Title
Antibody-based protection against HIV infection by vectored immunoprophylaxis

Journal
Nature, 481(7379)

ISSN
0028-0836

Authors
Balazs, Alejandro B
Chen, Joyce
Hong, Christin M
et al.

Publication Date
2012

DOI
10.1038/nature10660

Peer reviewed

HHS Public Access

Author manuscript

Nature. Author manuscript; available in PMC 2012 July 05.

Published in final edited form as:

Nature. ; 481(7379): 81–84. doi:10.1038/nature10660.

Antibody-based Protection Against HIV Infection by Vectored ImmunoProphylaxis

Alejandro B. Balazs[†], **Joyce Chen**[†], **Christin M. Hong**[†], **Dinesh S. Rao**[‡], **Lili Yang**[†], and **David Baltimore**[†,§]

[†]Division of Biology, California Institute of Technology, 1200 E. California Blvd., Pasadena, CA 91125

[‡]Dept. of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California at Los Angeles, 10833 Le Conte Ave., Los Angeles California, 90095

Abstract

Despite tremendous efforts, development of an effective vaccine against HIV has proved an elusive goal. Recently, however, numerous antibodies have been identified that are capable of neutralizing the vast majority of circulating HIV strains[1–5]. These antibodies all exhibit an unusually high level of somatic mutation[6], presumably due to extensive affinity maturation over the course of continuous exposure to an evolving antigen[7]. While substantial effort has focused on the design of immunogens capable of eliciting antibodies *de novo* that would target similar epitopes[8–10], it remains uncertain whether a conventional vaccine will be able to elicit analogs of the existing broadly neutralizing antibodies. As an alternative to immunization, vector-mediated gene transfer could be used to engineer secretion of the existing broadly neutralizing antibodies into the circulation. Here we describe a practical implementation of this approach, vectored immunoprophylaxis (VIP), which in mice induces lifelong expression of these monoclonal antibodies at high concentrations from a single intramuscular injection. This is achieved using a specialized adeno-associated virus (AAV) vector optimized for the production of full-length antibody from muscle tissue. We show that humanized mice receiving VIP appear to be fully protected from HIV infection even when challenged intravenously with very high doses of replication-competent virus. Our results suggest that successful translation of this approach to humans may produce effective prophylaxis against HIV.

[§]Direct all correspondence to: Dr. David Baltimore, California Institute of Technology, Dept. of Biology, M/C:147-75, 1200 E. California Blvd., Pasadena, CA 91125; phone: (626) 395-3580; fax: (626) 585-9495; baltimo@caltech.edu.

Author Contributions

A.B.B. and D.B. conceived the study with assistance from L.Y., A.B.B. designed the experiments. A.B.B., J.C., and C.M.H. carried out experiments. A.B.B., J.C., and C.M.H. analyzed the data. D.S.R. performed immunohistochemistry and analysis. A.B.B. and D.B. wrote the paper with contributions from all authors.

Author Information

Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature.

Keywords

HIV; antibody; prophylaxis; vaccine; AAV; humanized mice; T lymphocyte; VIP; engineered immunity

Previous efforts to engineer humoral immunity using AAV-based vectors resulted in modest antibody production[11] which was subsequently improved through the use of alternative capsids[12] and self-complementary AAV (scAAV) vectors[13] that increase expression at the expense of carrying capacity. Recently, scAAV vectors were employed to direct expression of SIV-neutralizing immunoadhesins consisting of small, artificially fused antibody fragments[14]. However, the efficacy of this prophylaxis was limited by an endogenous immune response directed against the immunoadhesin proteins. To ask whether newer capsid serotypes and vector configurations might support long-lived expression of full-length human antibodies from muscle, we produced AAV vectors with the capsid from serotype 8[15] that expressed either luciferase or 4E10 HIV neutralizing antibody driven from CMV promoters and administered them through a single injection of the gastrocnemius muscle (Fig. 1a). Within one week of vector administration, either luciferase or antibody gene expression was detectable (Supplementary Fig. 1a, left and right respectively). Expression continued to rise, achieving maximum levels after 12–16 weeks and then decreasing two- to three-fold before stabilizing for the duration of the 64-week study. Given the long-lived nature of this expression, it seemed possible that these vectors could be used to engineer lifelong humoral immunity provided by full-length, fully human antibodies. Hence, we carried out a systematic process of vector and transgene optimization to improve the expression characteristics of this system (Supplementary Information). The heavy and light chain variable regions of the HIV-neutralizing b12 antibody were cloned into the vector and AAV stock was produced for intramuscular administration of 1×10^{11} genome copies (GC) into the gastrocnemius muscle of two immunodeficient and two immunocompetent mouse strains: NOD/SCID/γc (NSG), Rag2/γc (RAG), B6, and Balb/C. Mice produced the encoded antibody at serum concentrations that were 100-fold higher than the levels achieved with the non-optimized vector and this level of expression persisted for at least 52 weeks (Fig. 1b compared to Supplementary Fig. 1a, right). In agreement with previous studies of AAV-induced tolerance in mice[16], we detected very limited mouse antibodies raised against human b12-IgG in B6 mice while Balb/C animals generated detectable mouse antibodies against the transgene (data not shown) that did not appear to impact human IgG levels.

To test the ability of VIP to protect mice from challenge *in vivo*, we adapted a previously described humanized mouse model[17] that exhibits CD4 cell depletion following challenge with replication-competent HIV (Supplementary Fig. 5). We administered vector expressing either luciferase or b12 antibody to NSG mice, producing stable serum b12 antibody concentrations of approximately 100μg/mL within six weeks (Fig. 1c). These mice were adoptively populated with expanded human peripheral blood mononuclear cells (huPBMCs), which engrafted over a period of two weeks. Mice were then challenged by intraperitoneal (IP) injection of the NL4-3 strain of HIV. Following HIV challenge, the

majority of mice expressing luciferase showed dramatic loss of CD4 cells while mice expressing b12 antibody showed no CD4-cell depletion (Fig 1d).

To compare the protective abilities of the historically available broadly neutralizing antibodies, vectors expressing b12, 2G12, 4E10, and 2F5 were produced and administered to NSG mice. Seven weeks after administration, NSG mice produced 20–250μg/mL of the indicated antibodies (Fig. 2a). Interestingly, *in vivo* serum concentrations of 4E10 and 2F5 were somewhat lower than b12 and 2G12 despite comparable expression in vitro (Supplementary Figure 3b), perhaps resulting from the previously described self-reactivity of these clones[18]. Transduced mice were adoptively populated with huPBMCs, challenged by intravenous (IV) injection with HIV, and sampled weekly to quantify CD4 cell depletion over time (Fig. 2b). Animals expressing b12 were completely protected from infection, while those expressing 2G12, 4E10 and 2F5 were partially protected. Groups demonstrating partial protection consisted of animals with delayed CD4 cell depletion as well as animals that maintained high CD4 cell levels throughout the course of the experiment. Interestingly, mice expressing 250μg/mL of the 2G12 antibody were only partially protected despite antibody levels being over 300-fold higher than previously established IC_{50} values for this antibody-strain combination *in vitro*[19]. Eight weeks post-challenge, mice were sacrificed and paraffin-embedded spleen sections underwent immunohistochemical staining for the HIV-expressed p24 antigen to quantify the extent of infection (Fig. 2c). Remarkably, mice expressing b12 had no detectable p24 expressing cells while those expressing other antibodies exhibited significant positive staining (Fig. 2d).

To determine the robustness of protection mediated by VIP, a large cohort of mice expressing b12 antibody were adoptively populated with huPBMCs. Prior to challenge, all mice expressed high levels of human IgG, presumably due to engrafted human B-cells (Supplementary Fig. 6a), but only those receiving the b12-expressing vector produced IgG specific for gp120, which reached 100μg/mL (Supplementary Fig. 6b). Mock-infected mice expressing either luciferase or b12 demonstrated consistent high-level CD4 cell engraftment throughout the course of the experiment, showing that transgene toxicity was not contributing to CD4 cell loss (Fig. 3). In contrast, mice expressing luciferase that received 1ng of HIV experienced rapid and extensive CD4 cell depletion. At higher doses, infection in luciferase-expressing mice became more consistent and resulted in depletion of CD4 cells below the level of detection in some cases (25, 125ng doses). Remarkably, all mice expressing b12 demonstrated protection from CD4 cell loss, despite receiving HIV doses over 100-fold higher than necessary to deplete seven out of eight control animals (Fig. 3).

As newer anti-HIV antibodies have become available, we have compared the relative efficacy of b12 to VRC01 antibody. VRC01 neutralizes over 90% of circulating HIV strains *in vitro*[1], making it an excellent candidate for human trials. We administered decreasing doses of vector expressing either b12 or VRC01 to NSG mice and monitored expression of the antibodies over time. For both antibodies, we observed clear dose-dependent expression at all time points analyzed (Supplementary Fig. 7a and Fig. 4 (left)). Mice expressing luciferase or antibodies at various levels were adoptively populated with huPBMCs. Just prior to challenge, a gp120-specific ELISA confirmed the effective antibody concentration in each group (Supplementary Fig. 7b and Fig. 4 (middle)). Following IV challenge with

10ng of HIV, CD4 cells were monitored to determine the impact of antibody concentration. An average b12 concentration of 34μg/mL and VRC01 concentration of 8.3μg/mL protected mice from infection (Supplementary Fig. 7c and Fig. 4 (right)). Groups expressing lower concentrations of b12 and VRC01 were only partially protected, with several animals showing no detectable loss of CD4 cells and others exhibiting delayed CD4 cell depletion.

Here we demonstrate that broadly neutralizing human antibodies expressed by VIP are capable of protecting animals from even high-dose HIV exposure *in vivo*. Human-to-human HIV transmission rates vary with behavior but do not generally exceed one per hundred heterosexual exposures[20], and recent studies have demonstrated that infections are generally initiated by a single founder strain[21]. Humanized mice with b12 serum concentrations of 100μg/mL were resistant to HIV infection at challenge doses 100-fold higher than necessary to infect the vast majority of animals, suggesting that this level of protection may far exceed what would be necessary to provide protection against HIV infection in humans. In contrast to previous approaches, VIP produces full-length antibodies that are identical in sequence to those produced by the immune system[22]. Recent experiments have suggested that full-length antibody structures possess superior *in vitro* neutralization activity as compared to modified architectures such as immunoadhesins[23]. Utilization of such naturally occurring antibody architectures should also reduce immune responses against the transgene, which were previously shown to reduce the effectiveness of prophylaxis against SIV[14].

Our results demonstrate that VIP administration results in long-lived production of human antibodies at super-prophylactic levels in immunocompetent animals. Clinical trials involving AAV have demonstrated remarkable success when targeting immunoprivileged sites such as retinal tissue[24], but transduction of liver resulted in an adaptive immune response against vector capsid[25]. Studies in non-human primates have shown that the elicitation of capsid-specific cytotoxic T-lymphocytes is limited to AAV capsids that exhibit heparin-binding activity[26]. Interestingly, serotypes lacking heparin-binding activity, including AAV8, did not induce CTL responses, suggesting that AAV8-based vectors, like the one we have used, may circumvent previously observed immunological barriers to long-term transduction. Additionally, in contrast to liver transduction, administration of AAV via intramuscular injection has been shown to result in very long-lived, albeit low-level, Factor IX expression[27], suggesting that the route of administration can significantly impact the duration of expression. While the expression level attainable in humans remains to be determined, it is worth noting that the significantly longer serum half-life of a human antibody in humans[28], as opposed to mice[29], may result in higher steady-state levels than those observed in the present study. Regardless of this, our results suggest that even if VIP administration in humans results in serum antibody concentrations 100-fold lower than those observed in mice, it may still confer protection against HIV infection.

Given the urgency that exists in combating the ongoing global HIV pandemic and the incremental progress towards a vaccine, novel paradigms of prophylaxis must be explored towards solving this global health crisis. Our work demonstrates the feasibility of directly translating the existing repertoire of broadly neutralizing antibodies into functional immunoprophylaxis with robust protective abilities *in vivo*. As more potent broadly neutralizing HIV antibodies are isolated[30], VIP can deliver these in concert with existing

antibodies to provide increased potency, broader coverage, and greater resistance to escape mutations. This approach may find broad utility in the rapid development of effective prophylaxis against any existing or future infectious disease for which broadly neutralizing antibodies can be isolated. Beyond infectious diseases, VIP can be applied to therapeutic regimens in which continuous production of monoclonal antibodies *in vivo* is desirable. Given the level of protection that VIP has demonstrated *in vivo*, we believe that highly effective prophylaxis through expression of existing monoclonal antibodies against HIV in humans is achievable.

Methods Summary

AAV2/8 was produced by transient transfection and purification from culture supernatant by PEG precipitation and cesium chloride ultracentrifugation. Virus was quantified by qPCR against CMV sequences and functionally validated *in vitro* to confirm gene expression prior to use *in vivo*. Mice were given single injections with purified vector in the gastrocnemius muscle. Antibody concentration in the serum was determined using an ELISA specific for either total human IgG or human IgG against HIV-gp120. Humanized mice expressing antibodies were produced by adoptive transfer of expanded huPBMCs into mice previously transduced with AAV vectors. HIV challenge was carried out via IP or IV injection and blood was sampled weekly to determine the ratio of CD4 to CD8 cells by flow cytometry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank James Wilson (University of Pennsylvania) for AAV8-related plasmids and assistance, Dennis Burton (Scripps Research Institute) for b12 and 2G12 expression plasmids, Gary Nabel (Vaccine Research Center) for 4E10, 2F5 and VRC01 expression plasmids, and the Caltech Protein Expression Center for providing purified antibodies. The following reagents were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: pNL4-3 from Dr. Malcolm Martin and TZM-bl cells from Dr. John Kappes and Dr. Xiaoyun Wu. The authors wish to thank Jesse Bloom, Darrell Kotton, Devdoot Majumdar, Gustavo Mostoslavsky, Ryan O'Connell and Alex Sigal for their helpful comments, and other members of the Baltimore lab for their assistance in carrying out this work. This project was supported by the Bill and Melinda Gates Foundation (Seattle, WA) through the Grand Challenges in Global Health Initiative (awarded to D.B.) Grand Challenge Grant #37866 and by the National Institutes of Health (HHSN266200500035C) through a contract from the National Institute of Allergy and Infectious Disease (NIAID). A.B.B. is supported by the amfAR postdoctoral research fellowship #107756-47-RFVA. D.S.R. is supported by career development award 1K08CA133521 from the NIH.

References

1. Wu X, et al. Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. Science. 2010; 329:856–861. [PubMed: 20616233]
2. Walker LM, et al. Broad and Potent Neutralizing Antibodies from an African Donor Reveal a New HIV-1 Vaccine Target. Science. 2009
3. Scheid JF, et al. Sequence and Structural Convergence of Broad and Potent HIV Antibodies That Mimic CD4 Binding. Science. 2011
4. Wu X, et al. Focused Evolution of HIV-1 Neutralizing Antibodies Revealed by Structures and Deep Sequencing. Science. 2011
5. Walker LM, et al. Broad neutralization coverage of HIV by multiple highly potent antibodies. Nature. 2011

6. Zhou T, et al. Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01. Science. 2010; 329:811–817. [PubMed: 20616231]
7. Scheid JF, et al. Broad diversity of neutralizing antibodies isolated from memory B cells in HIV-infected individuals. Nature. 2009; 458:636–640. [PubMed: 19287373]
8. Kwong PD, Wilson IA. HIV-1 and influenza antibodies: seeing antigens in new ways. Nature immunology. 2009; 10:573–578. [PubMed: 19448659]
9. Burton DR, et al. HIV vaccine design and the neutralizing antibody problem. Nature immunology. 2004; 5:233–236. [PubMed: 14985706]
10. Dormitzer PR, Ulmer JB, Rappuoli R. Structure-based antigen design: a strategy for next generation vaccines. Trends Biotechnol. 2008; 26:659–667. [PubMed: 18977045]
11. Lewis AD, Chen R, Montefiori DC, Johnson PR, Clark KR. Generation of neutralizing activity against human immunodeficiency virus type 1 in serum by antibody gene transfer. J Virol. 2002; 76:8769–8775. [PubMed: 12163597]
12. Fang J, et al. Stable antibody expression at therapeutic levels using the 2A peptide. Nat Biotechnol. 2005; 23:584–590. [PubMed: 15834403]
13. McCarty DM. Self-complementary AAV vectors; advances and applications. Mol Ther. 2008; 16:1648–1656. [PubMed: 18682697]
14. Johnson PR, et al. Vector-mediated gene transfer engenders long-lived neutralizing activity and protection against SIV infection in monkeys. Nat Med. 2009; 15:901–906. [PubMed: 19448633]
15. Gao GP, et al. Novel adeno-associated viruses from rhesus monkeys as vectors for human gene therapy. Proceedings of the National Academy of Sciences of the United States of America. 2002; 99:11854–11859. [PubMed: 12192090]
16. Breous E, Somanathan S, Wilson JM. BALB/c mice show impaired hepatic tolerogenic response following AAV gene transfer to the liver. Mol Ther. 2010; 18:766–774. [PubMed: 20068550]
17. Kumar P, et al. T cell-specific siRNA delivery suppresses HIV_1 infection in humanized mice. Cell. 2008; 134:577–586. [PubMed: 18691745]
18. Haynes BF, et al. Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. Science. 2005; 308:1906–1908. [PubMed: 15860590]
19. Binley JM, et al. Comprehensive cross-clade neutralization analysis of a panel of anti-human immunodeficiency virus type 1 monoclonal antibodies. J Virol. 2004; 78:13232–13252. [PubMed: 15542675]
20. Wawer MJ, et al. Rates of HIV-1 transmission per coital act, by stage of HIV-1 infection, in Rakai, Uganda. J Infect Dis. 2005; 191:1403–1409. [PubMed: 15809897]
21. Salazar-Gonzalez JF, et al. Deciphering human immunodeficiency virus type 1 transmission and early envelope diversification by single-genome amplification and sequencing. J Virol. 2008; 82:3952–3970. [PubMed: 18256145]
22. Fang J, et al. An antibody delivery system for regulated expression of therapeutic levels of monoclonal antibodies in vivo. Mol Ther. 2007; 15:1153–1159. [PubMed: 17375065]
23. West AP Jr, Galimidi RP, Gnanapragasam PNP, Bjorkman PJ. Single chain Fv-based anti-HIV proteins: Potential and Limitations. J Virol. 2011 Accepted.
24. Maguire AM, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. The New England journal of medicine. 2008; 358:2240–2248. [PubMed: 18441370]
25. Manno CS, et al. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. Nat Med. 2006; 12:342–347. [PubMed: 16474400]
26. Vandenberghe LH, et al. Heparin binding directs activation of T cells against adeno-associated virus serotype 2 capsid. Nat Med. 2006; 12:967–971. [PubMed: 16845388]
27. Jiang H, et al. Evidence of multiyear factor IX expression by AAV-mediated gene transfer to skeletal muscle in an individual with severe hemophilia B. Mol Ther. 2006; 14:452–455. [PubMed: 16822719]
28. Morell A, Terry WD, Waldmann TA. Metabolic properties of IgG subclasses in man. The Journal of clinical investigation. 1970; 49:673–680. [PubMed: 5443170]

29. Petkova SB, et al. Enhanced half-life of genetically engineered human IgG1 antibodies in a humanized FcRn mouse model: potential application in humorally mediated autoimmune disease. International immunology. 2006; 18:1759–1769. [PubMed: 17077181]

30. Diskin R, et al. Increasing the Potency and Breadth of an HIV Antibody using Structure-Based Rational Design. Science. 2011 Accepted.

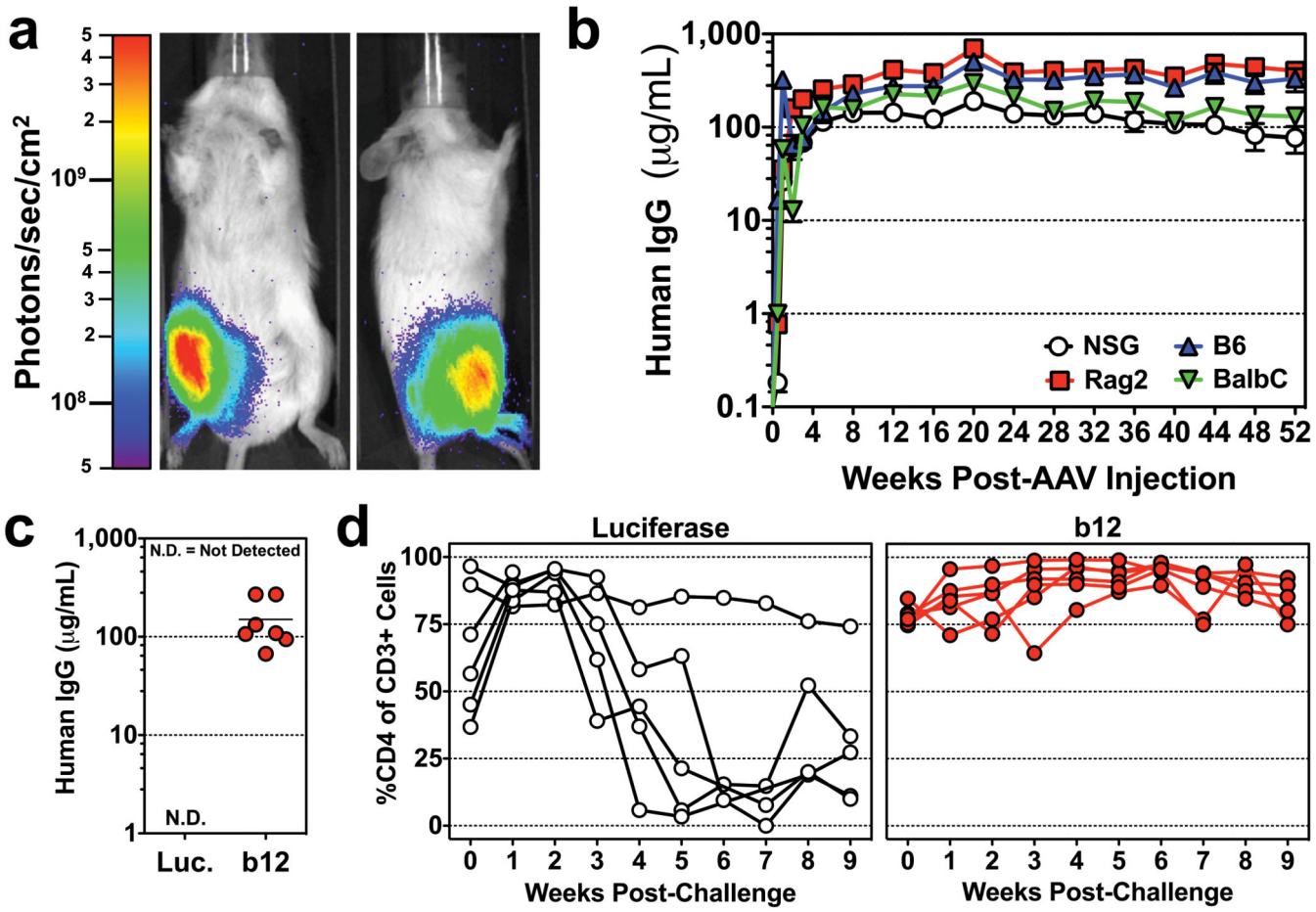

Figure 1. VIP protects against HIV-mediated CD4 cell depletion in humanized mice

a, Xenogen imaging of a representative Rag2$^{-/-}\gamma c^{-/-}$ mouse 15 weeks after intramuscular injection of 1×10^{10} genome copies (GC) of AAV2/8 expressing luciferase. **b,** Quantitation of human IgG by ELISA following intramuscular injection of 1×10^{11} GC of the optimized expression vector producing b12-IgG in either immunodeficient NOD/SCID/$\gamma c^{-/-}$(NSG) and Rag2$^{-/-}\gamma c^{-/-}$ (Rag2) or immunocompetent c57BL/6 (B6) and Balb/C mice (plot shows mean and standard error, n=4). **c,** Concentration of human IgG in circulation as measured by total human IgG ELISA on serum samples taken 6 weeks after intramuscular injection of vector expressing either luciferase or b12-IgG (N.D. = not detected). **d,** Depletion of CD4 T-cells in humanized mice following intraperitoneal (IP) challenge with 10ng p24 NL4-3 into animals that received AAV2/8 vectors expressing luciferase (left) or b12-IgG1 (right) 6 weeks earlier (n=6).

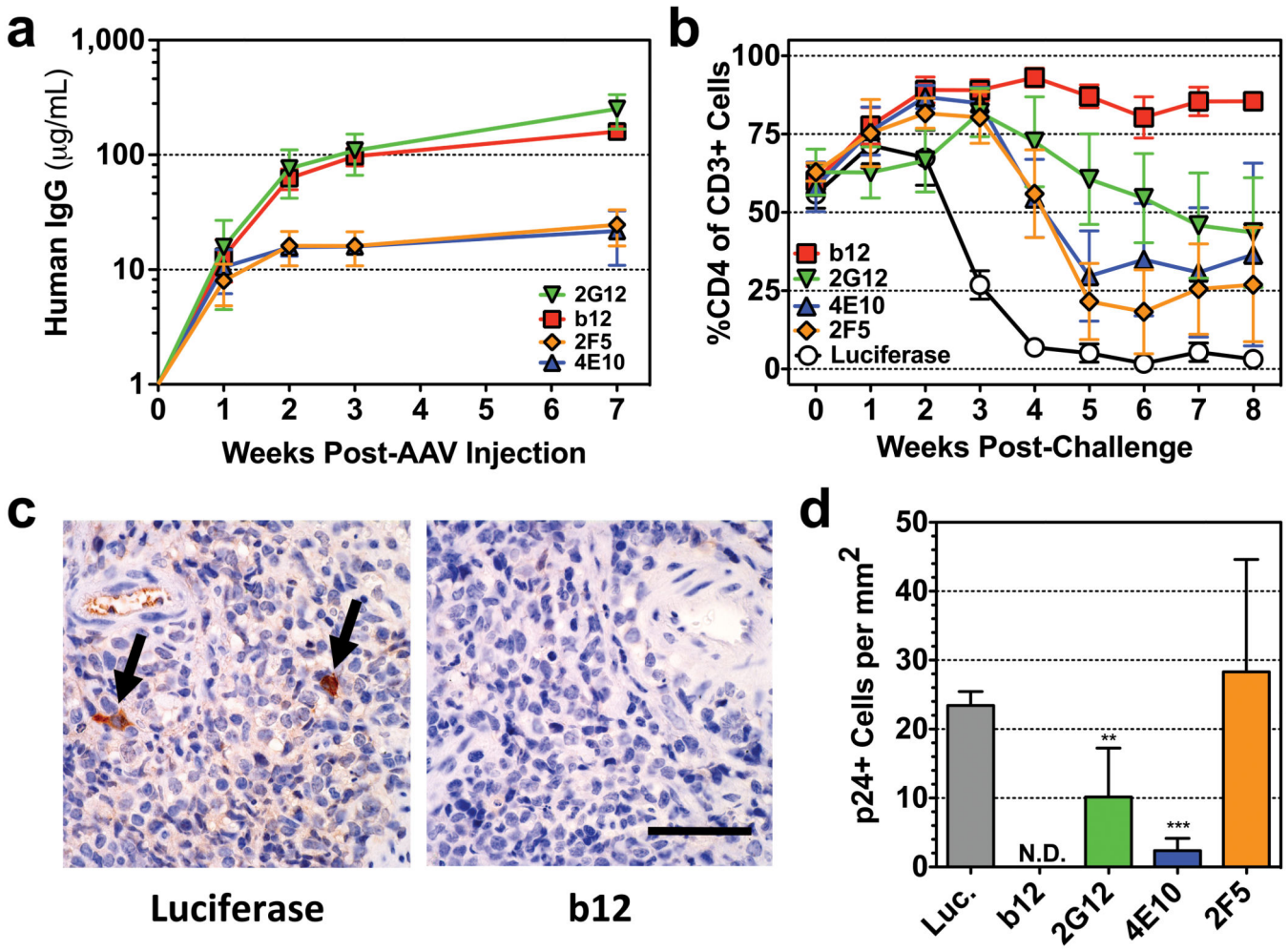

Figure 2. Comparison of protection mediated by various broadly neutralizing HIV antibodies

a, Concentration of antibody in circulation as measured by total human IgG ELISA on serum samples taken after intramuscular injection of vectors expressing four broadly neutralizing HIV antibodies (n=8). **b,** Comparison of the relative effectiveness of four broadly neutralizing HIV antibodies in protecting HuPBMC-NSG humanized mice against CD4 cell depletion following intravenous HIV challenge with 5ng p24 NL4-3 (n=8). **c,** HIV p24 detection by immunohistochemical (IHC) staining of sections taken from spleens 8 weeks post-challenge. Arrows indicate cells scored as positive for p24 expression. Scale bar represents 40 micrometers. **d,** Quantitation of IHC staining of spleen denoting the relative frequency of p24 expressing cells in spleens of infected animals. Asterisks indicate outcomes significantly different than luciferase control mice versus mice expressing antibodies by two-tailed t test (n=4–6, N.D.= Not Detected) **P<0.01, ***P<0.0001. Plots **a** and **b** show mean and standard error, Plot **d** shows mean and s.d.

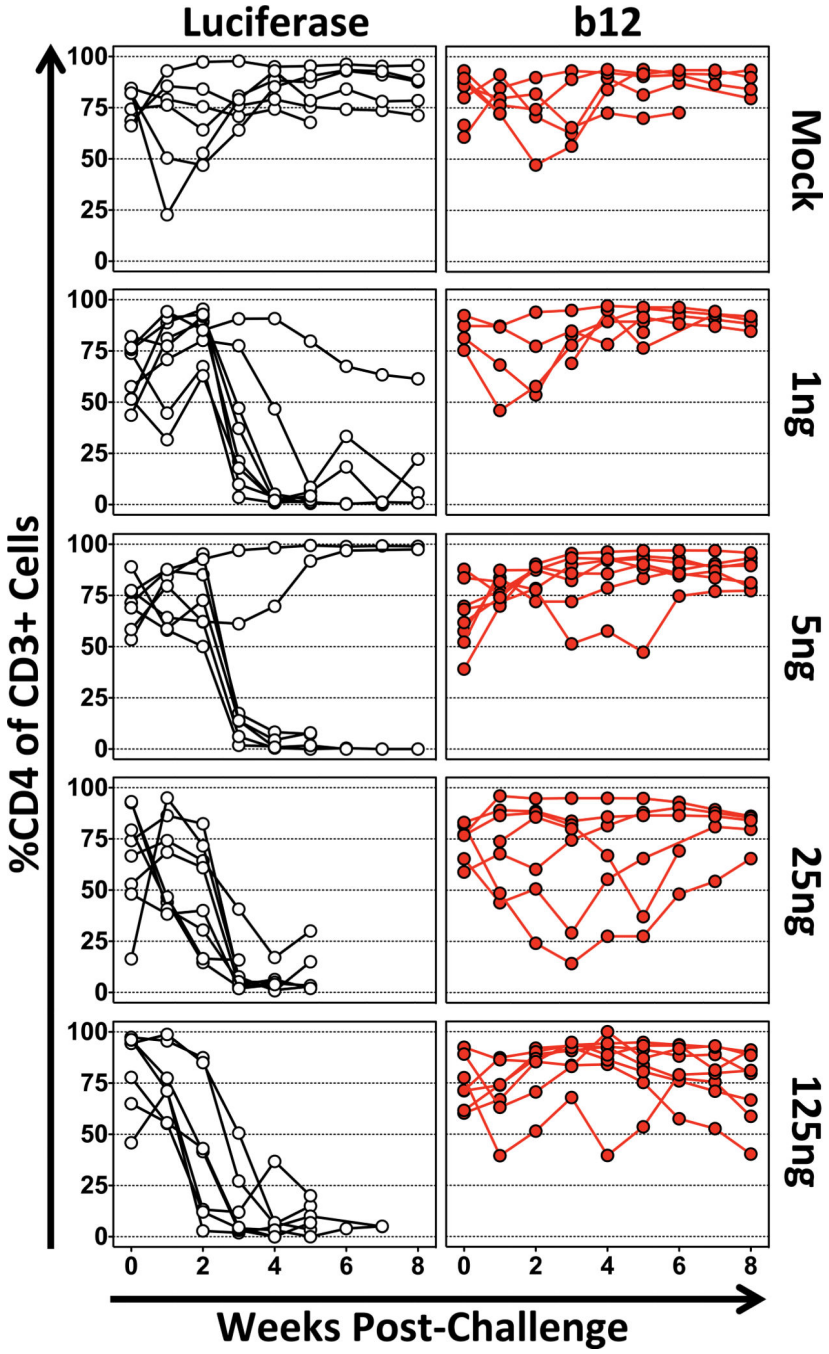

Figure 3. Robustness of CD4 cell protection mediated by b12 antibody

CD4 cell depletion in HuPBMC-NSG humanized mice as a result of intravenous challenge with the dose of NL4-3 indicated on the far right. Mice expressing luciferase (left plots) were susceptible to CD4 cell loss whereas those expressing b12 (right plots) demonstrated protection from HIV at all doses (n=8).

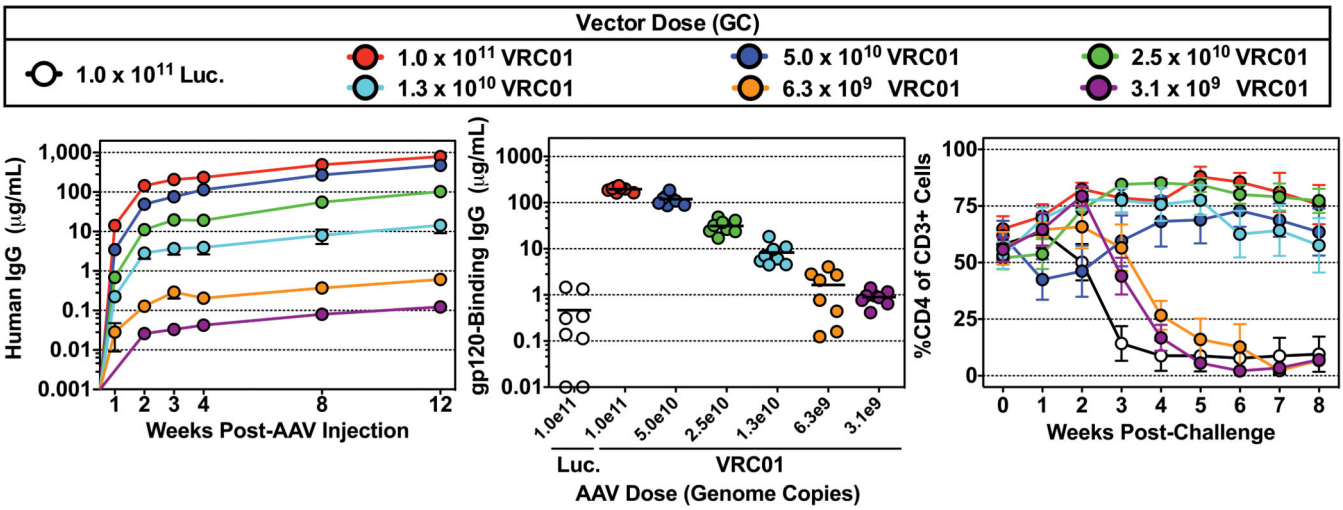

Figure 4. Determination of the minimum protective dose of VRC01 *in vivo*

(left) VRC01 expression over time as a function of dose as determined by total human IgG ELISA on serum samples taken following AAV administration (n=8). Mice receiving luciferase-expressing vector exhibited no detectable human antibodies (n=12). (middle) Concentration of VRC01 in serum one day prior to challenge, 3 weeks after adoptive transfer of human PBMCs and 15 weeks after intramuscular administration of the indicated dose of AAV as determined by a gp120-specific ELISA to measure the fraction of antibodies capable of binding HIV (n=8–12). (right) CD4 cell depletion in HuPBMC-NSG humanized mice as a result of intravenous challenge with 10ng of NL4-3 into animals expressing a range of VRC01, demonstrating the minimum dose of antibody necessary to protect against infection. Left and right plot show mean and standard error, middle plot shows individual animals and mean (n=8–12).