it specifically captures a previously undescribed novel epitope that is unique to HIV prefusion intermediates. As expected, cells that were fixed prior to mixing did not show receptor engagement, (IF negative with 5E1). We also obtained convincing trifluorophore IF staining patterns of DAG cells and H3B cells by mAb 8B3 or by mAb 5E1 (see below).

Further confirmation of DAG specificity with HIV patient’s serum and mAbs using confocal trifluorophore IF

The novel DAG specificity was further confirmed with confocal IF staining using HIV patient’s serum, mouse mAb 5E1 and 8B3 and rat anti-human CD4. Figure 5a shows 5E1-specific staining (green) against DAG19 but not H3B cells—likely directed against novel, prefusion DAGs. The patient’s serum showed specific fluorescence (blue) with DAG19 and H3B cells, which were virus positive. As expected, staining with rat anti-CD4 showed specific fluorescence (red) with the HuT78 cells (CD4+), but not against H3B cells, which are CD4 negative. Colocalized imaging showed blue, red and green fluorescence against the DAG19 cell but only reaction of patient’s serum, mouse mAb 5E1 and 8B3 with DAG19 and H3B cells when reacted with 8B3 mAb—likely directed against static/native HIV antigens. The HIV patient’s serum showed specific fluorescence against DAG19 and H3B cells, both of which contain the virus. Similarly, anti-CD4 showed specific fluorescence (red) against HuT78 (CD4 pos) but not H3B cells (CD4 neg). Colocalized imaging showed blue, red and green fluorescence against DAG19 cells but only...
blue (patient’s serum) and green fluorescence (8B3) against the virus in H3B cells. Table 1 summarizes the results of the IF staining (Figures 5a and b) of DAG19 and H3B cells.

The results of the IF staining and colocalized imaging with the four antibodies (5E1, 8B3, patient’s HIV serum and anti-CD4) against DAG19 and H3B cells provide further confirmation of the novel DAG specificity.

**DISCUSSION**

The work described in this paper has shown the successful production of HIV DAGs using a synchronous one-step, cell-to-cell transmission model in which the viral donor H3B cells (B-clade HIV) were co-cultured with viral acceptor HuT78 T-cells. At selected temperature-arrest states, transiently formed and exposed epitopes—as a result of the interaction between HIV gp120, CD4 and co-receptor CXCR4—were fixed with 0.05% formaldehyde prior to viral fusion. Using indicator cells and fusion assays, Mkrtchyan et al.26 showed that HIV prefusion intermediate complexes, composed of viral envelope proteins gp120/gp41 and CD4 and CCR5/CXCR4, could be arrested at 23 °C. Poon et al.29 have also showed that formaldehyde-stabilized, heat-inactivated clade B virions could produce bNAb responses against heterologous HIV from clades A and C.

Our results suggest that the HIV prefusion intermediates can be induced and arrested in vitro by using the high multiplicity of infection model that has been successfully used in our laboratory to study HIV replication for more than 20 years.23 We have shown that the viral fusion process can be ‘frozen’ and viral prefusion intermediates can be arrested at various temperatures below 25 °C. This TAS does not allow fusion-mediated HIV infection to progress further despite prolonged co-incubation of virus donor and acceptor cells. The transiently formed epitopes were fixed as the DAGs—which were subsequently used to produce mouse mAb. The specific 5E1 mAb showed reaction with the DAG 19 cells but not against the static/native viral or cellular antigens on H3B or HuT78 cells, unlike the 8B3 mAb (see Figures 5a and b).

This unorthodox approach of using fixed, temperature-arrested HIV prefusion intermediates as novel ‘designer antigens’ can be used for the induction of potential bNAbs against HIV. bNAbs are a crucial protective immune correlate in controlling HIV but traditional approaches have so far failed to induce them (see review by McCoy and Weiss30). The human body “…is indeed capable of producing potent, broadly neutralizing antibodies;31 and epitopes following CD4 binding have been shown as promising targets.6,22,32,33 In the natural course of HIV infection at body temperature (37 °C), these epitopes are only transiently exposed, thus the host immune system does not have sufficient time to recognize them and to produce specific antibodies. On the contrary, the DAGs reported here, in fixed form, can be used to induce potential ‘designer HIV bNAbs’—once present in adequate concentrations, the antibodies would be able to react with the epitopes transiently expressed (induced) on the HIV prefusion intermediates and may halt the fusion process—thus inhibit HIV infection. The HIV DAGs can be used for quantity immune sera production in large farm animals. The resulting immune sera, as biological antiviral agents, potentially would contribute to current efforts in formulating non-abrasive anti-HIV topical microbicides.
Production of mouse mAbs
Six to 8-week-old female BALB/c mice were immunized with 5 × 10^6 cells with an equal volume of complete Freund’s adjuvant in each mouse via the subcutaneous route. The second dose was given via the intraperitoneal route with 10^6 cells in an equal volume of Freund’s incomplete adjuvant 5 to 6 weeks post first immunization. The final dose was given with 5 × 10^6 cells in PBS via the intravenous route 4 to 5 weeks post second dose. The spleens were then extracted from the immunized mice 4 days later according to the SA Pathology Animal Ethics Committee (AEC) guidelines (www.sapathology.sa.gov.au). The immune spleen cells were fused with Sp2/O-Ag14 myeloma cells (www.cell-lines-service.de or www.sigmaaldrich.com/australia.html) in the ratio of 5:1 respectively, using polyethylene glycol MW1500, according to standard protocols.24

Selection and characterization of DAG specific antibody
Cultures that showed the presence of hybridoma colonies were tested by the IF test for DAG-specific antibodies. The culture supernatant fluids were reacted overnight at 4°C against DAG, H3B and HuT78 cells, which were fixed with 0.05% formaldehyde onto silane-coated glass slides. The unreacted antibodies were washed three times with PBS and then reacted with fluorescein isothiocyanate-goat anti-mouse immunoglobulin A, G and M (www.abcam.com). The latter were incubated at 37°C for 30 min. and then washed three times with PBS to remove unreacted fluorescein isothiocyanate conjugate. Fluorescent mounting fluid (Dako, www.dako.com/au) was then added to the stained cells and viewed with an Olympus BX51 microscope equipped with LED fluorescence excitation. Hybridoma fluids that stained the DAG but not H3B or HuT78 cells were selected for further passage and subcloning by limiting dilution. Anti-human CD4 mAb (rat) was purchased from www.lifetechnologies.com. Characterization of DAG-specific mAb was described in Results.

Production of DAG antibodies in guinea pigs
DAGs ca. 2–3 × 10^6 cells (prepared at 16, 19 and 23°C) were mixed with an equal volume of complete Freunds adjuvant and immunized subcutaneously in 12-week-old guinea pigs (www.sahmri.com). The second, third and fourth inoculations (5–10 × 10^5 DAG cells with Freunds incomplete adjuvant) were given subcutaneously at 3 to 4 week intervals. Sera from the immunized guinea pigs were obtained by cardiac puncture one week after the final inoculation, according to the AEC guidelines (as above).

Table 1 Confocal immunofluorescence staining of DAG19 and H3B cells with 5E1 mAb, 8B3 mAb, patient’s HIV serum and anti-human CD4 with three fluorophores

| Primary Ab                  | Secondary Ab (conjugate)                  | DAG19  | H3B  |
|-----------------------------|------------------------------------------|--------|------|
| Patient’s anti-HIV          | Goat anti-human IgG (blue)               | Pos    | Pos  |
| Rat Anti-human CD4          | Goat anti-rat (red)                      | Pos    | Neg  |
| 5E1 mAb anti-novel epitope  | Goat anti-mouse immunoglobulins (green)  | Pos    | Neg  |
| 8B3 mAb anti-HIV            | Goat anti-mouse immunoglobulins (green)  | Pos    | Pos  |

Abbreviations: Ag, antigen; DAG, designer antigen; HIV, human immunodeficiency virus; mAb, monoclonal antibody; Neg, negative; Pos, positive. 1HIV pos, human CD4 pos and novel epitope neg. 2Alexa Fluor 350 3Alexa Fluor 594. 4Fluorescein isothiocyanate (www.lifetechnologies.com). 5Static/native antigen

METHODS
Production of DAGs
HIV donor H3B cells (CD4 negative) were co-cultured with HuT78 viral acceptor cells (CD4+) at a ratio of 1:10, respectively. H3B cells on average contain two HIV proviral DNA copies per cell and a culture of 5 × 10^7 cells produces a virus titre of 5 × 10^5 TCID50 ml^-1.22 The co-culture mix of H3B and HuT78 cells (1–3 × 10^6 ml^-1 in phosphate-buffered saline (PBS)) was incubated for 3 h at selected temperatures 4, 10, 15, 19 or 23°C (temperature-arrested state, TAS). An equal volume of 0.1% formaldehyde (Ultrapure, methanol-free) pre-equilibrated at the selected temperature was then added to the culture, mixed gently and incubated at the same temperature for 1 h. The DAG cells (viz. DAG4, DAG10, DAG15, DAG19 or DAG23) were then washed twice with PBS and stored in 0.05% formaldehyde (15.6 mM) in PBS containing 0.05% merthiolate as preservative. To test for possible viable virus in the DAGs, the absence of viable HIV. A similar mix of the DAG cells that were twice with PBS and stored in 0.05% formaldehyde was inoculated into TZM-bl indicator cells (NIH AIDS Research and Reference Reagent Program, www.aidsreagent.org). There were no positive indicator cells observed in the HIV single cycle infectivity assay (see below), which confirmed the absence of viable virus.
HIV single cycle infectivity assay

Tissue culture 48-well microplate with $2 \times 10^4$ TZM-bl cells was infected with HIV-HXB2 cell free virus stock (H3B cell culture supernatant—prepared as described in Kok et al.). The virus was treated with an equal volume of 1/5 dilution of normal guinea pig serum, or one of the ‘absorbed sera’, or one of the ‘depleted sera’ for 1 h at 4 °C prior to infection. The nominal multiplicity of infection used was 0.002. At 24 and 48 h post-infection, the TZM-bl cells were fixed with 1% formaldehyde in PBS and stained with X-gal according to established laboratory protocols. HIV-infected cells (blue) were counted after 90 min staining.36,37

ACKNOWLEDGEMENTS

Part of this work was supported by an Australian Centre for HIV and Hepatitis Research Grant.

1 Burton DR, Weiss RA. AIDS/HIV. A boost for HIV vaccine design. Science 2010; 329: 770–773.
2 Michaelson A, Picker LJ, Moore JP, Burton DR. Another HIV vaccine failure: where to next? Nat Med 2013; 19: 1576–1577.
3 McMichael AJ. In pursuit of an HIV vaccine: an interview with Andrew McMichael. BMC Biol 2013; 11: 60.
4 Virgin HW, Walker BD. Immunology and the elusive AIDS vaccine. Nature 2010; 464: 224–231.
5 Mascola JR, Montefiori DC. The role of antibodies in HIV vaccines. Annu Rev Immunol 2010; 28: 413–444.
6 Ruprecht CR, Kranap A, Reynell L, Mann AM, Brandenberg DF, Berlinger L et al. MPER-specific antibodies induce gp120 shedding and irreversibly neutralize HIV-1. J Exp Med 2011; 208: 439–454.
7 de Mulder M, York VA, Wijnia AA, Michaud HA, Nixson DF, Holquin A et al. HIV-1 drug resistance prevalence, drug susceptibility and variant characterization in the Jacobs Medical Center paediatric cohort, Bronx, NY, USA. HIV Med 2014; 15: 135–143.
8 Megens S, Laethem KV. HIV-1 genetic variation and drug resistance development. Expert Rev Anti Infect Ther 2013; 11: 1159–1178.
9 Siliciano JD, Siliciano RF. Recent trends in HIV drug resistance.Curr OpinViro l2013; 3: 487–494.
10 Kwong PD, Doyle ML, Casper DJ, Cicala C, Levort SA, Maedje S et al. HIV-1 exodes antibody-mediated neutralization through conformational masking of receptor-binding sites. Nature 2002; 420: 678–682.
11 Weiss RA, Clapham PH, Cheingsong-Popov R. Neutralization of human T-lymphotropic virus type III by sera of AIDS and AIDS-risk patients. Nature 1985; 316: 69–72.
12 Burchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Moggi R, Li D et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, concept-test trial. Lancet 2008; 372: 1881–1893.
13 Ubera K. HIV virus development in the aftermath of the STEP study: re-focus on occult HIV infection? PLoS Pathog 2008; 4: e1000114.
14 Kerks-Ng S, Pitsulitthum P, Nitaasaphan S, Kaewkungwal J, Chiu J, Paris R et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. New Engl J Med 2009; 361: 2209–2220.
15 Doria-Rose NA, Schnarrn CA, Gorman J, Moore PL, Bhiman JN, Dekosky BJ et al. Developmental pathway for potent V1V2-neutralizing HIV-1 antibodies. Nature 2014; 509: 55–62.
16 Olek G, McKee K, Yang Y, Yang ZY, Skinner J, Guanaga FJ et al. Relationship between antibody 2F5 neutralization of HIV-1 and hydrophobicity of its heavy chain third complementarity-determining region. J Virol 2010; 84: 2955–2962.
17 Pancera M, Maedje S, Ban YE, Chen L, Huang CC, Kong L et al. Structure of HIV-1 gp120 with gp41-interacting region reveals layered envelope architecture and basis of conformational mobility. Proc Natl Acad Sci USA 2010; 107: 1166–1171.
18 Wu X, Yang ZY, Li Y, Hogeckom CM, Schief WR, Seaman MS et al. Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. Science 2010; 329: 856–861.
19 Walker LM, Phugat SK, Chan-Hui PY, Wagner D, Phuang P, Goss JL et al. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. Science 2009; 326: 285–289.
20 Frey G, Peng H, Riba-Volloch S, Morell M, Cheng Y, Chen B. A fusion-intermediate state of HIV-1 gp41 targeted by broadly neutralizing antibodies. Proc Natl Acad Sci USA 2008; 105: 3739–3744.
21 Markovic I, Cloose KA. Recent advances in understanding the molecular mechanisms of HIV-1 entry and fusion: revisiting current targets and considering new options for therapeutic intervention. Curr HIV Res 2004; 2: 223–234.
22 Zhou T, Georgiev I, Wu X, Yang ZY, Dai K, Fina A et al. Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01. Science 2010; 329: 811–817.
23 Li P, Burrell CJ. Synthesis of human immunodeficiency virus DNA in a cell-to-cell transmission model. AIDS Res Hum Retroviruses 1992; 8: 253–259.
24 Sattentau Q. Avoiding the void: cell-to-cell spread of human viruses. Nat Rev Microbiol 2006; 6: 815–826.
25 Sattentau QJ. Cell-to-cell spread of retroviruses. Viruses 2010; 2: 1306–1321.
26 Mertchyan SR, Markosyan RM, Eaadon MT, Moore JP, Melejyan GB, Cohen FS. Ternary complex formation of human immunodeficiency virus type 1 Env, CD4, and chemokine receptor captured as an intermediate of membrane fusion. J Virol 2005; 79: 11161–11169.
27 Li P, Stephenson AJ, Kuijper LJ, Burrell CJ. Double-stranded strong-stop DNA and the second template switch in Human Immunodeficiency Virus (HIV) DNA synthesis. Virology 1993; 194: 82–88.
28 Henderson HI, Hope TJ. The temperature arrested intermediate of virus-cell fusion is a functional step in HIV infection. Virology 2006; 33: 36.
29 Poon B, Hsu JF, Gudeman V, Chen IS, Grovit-Fertas K. Formaldehyde-treated, heat-inactivated virions with increased human immunodeficiency virus type 1 env can be used to induce high-titer neutralizing antibody responses. J Virol 2005; 79: 10210–10217.
30 McCoy LE, Weiss RA. Neutralizing antibodies to HIV-1 induced by immunization. J Exp Med 2013; 210: 209–223.
31 Johnston MI, Fauci AS. HIV vaccine development—improving on natural immunity. New Engl J Med 2011; 365: 873–875.
32 Bianchi E, Joyce JG, Miller MD, Finnefrock AC, Liang X, Finotto M et al. Vaccination with peptide mimetics of the gp41 prehaeirpin fusion intermediate yields neutralizing antisera against HIV-1 isolates. Proc Natl Acad Sci USA 2010; 107: 10655–10660.
33 Rusert P, Kranap A, Magnus C, Brandenberg DF, Weber J, Ehlert AK et al. Interaction of the gp120 V1V2 loop with a neighboring gp120 unit shields the HIV envelope trimmer against cross-neutralizing antibodies. J Exp Med 2011; 208: 1419–1433.
34 Greenfield EA. Antibodies: A Laboratory Manual, Second edition 2nd edn. Cold Spring Harbor Laboratory Press: New York, 2014.
35 Kok T, Li P, Burrell CJ. Cell-to-cell transmission of human immunodeficiency virus infection induces two distinct phases of viral RNA expression under separate regulatory control. J Gen Virol 1993; 74 (Pt 1): 33–38.
36 Carr JM, Coolen C, Davis AJ, Burrell CJ, Li P. Human immunodeficiency virus 1 (HIV-1) virion infectivity factor (Vif) is part of reverse transcription complexes and acts as an accessory factor for reverse transcription. Virology 2008; 372: 147–156.
37 Kimpton J, Emerman M. Detection of replication-competent and pseudotyped human immunodeficiency virus with a sensitive cell line on the basis of activation of an integrated beta-galactosidase gene. J Virol 1992; 66: 2232–2239.
Author/s:
Kok, T; Gaeguta, A; Finnie, J; Gorry, PR; Churchill, M; Li, P

Title:
Designer antigens for elicitation of broadly neutralizing antibodies against HIV.

Date:
2014-09

Citation:
Kok, T., Gaeguta, A., Finnie, J., Gorry, P. R., Churchill, M. & Li, P. (2014). Designer antigens for elicitation of broadly neutralizing antibodies against HIV.. Clin Transl Immunology, 3 (9), pp.e24-. https://doi.org/10.1038/cti.2014.22.

Persistent Link:
http://hdl.handle.net/11343/262317

File Description:
Published version

License:
CC BY-NC-SA