2. Gritz, E.R. *et al*. (2013) Long-term outcomes of a cell phone-delivered intervention for smokers living with HIV/AIDS. *Clin. Infect. Dis.* **57**, 608–615. doi:10.1093/cid/cit349

3. Lo, J. and Plutzky, J. (2012) The biology of atherosclerosis: general paradigms and distinct pathogenic mechanisms among HIV-infected patients. *J. Infect. Dis.* **205**(Suppl 3), S368–S374. doi:10.1093/infdis/jis201

4. Kuller, L.H. *et al*. (2008) Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. *PLoS Med.* **5**, e203. doi:10.1371/journal.pmed.0050203

5. Kaplan, R.C. *et al*. (2011) T cell activation and senescence predict subclinical carotid artery disease in HIV-infected women. *J. Infect. Dis.* **203**, 452–463. doi:10.1093/infdis/jiq071

6. Westhorpe, C.L. *et al*. (2013) Associations between surface markers on blood monocytes and carotid atherosclerosis in HIV-positive individuals. *Immunol. Cell Biol.* **92**, 133–138.

7. Neuhaus, J. *et al*. (2010) Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. *J. Infect. Dis.* **201**, 1788–1795. doi:10.1086/652749

8. Friis-Moller, N. *et al*. (2003) Combination antiretroviral therapy and the risk of myocardial infarction. *N. Engl. J. Med.* **349**, 1993–2003. doi:10.1056/NEJMoa030218

9. Worm, S.W. *et al*. (2010) Risk of myocardial infarction in patients with HIV infection exposed to specific individual antiretroviral drugs from the 3 major drug classes: the data collection on adverse events of anti-HIV drugs (D:A:D) study. *J. Infect. Dis.* **201**, 318–330. doi:10.1086/649897

10. Trevillyan, J.M. *et al*. (2013) Abacavir exposure and cardiovascular risk factors in HIV-positive patients with coronary heart disease: a retrospective case-control study. *Sex. Health* **10**, 97–101. doi:10.1071/SH12081

11. Falcinelli, E. *et al*. (2013) *In vivo* platelet activation and platelet hyperreactivity in abacavir-treated HIV-infected patients. *Thromb. Haemost.* **110**, 349–357. doi:10.1160/TH12-07-0504

12. Rose, H. *et al*. (2008) HIV infection and high density lipoprotein metabolism. *Atherosclerosis* **199**, 79–86. doi:10.1016/j.atherosclerosis.2007.10.018

13. Penzak, S.R. and Chuck, S.K. (2000) Hyperlipidemia associated with HIV protease inhibitor use: pathophysiology, prevalence, risk factors and treatment. *Scand. J. Infect. Dis.* **32**, 111–123. doi:10.1080/003655400750045196

Biographies

Jennifer Hoy is Professor Director of HIV Medicine at the Alfred Hospital and Monash University. She has over 25 years experience in both research and care in HIV infection. Her main research interest is in non-AIDS morbidity, including the pathogenesis of cardiovascular disease in HIV, dissecting the roles of traditional risk factors, HIV and chronic inflammation as well as the role of antiretroviral therapy.

Dr Janine Trevillyan is an Infectious Diseases Physician at the Alfred Hospital, Melbourne. She is currently completing a PhD with Monash University investigating the pathogenesis and prevention of cardiovascular disease in people living with HIV.

Therapeutic vaccination in HIV infection

Mina John

Department of Clinical Immunology
Royal Perth Hospital
Wellington Street
Perth, WA 6000, Australia
Tel: +61 8 9224 2899
Fax: +61 8 9224 2920
Email: M.John@iiid.com.au

The concept of using a vaccine to induce or enhance HIV-specific immune responses in those with established HIV infection has been through alternating waves of enthusiasm and skepticism over the past three decades of the HIV epidemic. The earliest therapeutic vaccination trials were conducted in subjects with uncontrolled viremia or suboptimal therapy and the highly activated and dysfunctional immune environment of such individuals would significantly impede the efficacy of any immunostimulatory approaches. Contemporary antiretroviral treatment assures potent and sustained inhibition of HIV replication, reduces immune activation and restores a variety of specific immune functions. This provides the context for a renewed level of scientific interest in the possibility of inducing HIV-specific immunity that could clear virally infected cells, retain long lived memory, and help reduce latent reservoirs of HIV that cannot be eliminated by current drugs or intensification strategies.

To date the most studied approaches for vaccination have been based on viral vectors, plasmid DNA and more recently, autologous dendritic cells (DCs). Among the vectored vaccines, the recombinant canarypox vaccine ALVAC is the most extensively studied across more than 15 preventative vaccine trials, including as the prime to subtype B/E gp120 protein boost in the RV144 'Thai' trial that reduced HIV acquisition by 31%[1]. Notably, there was no effect on levels of viremia and/or CD4 T cell count in vaccinated subjects who ultimately acquired HIV and in the therapeutic vaccination setting, results with ALVAC based vaccines have been contradictory. While an open label single arm study found that up to 11% of patients who stopped their antiretroviral treatment after vaccination could remain off treatment for 44 weeks[2], the largest randomised, placebo controlled studies have shown either no effect on the level of

rebound viremia after drug treatment interruption (TI)[3] or even increased viral rebound and decreased time to resumption of treatment[4]. A trial of a recombinant fowl pox vectored vaccine expressing subtype B Gag/Pol did show a lower viral rebound after TI but this was specifically associated with the vaccine's inclusion of human IFNγ and production of IgG2 antibodies to p24 Gag[5]. Constructs based on modified vaccinia ankara (MVA) or adenovirus showed promise in terms of eliciting CD8 and CD4 T cell responses with modest reductions of rebound viremia[6,7]. One of the key risks of vectored approaches is anti-vector immunity, and the related possibility that broad non-specific immune activation can be harmful if it increases the susceptibility of CD4 T cells to HIV infection. Vector immunity played a role in the terminated 'STEP' prevention trial of a recombinant Ad5 HIV-1 gag/pol/nef vaccine, in which pre-existing immunity to adenovirus serotype 5 (Ad5) was a risk factor for early HIV acquisition in vaccinees[8]. In this and two other trials of the same vaccine, HVTN 503 and 505, vaccination failed to reduce viral load in newly infected subjects[9]. The vaccine induced far weaker magnitude T cell responses than that seen in average HIV controllers and responses were directed against very few epitopes in each subject. DNA-based vaccines avoid anti-vector immunity, can be administered repeatedly and can incorporate molecular adjuvants to be co-expressed with the HIV antigens to alter the magnitude, quality and even tissue homing pathways of HIV-specific effector T cells[10]. Despite these theoretical advantages, human studies have been disappointing. The VRC HIV DNA 009-00-VP consisting of four plasmids encoding a subtype B Gag-Pol-Nef fusion protein was poorly immunogenic and failed to impact rebound viremia after TI[11]. While eliciting strong HIV-specific CD8 T cell responses is the central goal of all these approaches, recent studies of a four HIV antigen (F4) protein vaccine formulated with AS01 adjuvant has demonstrated successful induction of durable, polyfunctional CD4+ T-cell responses in HIV-1-infected subjects[12]. Notably these responses were stronger and more persistent in those on antiretroviral therapy compared to treatment-naïve individuals. This raises the general point that it is problematic to extrapolate immunogenicity correlates from infected vaccinees in prevention trials to the therapeutic setting, where negative impacts of HIV viremia should be suppressed by well established drug treatment prior to vaccination.

These more 'classical' approaches to vaccination rely on natural mechanisms of antigen presentation, which may be intact in the healthy individual receiving a preventative vaccine, but are likely to be impaired in a subject with established HIV infection. HIV has negative effects on differentiation and maturation of monocyte-derived dendritic cells that would impact innate and adaptive responses[13,14]. There have been 13 clinical studies of DC based therapeutic vaccination in humans to date. These are autologous DCs, matured and manipulated to express HIV antigens in any one of several ways *ex vivo*. Antigen can be loaded into DCs as peptides, recombinant whole proteins or apoptotic cells, or transfected into DCs with viral vectors in DNA or as mRNA. Two small studies have shown that vaccination with peptide or protein-pulsed DCs is safe but has no effect on viremia during TI[15,16]. Garcia *et al* have used DC pulsed with heat-inactivated autologous virus in treated patients and found viral load reductions greater than 1 log at 12 and 24 weeks post analytic TI in 55% and 35% of vaccinees compared to 9% and 0% of controls respectively[17,18]. A number of more recent studies using DC electroporated with mRNA encoding HIV-1 antigens have shown this to be a highly efficient loading mechanism, with successful induction of CD8 T cell responses, partial viral control *in vivo* and/or viral inhibition *in vitro*[19–21]. Notably, the most positive results have been associated with use DCs matured *ex vivo* and combined with autologous HIV antigens, which suggests that suppression of particular variants unique to each individual, including immune escape variant epitopes, is important for antiviral control.

The future role of therapeutic vaccination in functional cure still remains closely tied to the success of strategies to limit or purge the latent reservoir, which are necessary to make HIV visible to immune recognition and clearance. It remains to be seen whether achieving immune control in the absence of antiretroviral drugs, even if not permanent, could find 'niche' applications in current models of life-long clinical care, such as a 'one-off' intervention available to patients at particular times when oral therapy would be otherwise interrupted for adherence or medical reasons or in paediatric patients where the challenge of life-long adherence is most burdensome. Key challenges include the need to improve the quality of vaccine-induced CD8 T cell responses in terms of central and effector-memory phenotypes, cytolytic ability and polyfunctionality. The fact that viral escape occurs in acute infection, or can be even transmitted in founder viruses, suggests that the precise epitope-specificity of vaccine-induced cytotoxic T cells is important and should not merely recapitulate responses to which vaccinees have already escaped *in vivo*. In this regard, consideration of immunogens that are more likely to promote responses against epitopes functionally or structurally constrained against mutation should be explored. Analytic treatment interruptions will be increasingly problematic as an outcome measure in clinical trials because of the known risks to both vaccine and placebo groups and therefore, vaccine efficacy will need to be measured by reliable surrogate markers of immunogenicity and viral burden and the field should standardise which assays and markers will be used to compare different platforms and products. For the moment, therapeutic vaccination remains in the realm of test-of-concept. However, in

incrementally helping to improve our understanding of the requirements for functional cure, research into therapeutic vaccination could perhaps be approached with neither wild enthusiasm nor undue scepticism but with a tempered optimism.

References

1. Rerks-Ngarm, S. *et al*. (2009) Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N. Engl. J. Med.* **361**, 2209–2220. doi:10.1056/NEJMoa0908492
2. Tubiana, R. *et al*. (2005) Therapeutic immunization with a human immunodeficiency virus (HIV) type 1-recombinant canarypox vaccine in chronically HIV-infected patients: the Vacciter Study (ANRS094). *Vaccine* **23**, 4292–4301. doi:10.1016/j.vaccine.2005.04.013
3. Kinloch-de Loes, S. *et al*. (2005) Impact of therapeutic immunization on HIV-1 viremia after discontinuation of antiretroviral therapy initiated during acute infection. *J. Infect. Dis.* **192**, 607–617. doi:10.1086/432002
4. Autran, B. *et al*. (2008) Greater viral rebound and reduced time to resume antiretroviral therapy after therapeutic immunization with the ALVAC-HIV vaccine (vCP1452). *AIDS* **22**, 1313–1322. doi:10.1097/QAD.0b013e3282fdce94
5. French, M.A. *et al*. (2010) Vaccine-induced IgG2 anti-HIV p24 is associated with control of HIV in patients with a 'high-affinity' FcgammaRIIa genotype. *AIDS* **24**, 1983–1990. doi:10.1097/QAD.0b013e32833c1ce0
6. Dorrell, L. *et al*. (2007) Safety and tolerability of recombinant modified vaccinia virus Ankara expressing an HIV-1 gag/multiepitope immunogen (MVA.HIVA) in HIV-1-infected persons receiving combination antiretroviral therapy. *Vaccine* **25**, 3277–3283. doi:10.1016/j.vaccine.2007.01.005
7. Schooley, R.T. *et al*. (2010) AIDS clinical trials group 5197: a placebo-controlled trial of immunization of HIV-1-infected persons with a replication-deficient adenovirus type 5 vaccine expressing the HIV-1 core protein. *J. Infect. Dis.* **202**, 705–716. doi:10.1086/655468
8. Duerr, A. *et al*. (2012) Extended follow-up confirms early vaccine-enhanced risk of HIV acquisition and demonstrates waning effect over time among participants in a randomized trial of recombinant adenovirus HIV vaccine (Step Study). *J. Infect. Dis.* **206**, 258–266. doi:10.1093/infdis/jis342
9. Gray, G. *et al*. (2010) Overview of STEP and Phambili trial results: two phase IIb test-of-concept studies investigating the efficacy of MRK adenovirus type 5 gag/pol/nef subtype B HIV vaccine. *Curr. Opin. HIV AIDS* 357–361. doi:10.1097/COH.0b013e32833d2d2b
10. Kraynyak, K.A. *et al*. (2010) Systemic immunization with CCL27/CTACK modulates immune responses at mucosal sites in mice and macaques. *Vaccine* **28**, 1942–1951. doi:10.1016/j.vaccine.2009.10.095
11. Rosenberg, E.S. *et al*. (2010) Safety and immunogenicity of therapeutic DNA vaccination in individuals treated with antiretroviral therapy during acute/early HIV-1 infection. *PLoS ONE* **5**, e10555. doi:10.1371/journal.pone.0010555
12. Harrer, T. *et al*. (2013) Safety and immunogenicity of an adjuvanted protein therapeutic HIV-1 vaccine in subjects with HIV-1 infection: a randomised placebo-controlled study. *Vaccine* doi:10.1016/j.vaccine.2013.10.030
13. Miller, E.A. *et al*. (2012) Plasma factors during chronic HIV-1 infection impair IL-12 secretion by myeloid dendritic cells via a virus-independent pathway. *J. Aacquir. Immune Defic. Syndr.* **61**, 535–544. doi:10.1097/QAI.0b013e31826afbce
14. Granelli-Piperno, A. *et al*. (2006) HIV-1 selectively infects a subset of nonmaturing BDCA1-positive dendritic cells in human blood. *J. Immunol.* **176**, 991–998.
15. Kundu, S.K. *et al*. (1998) A pilot clinical trial of HIV antigen-pulsed allogeneic and autologous dendritic cell therapy in HIV-infected patients. *AIDS Res. Hum. Retroviruses* **14**, 551–560. doi:10.1089/aid.1998.14.551
16. Ide, F. *et al*. (2006) Peptide-loaded dendritic-cell vaccination followed by treatment interruption for chronic HIV-1 infection: a phase 1 trial. *J. Med. Virol.* **78**, 711–718. doi:10.1002/jmv.20612
17. García, F. *et al*. (2005) Immunotherapy and therapeutic vaccines in HIV infection. *Enferm. Infecc. Microbiol. Clin.* **23**(Suppl 2), 95–104. doi:10.1016/S0213-005X(05)75165-X
18. Garcia, F. *et al*. (2013) A dendritic cell vaccine elicts T cell responses associated with control of HIV-1 replication. *Sci. Transl. Med.* **5**, 166ra2.
19. Routy, J.P. *et al*. (2010) Immunologic activity and safety of autologous HIV RNA-electroporated dendritic cells in HIV-1infected patients receiving antiretroviral therapy. *Clin. Immunol.* **134**, 140–147. doi:10.1016/j.clim.2009.09.009
20. Routy, J.P. and Nicolette, C. (2010) Arcelis AGS-004 dendritic cell-based immunotherapy for HIV infection. *Immunotherapy* **2**, 467–476. doi:10.2217/imt.10.28
21. Van Gulck, E. *et al*. (2012) mRNA-based dendritic cell vaccination induces potent antiviral T-cell responses in HIV-1-infected patients. *AIDS* **26**, F1–F12. doi:10.1097/QAD.0b013e32834f33e8

Biography

Mina John is a clinical immunologist, Royal Perth Hospital, and Associate Professor, Institute of Immunology and Infectious Diseases (IIID), Murdoch University, Western Australia.

A spectrum of (avoidable) HIV latency?

Miles Davenport

Complex Systems in Biology Group
Centre for Vascular Research
University of New South Wales
Sydney, NSW 2052, Australia
Tel: +61 2 9385 2762
Fax: +61 2 9385 1797
Email: m.davenport@unsw.edu.au

Long-lived latently HIV-infected cells present a major barrier to the eradication of the virus under ART. Current strategies are aimed at eliminating this reservoir of cells once it is established. However, it may be easier to prevent the formation of the reservoir rather than eliminate it.

Current anti-retroviral therapies are able to effectively suppress HIV replication and reduce viral levels. However, they are unable to eliminate a pool of cells that are infected with virus, but remain dormant after infection. This pool includes different cell types, such as CD4+ T cells and macrophages, that have integrated virus, but fail to express viral proteins for a prolonged period, and are thus designated 'latently infected'. Our current concept of latency is very much shaped by the problems caused by these extremely long-lived infected cells, persisting at fairly stable levels for many years on therapy and requiring the continuous administration of ART.

This particular view of latency has not always been the case. The observation that some cells only expressed viral antigens after a