Immune Correlates of Vaccine-derived protection: the case of HIV/AIDS

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Introduction

• The search for an AIDS vaccine started 25 years ago, and to the (limited) exception of the recent Thai RV144 trial, has not yet led to any promising product, whether based on neutralizing antibody or T cell responses.

• “We still don’t know how, why or if a body makes a robust neutralizing antibody and T cell response that can block acquisition and/or prevent disease progression. The reason we don’t know this, is because the body doesn’t do it in natural infection.....

“With other viruses, nature tells us just follow me and I’ll lead you to a vaccine. With HIV, nature is telling us if you follow me, you’re going to be in trouble » (A Fauci, IAVI Report 2009).
Why has it been so difficult to develop an HIV/AIDS vaccine?

1. **Virus variability is immense** and the virus constantly escapes both the humoral and the cellular immune responses of the host. In addition, it remains **latent** in reservoir cells.

2. **Correlates of protection are unknown**
   
   We still do not know the **nature, quality and quantity** of the immune responses to be induced.

3. **Animal models are imperfect**: “Mice lie and monkeys exaggerate”. The best animal model at this time remains the SIV/macaque model. But monkeys are expensive, so experiments are run on small numbers of animals.
HIV/AIDS vaccines: an historical retrospective of human trials

1) Gp120 approach: VaxGen Phase III trials with gp120 clade B or E → Only type-specific neutralizing Ab (NAb) responses, no broad NAb → no protection

2) CMI approach: Merck Phase IIb STEP trial with Ad5-HIV recombinants → No protection against infection, no protection against disease, but an unexpected increase in infectibility in uncircumcised vaccinees with preexisting immunity to the Ad5 vector.

3) Combined approach: Thai RV144 Phase III trial with ALVAC prime and gp120 boosts → No NAb, no CTL, but 31% protection against infection (39% in women, 26% in men).
The trial, which took place in the United States, Puerto Rico, Canada, and the Netherlands, has strong reservations about subset analyses in general. “Subset analyses are notoriously difficult to interpret, and they’re doubly difficult when the overall result is nil, which is the case here,” says Self.

Seth Berkley, head of the International AIDS Vaccine Initiative, a nonprofit organization that bankrolls development of products, is more blunt. He notes that VaxGen’s subanalysis hinged on “just 13 infections”...
The Merck Ad5-HIV gag, pol, nef Phase IIb « STEP » trial

An Ad5-HIV gag,pol, nef vaccine was administered three successive times to >1000 volunteers at risk. Some volunteers were pre-immune to the vector (Ad5)

The trial eventually had to be stopped because of an increased number of infections in the uncircumcised volunteers with previous immunity to the Ad5 vector: 29/532 infections scored in the Ad5 + vaccinated group vs 13/528 in the Ad5 + placebo group (Schoenly, Weiner J Virol 2008)

...then came September 2009

- "Vaccin contre le SIDA: enfin des résultats encourageants!"  
  (Le Figaro, vendredi 25 septembre 2009)

- "6 year study with 16,000 participants, costing $100M: For first time, AIDS vaccine shows some success"  
  (Donald McNeil, September 25, 2009)
The RV144 Phase III trial (September 2009)

- ALVAC-HIV env, gag, pol + gp120 clade E: prime-boost regimen tested on 16,400 volunteers in Thailand: 51 documented infections in the vaccinated group versus 74 in the placebo group, i.e. a (statistically significant) 31% reduction in the number of infections. Protection actually was 61% at year 1 and appeared to progressively wane with time.

- No difference was observed in virus loads between infected placebos and infected vaccinees, showing the vaccine elicited no protection against disease (no cellular immunity effect).

- No broadly neutralizing Ab were elicited.

- So, what was the basis for protection?
Possible correlates of protection against HIV/SIV infection/disease

1. **Neutralizing antibodies**: cross-clade, broadly neutralizing Abs

2. **Cytotoxic T cells**: CD8+ CTLs

3. **Nonneutralizing antibodies**:  
   A. antibody-dependent cellular cytoxicity (ADCC)  
   B. transcytosis-blocking antibodies (mucosal IgAs)

4. **Innate immunity**: NK cells; APOBEC3G (A3G)
1. The role of CD8+ T cells in protection

Initial demonstration of the protective role of CD8+ T cells was in the SIV/macaque model: experimental depletion of CD8+ T cells by anti-CD8 Mabs in SIV-infected animals → large increase in virus loads and accelerated disease progression with premature death (Schmitz, Science 1999; Letvin, Immunity 2007)
Role of CD8 CTLs in HIV-1-infected humans

• Human «elite controllers», whose viral load remains <75 copies/mL in the absence of antiviral treatment, show potent, multi-functional, viral infection-suppressing CD8+ CTL responses (Almeida, J Exp Med 2007; Migueles, Immunity 2008).

• Polyfunctional CD8+ T cells are also found in HIV controllers (<2000 copies/mL), including in mucosal tissues. Controllers frequently have a B27, B52 or B57 HLA haplotype (Betts, Blood 2006; Saez-Cirion, PNAS 2007; Emu J Virol 2008; Ferre, Blood 2009).
Perforin expression by CD8+ T cells, a new correlate of HIV elite control

(Hersperger et al, PLoS Pathog 2010)
CD8+ T cells in mucosal tissues

- HIV-specific CD8+ CTL have also been found in the cervical tissue in HIV-1-exposed, persistently seronegative (‘HEPS’) commercial sex workers (Rowland-Jones, 2000).
Role of CTLs in vaccine protection against rectal SIV challenge in monkeys

- Macaques were immunized intracolorectally using a SIV peptide prime/MVA-gag, pol, env, rev, tat and nef boost protocol.
- Both peptides and MVA were mixed together with Poly I:C, CpG and IL-15 as adjuvants
- Seven weeks after the last immunization, the animals were challenged with a low dose SIV mac251 by the colorectal route
- SIV RNA levels were monitored for 185 days.
  (Sui et al, Proc Nat Acad Sci USA 2010)
Viral loads upon rectal SIV challenge as a function of time (Sui et al, PNAS 2010)
Protection against rectal SIV challenge correlated with polyfunctional T cells

- Plasma VL upon rectal SIV challenge was inversely correlated with polyfunctional T cells, measured in PBMCs using intracellular cytokine staining (ICS) for IFNγ, IL-2 and TNF but in a non linear fashion (threshold=2%)
- No correlation was however observed between VLs and the frequency of tetramer-positive central and effector memory T cells in the colon
- Protective polyfunctional T cells stain positive for IL-2, TNF, MIP-1β, IFN-γ and granzyme; they actively suppress viral replication through cell killing

(Sui et al, Proc Nat Acad Sci USA 2010)
CD8+ T cells and vaccine protection against vaginal SIV challenge

- Immunization of macaque monkeys using attenuated SHIV 89.6 consistently elicited protection against intravaginal SIV challenge
- Protected animals showed polyfunctional, degranulating SIV-specific CD4+ and CD8+ T cells in the vaginal mucosa (Genescà, Mucosal Immunol 2008 and J Intern Med 2009)
- Furthermore, depletion of CD8+ T cells completely abrogated protection, showing that the protection from vaginal SIV challenge was mediated by effector T cell responses (Genescà, J Virol 2008)
2. The role of nonneutralizing antibodies in protection

A. **Fc-mediated anti-viral effector functions**: 
   
   • **Antibody-dependent cellular cytotoxicity** (ADCC) occurs when an Ab molecule bound by its **Fab segment** to a cognate viral Ag on the surface of an infected target cell interacts through its **Fc portion** with the Fc receptors of an effector cell (NK cell, monocyte), leading to death of the target cell. 
   
   • **Antibody-dependent cell-mediated viral inhibition** (ADCVI) is similar to ADCC but the read-out is the **inhibition of virus production** rather than the death of the target cell. 

(Forthal & Moog, Curr Opin HIV AIDS 2009)
HIV-specific ADCC/ADCVI in humans

• **ADCC** activity in serum decreases with CD4+ T cell loss and disease progression in HIV-infected individuals; it is **higher in elite controllers** (Lambotte, AIDS 2009)

• A high vaccine-induced ADCVI activity was associated with **lower rate of sexually-acquired HIV infection** in gp120-vaccinees (Forthal & Moog, J Immunol 2007)

• **ADCC** was reported following immunization with gp120 in the **Thai RV144 Phase III trial** (Karnasuta, Vaccine 2005) and in a Phase I/II trial of a HIV multiprotein subunit vaccine (Goepfert Vaccine 2007). Its possible correlation with protection was unfortunately **not assessed** in these trials
ADCC/ADCVI activities in passive immunization NHP models

• Protection of newborn monkeys against oral SIV infection by passive immunization with a nonneutralizing anti-SIV serum strongly correlated with ADCVI activity of the serum (Van Rompay, J Infect Dis 1998)

• Passive immunization with broadly neutralizing MAb b12 induced protection in 8/9 monkeys against vaginal SHIV challenge. A variant of IgG1 b12 that bound poorly to FcR retained full neutralizing activity but protected only about 50% of the animals, implying ADCC/ADCVI as an important mechanism in protection (Hessell, Nature 2007; Hessell Nat Med 2009)
ADCC/ADCVI activities in active immunization SIV/macaque model

• Rhesus macaques were immunized using a Ad5 hr-SIV recombinant /SIV enveloppe subunit prime-boost regimen then challenged intrarectally with SIV mac251 (Patterson, J Virol 2003 and 2004).

• A strong level of protection was achieved in the monkeys in spite of total absence of neutralizing Ab induction

• A significant correlation was found between ADCC activity in serum and mucosal secretions and protection against challenge (Gomez-Roman J Immunol 2005; Hidajat, J Virol 2009)
ADCC/ADCVI activities in the SHIV model

- A very similar observation was made using the SHIV model: monkeys were immunized using a prime-boost vaccination regimen with Ad5 hr-HIV recombinant followed by HIV gp140
- The animals were challenged by the intravenous route with SHIV 89.6P
- Both ADCC and ADCVI activities were observed to correlate with reduced acute and chronic viremia following the SHIV challenge (Xiao, J Virol 2010)
2. The role of nonneutralizing antibodies in protection

B. Transcytosis inhibition:

- Multiple mechanisms for HIV to pass through the mucosal barrier have been proposed that include:
  - **transcytosis** of HIV-1 across **simple columnar epithelial layers** (endocervix, rectum, GI tract)
  - Uptake of HIV-1 by **intraepithelial Langerhans cells (LC) or dentritic cells (DC)** in **stratified squamous epithelia** (oral cavity, oesophagus, anus, vagina and exocervix)
HIV may cross the mucosal barrier by:
1. DC transportation
2. Transcytosis through epithelial cells
3. Transcytosis through M cells

Trancytosis: translocation of HIV across the host’s epithelial membranes of the genital & intestinal tracts

HIV produced by the infected cells

Migration of HIV-infected cells to proximal lymph nodes

HIV replication in lymph nodes

Virus spread into the host

Adapted from Hakim Hocini & Morgane Bomsel
J. Infect Dis 179:S448-453, 1999
The role of mucosal secretory IgAs

• Mucosal IgAs specific for HIV-1 gp41 MPER have been shown to block HIV-1 transcytosis across epithelial barriers in vitro (Alfsen, J Immunol 2001; Nguyen, J AIDS 2006; Shen, J Immunol 2010). Such IgAs can be found in cervicovaginal secretions of highly exposed persistently IgG seronegative women.

• Cervical B cells from these women were used to generate libraries of monoclonal IgAs that inhibit HIV transcytosis and prevent infection of T cells or macrophages in vitro (Tudor, Mucosal Immunol 2009; Tudor, Nat Immunol 2009).
GP41-virome immunization
(Bomsel et al, Submitted)

- Female macaque monkeys were immunized with rgp41 and an MPER peptide (P1) grafted onto virosomes: either 4 times by the IM route or twice IM then twice intranasally (IN).
- The animals were then challenged 13 successive times by the vaginal route with a low dose (30 TCID50) of SHIV SF162P3 (once- or twice-a-week)
- None of the IM/IN immunized females, and only 3/6 IM immunized females, became infected (vs 6/6 placebos)
- All the protected animals developed transcytosis-blocking IgAs in their vaginal secretions. None showed evidence of NAbs in their serum
Mucosal transcytosis-blocking Abs as a correlate of mucosal protection

- Mucosal transcytosis-blocking Abs were also detected in the Ad5hr/env subunit prime-boost SIV immunization and rectal SIV challenge experiment (Patterson, J Virol 2003 and 2004)

- The transcytosis-inhibition activity correlated with reduced chronic viremia after challenge (Xiao, J Virol 2010)
3. Innate immune responses: APOBEC3G as a possible correlate of protection

• APOBEC3G (A3G) is a potent cellular host factor of innate immunity that can restrict lentivirus infection through cytidine deamination of proviral DNA, leading to G to A hypermutations (Sheehy, Nature 2002; Mangeat, Nature 2003; Zhang, Hum Mol Genet 2004)

• HIV counters A3G activity by using the Vif protein to bind and target A3G protein for enhanced degradation through proteasomal pathways (Cullen, J Virol 2006)

• Upregulation of A3G can prevent cells from HIV or SIV infection in vitro (Pido-Lopez, J Immunol 2007)
A3G levels in the SIV model

• In the SIV vaccination/challenge experiments of Sui et al (PNAS 2010), another correlate of protection was found: A3G mRNA levels in mesenteric lymph nodes.

• There was a significant inverse correlation between viral loads at set point and A3G mRNA levels in DCs, CD4 T cells and monocyte/ macrophages in the immunized monkeys which became infected upon challenge.

• Adjuvant alone (Poly I:C, CpG and IL-15) without vaccine antigen also upregulated the level of A3G and elicited partial protection.
Correlation between set-point plasma VL and A3G expression levels in the colon IEL
APOBEC3G (A3G) mRNA levels in HIV-1 infected persons

• A3G mRNA levels (measured by PCR) are elevated in PBMCs of HIV-1 controllers (long-term nonprogressors) (Jin, J Virol 2005; Jin, Retrovirology 2007)

• They also are elevated in PBMCs and cervical tissues of HIV-exposed seronegative (« HEPS ») individuals (Biasin, J Infect Dis 2007)

• They seem to correlate positively with CD4+ T cell number and negatively with HIV-1 viral loads in infected individuals (Zhao , J Acquired Immune Defic Syndr 2010)
A3G mRNA levels in HIV infection
Conclusion-1

1. Control of viremia in SIV/SHIV/HIV infection correlates with and is dependent on CD8+ CTLs in macaques and chimpanzees (Belyakov Blood 2006 and J Immunol 2007)

Mucosal protection against SIV/SHIV infection in NHP models correlates with high-avidity, polyfunctional, degranulating mucosal CD8+ CTLs

There is indirect evidence for the role of such high avidity, polyfunctional, anti-HIV CD8+ T cells in protection against HIV disease progression in humans.
• Mucosal antibodies (essentially gp41-specific IgAs) that inhibit HIV/SIV transcytosis across an intact epithelial cell layer correlated with reduced chronic viremia after rectal SIV challenge or full protection against vaginal SHIV challenge in NHP models. These Ab may play an important role in mucosal protection in humans

• Nonneutralizing antibodies (mostly Env-specific IgGs) can also play a major role in protection through ADCC and ADCVI, as seen in a variety of SIV and SHIV vaccine protection experiments in rhesus macaques
Conclusion-3

• Elevated A3G-mediated intrinsic resistance of DCs can counteract SIV infection at the mucosal portal of entry. Levels of A3G expression can be upregulated by certain combinations of adjuvants and correlate with protection against mucosal SIV challenge (Pion, J Exp Med 2006; Wang, Vaccine 2009; Sui, PNAS 2010)
A model of vaccine protection: the live attenuated SIVΔnef

- **SIV Δnef**: Single (Δnef) or multiple (Δnef, Δvif, etc..) deletions of accessory genes in the SIV genome attenuate its virulence for rhesus monkeys (Daniel, Science 1992; Wyand, J Virol 1999).
- The virus is still, however, **virulent for newborn monkeys**; in adult monkeys, it establishes a **permanent low-level lentivirus infection**
- **SIV Δnef** has been used as an **attenuated virus vaccine**, as it provides protection against infection with virulent SIV strains
- **Note that protection is of a non-sterilizing kind**:
  - 3 logs decrease in **viral load** after homologous challenge;
  - 2 logs decrease after heterologous challenge

**Correlates of protection by SIV Δnef remain unknown**
(Mansfield, J Virol 2008)