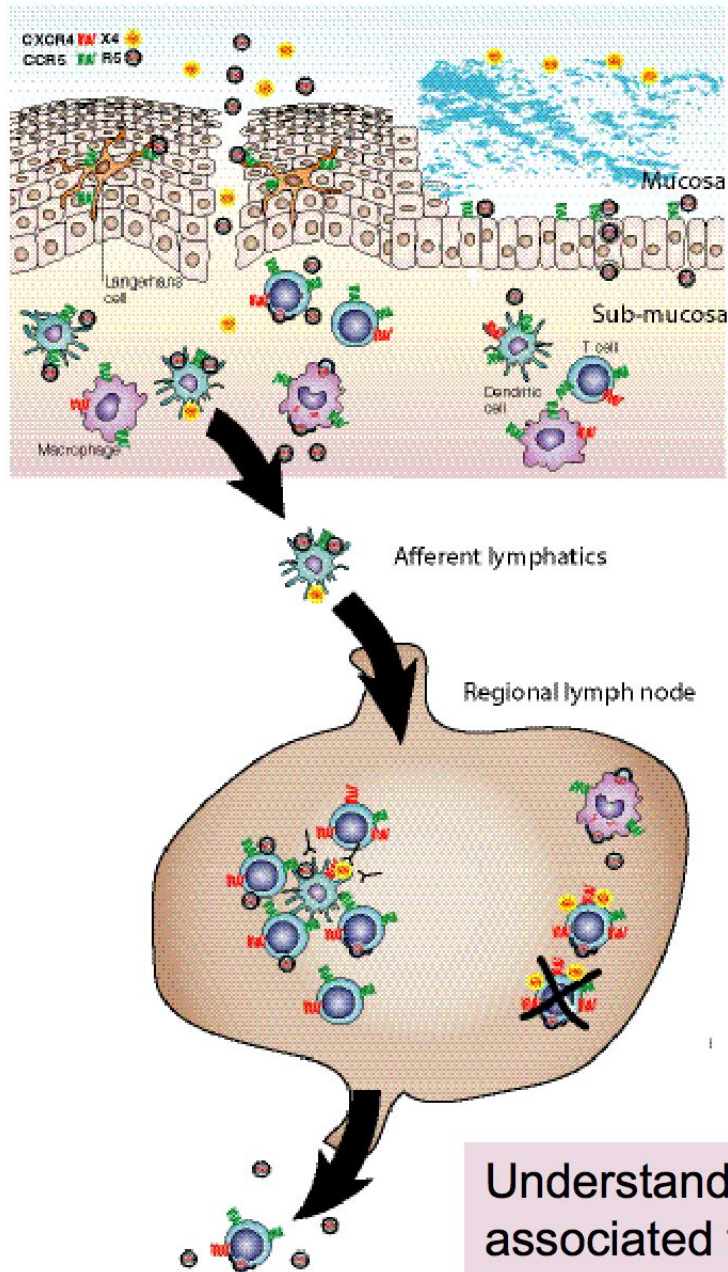


*Mucosal protection to HIV-1:
what to induce and how to
measure it?*

AIDS Vaccine 07, Seattle, August 20-23, 2007

Robin Shattock
St George's, University of London

The time to act is short !



Exposure: 30-60 mins

DC-T cell transfer 1-4 hours
(virological synapse)

Localized infection: 16-72 hours

Dissemination to draining LN: 24-72 hours
(virological synapse)

Induction of memory responses: 3-5 days

Understanding the relative efficiency of cell free vs cell-associated transmission in immunized animals

A number of critical questions remain unanswered

- The relative role of cell free vs. infected cells in mucosal transmission,
- The potential mechanism of viral transport across mucosal surfaces,
- The identity, frequency, location and role of the primary targets,
- The relative importance of these by mucosal route,
- The relative impact of mucosal responses on these different pathways.

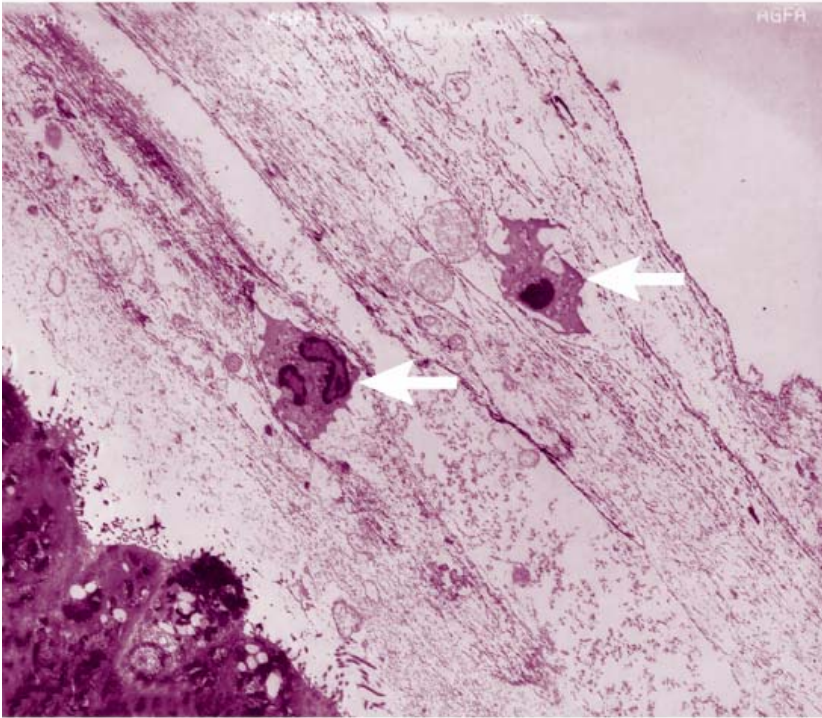
Langerhans Cells in Ectocervix

HIV can be found associated with Langerhans cells located in the interstitial space of the squamous epithelium

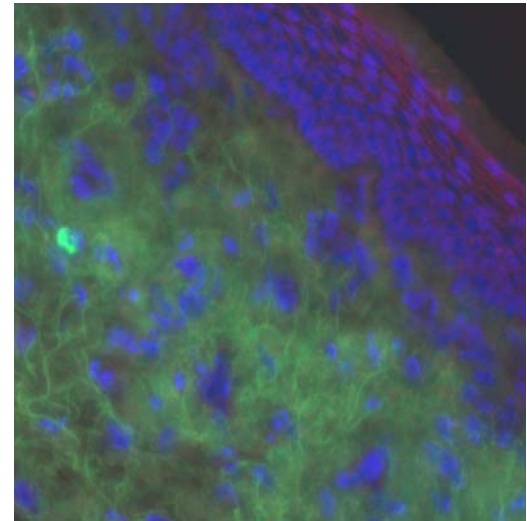
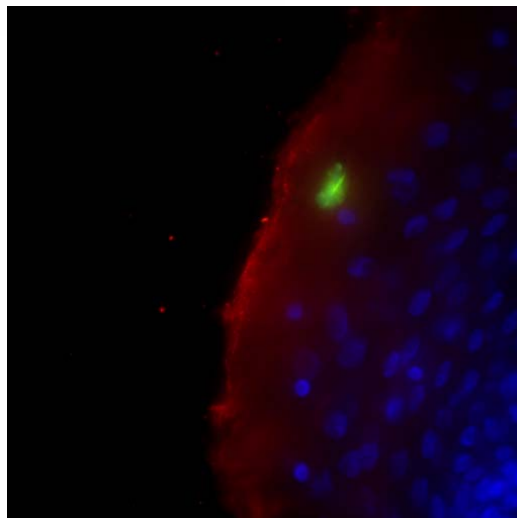
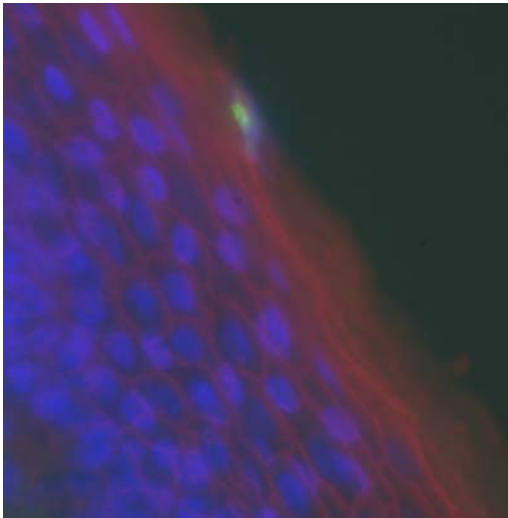
T. Hope

CD1a (Langerhans cells)

GFP-VPR labeled HIV after photoactivation



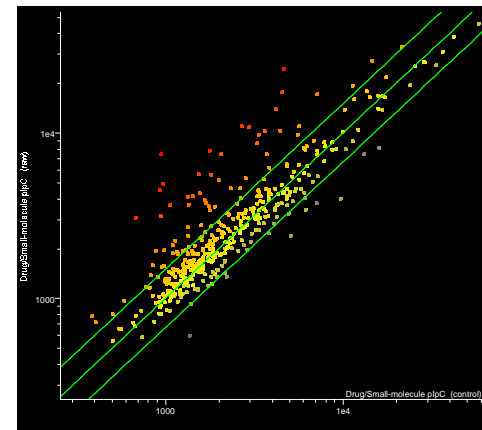
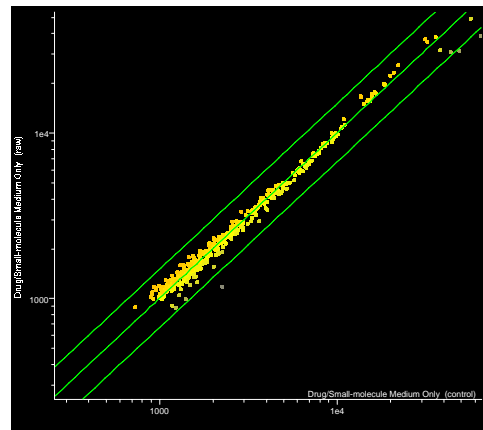
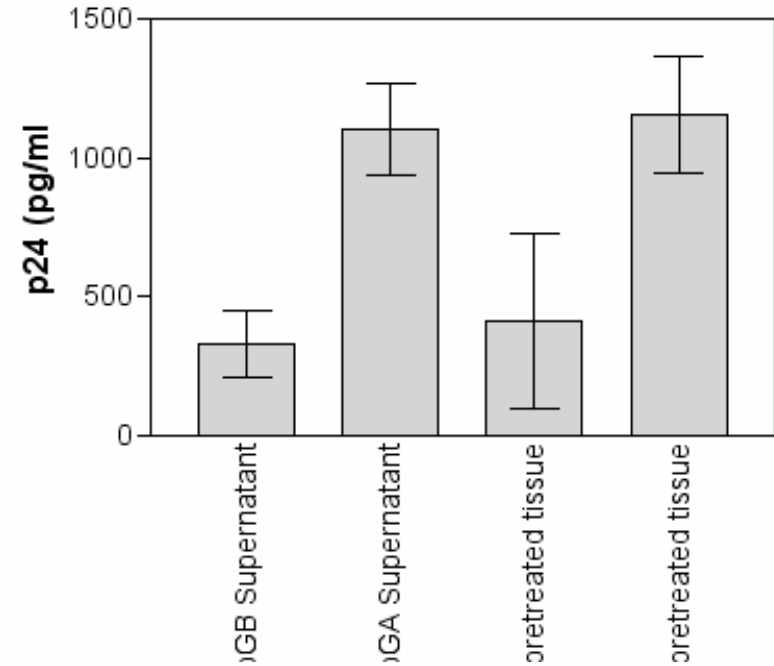
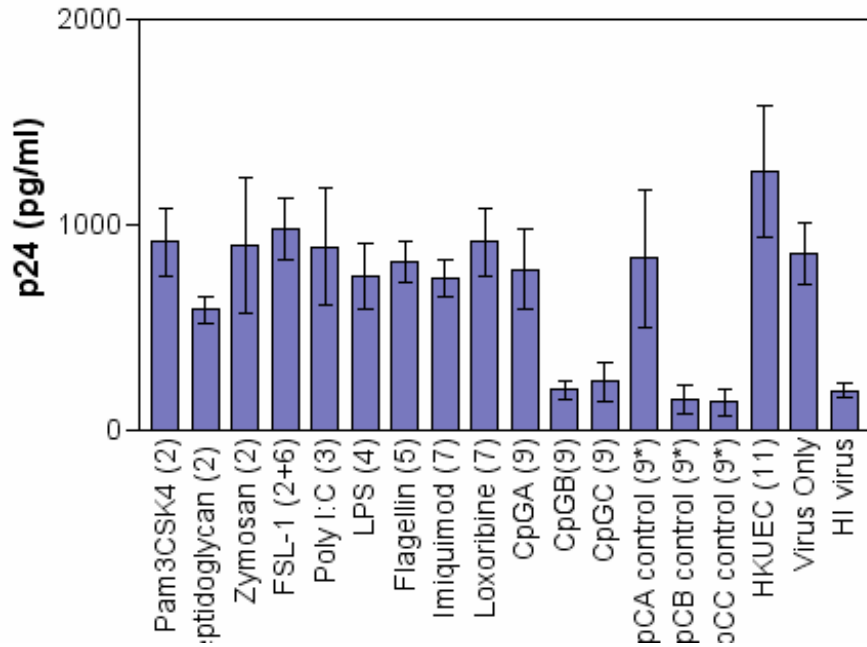
Cell free or cell associated virus?



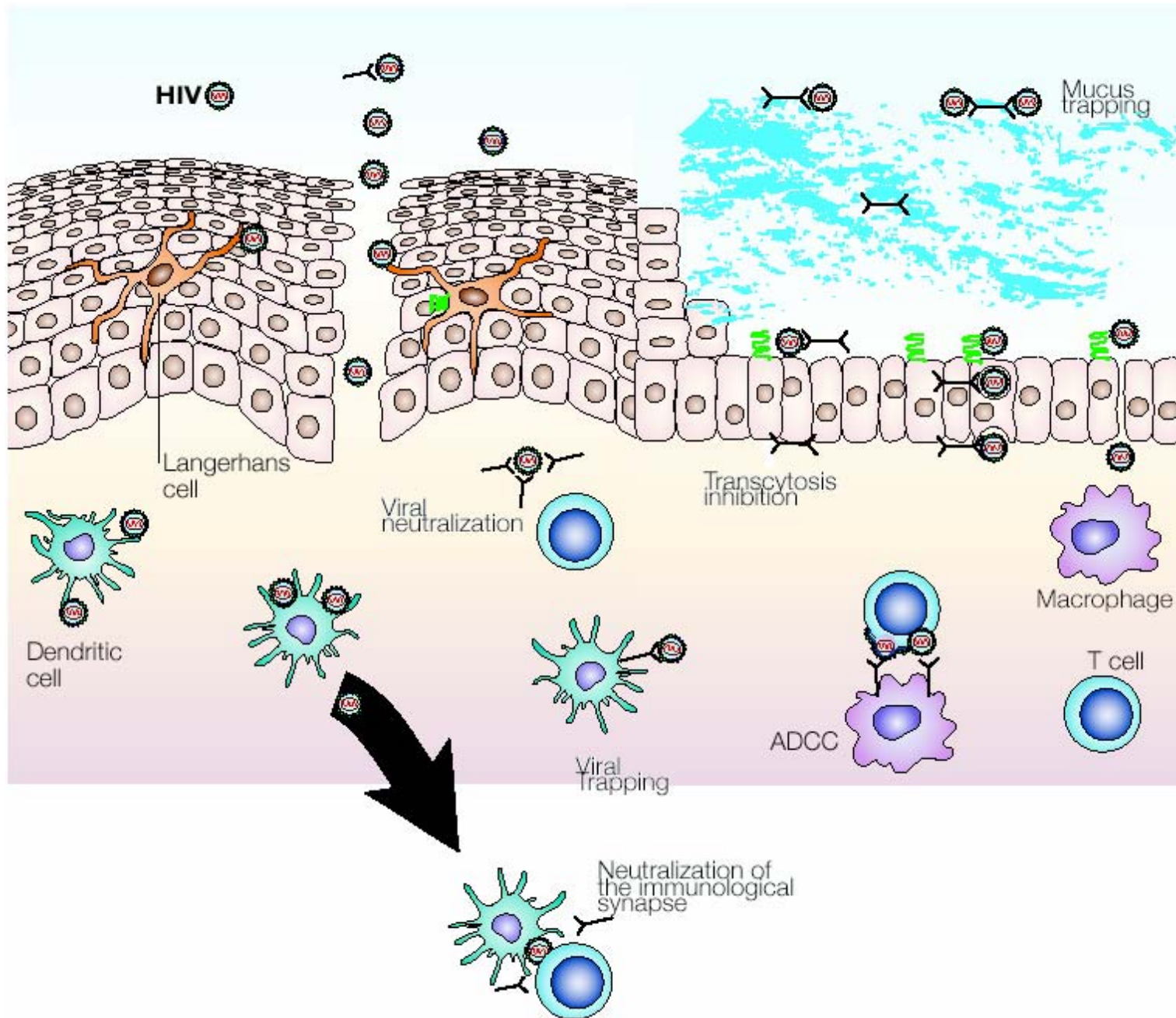
Does innate immunity modulate mucosal HIV transmission?

- Innate immune responses occur within minutes or hours of pathogen entry
- Innate antiviral factors (intracellular and secreted) modulate viral replication
- Their role in modulating transmission/susceptibility is not fully understood
- The innate responses to viral exposure may be critical in influencing primary and secondary adaptive immune response
- TLRs and non-TLR pattern recognition receptors modulating the strength, quality and persistence of adaptive immune responses.

Can innate immunity control viral replication in mucosal tissue?



What humoral responses needed at the time of infection



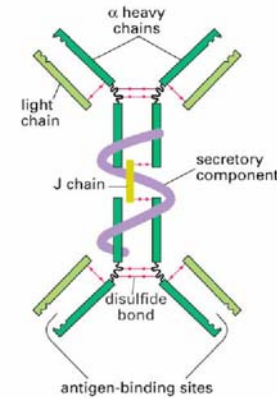
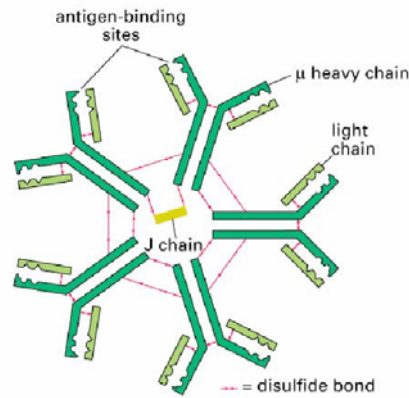
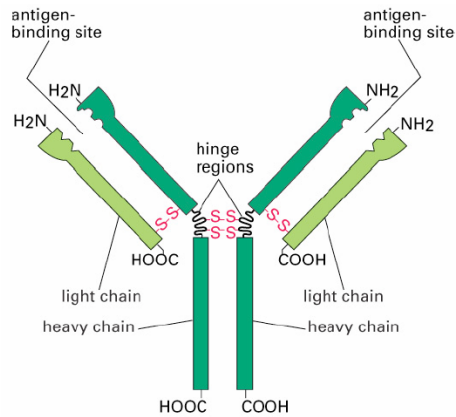
What needs to be measured and how?

- Are neutralizing antibodies the only responses contributing to robust mucosal protection?
- Do other functional characteristics have equal or additional importance?
- How is this modulated by antibody isotype?
- What is the role of locally produced antibodies?
- How much spill over of systemic antibodies is there into mucosal compartments?
- Is this changed by sexual arousal?
- Are luminal antibodies important?

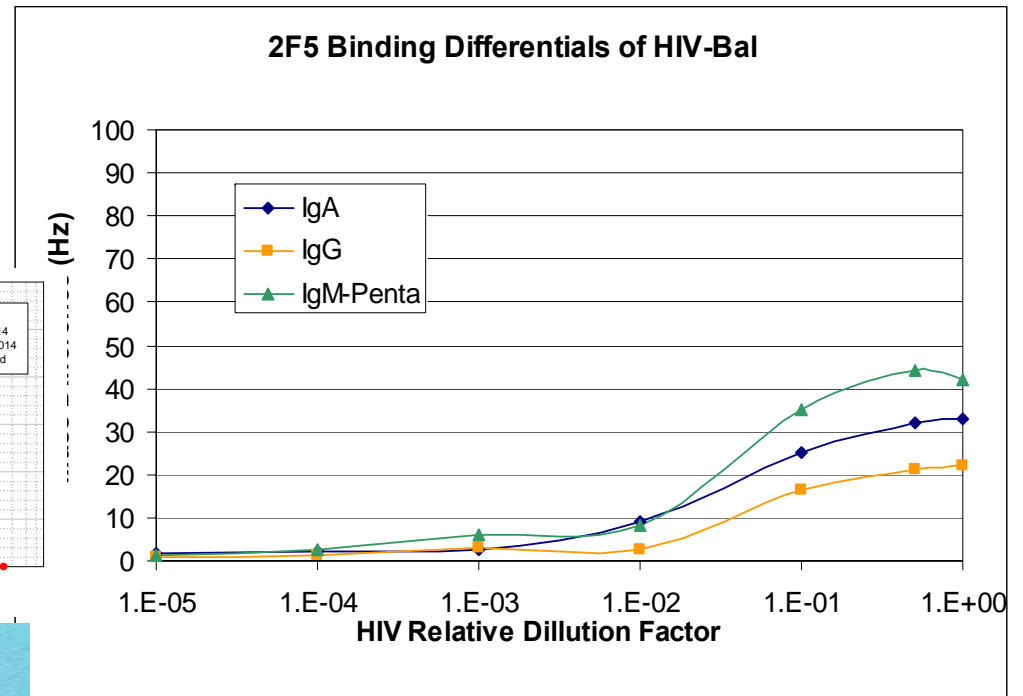
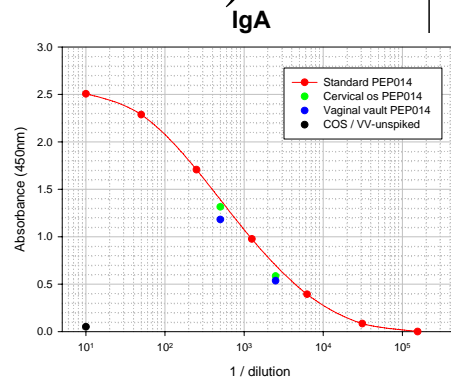
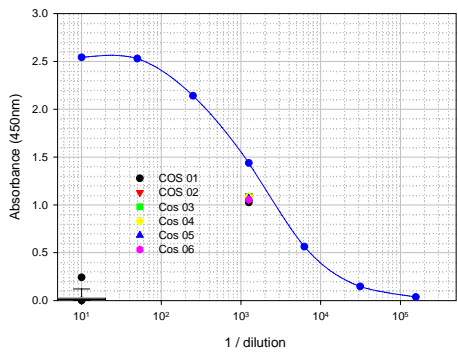
Are local events important for mucosal protection?

- Passive transfer of b12 Mab requires 25mg/kg for sterile protection against vaginal challenge.
- *Parren...Burton et al. J Virol, 2001. 75:8340-8347.*
- Greater protection was achieved against vaginal vs. IV challenge - *Mascola et al. Nat Med, 2000. 6:207-10*
- Topically applied b12 Mab (5mg) provides 80% protection (4/5) - *Veazey et al. Nat Med, 2003, 9:343-346*
- IgA secreting cells in the iliac lymph nodes of TILN immunized macaques correlate significantly with protection from infection - *Lehner et al. Nat Med. 1996, 2:1054-5.*

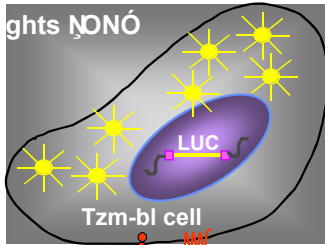
What are the correlates of protective antibody responses?



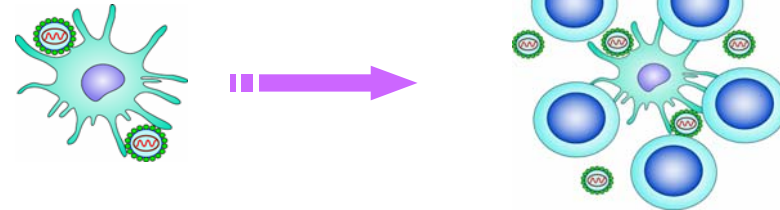
- neutralizing antibodies?
- IgG, sIgA, IgM?
- Systemic vs local?
- Kinetics (when and where)?



Greater emphasis on development of functional mucosal antibody assays.



Neutralization assays – cell-free infection
TZM-BI, PBMC & M0

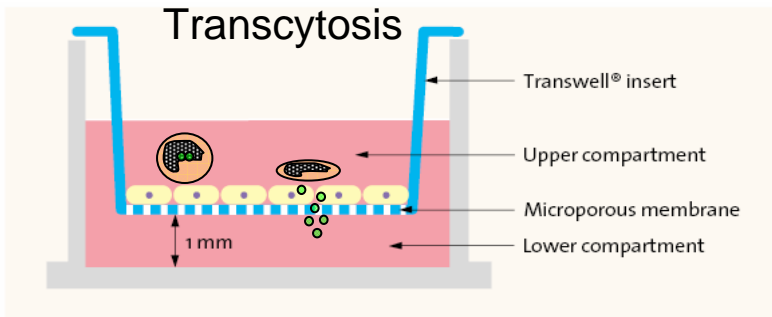


Cell-cell transmission

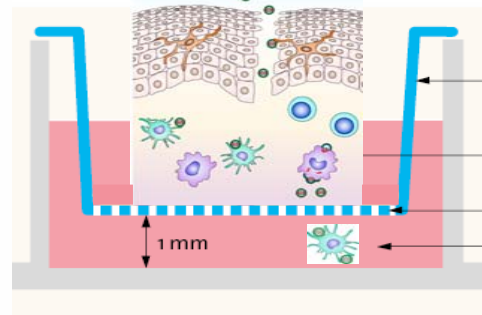
Moog - P10-1 & 16
OA05-03

Defining predictive Mucosal assays

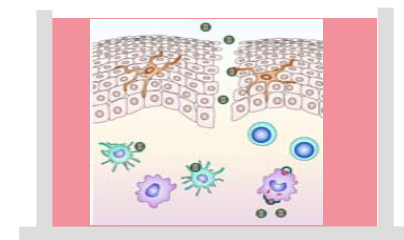
Blockage of Transcytosis



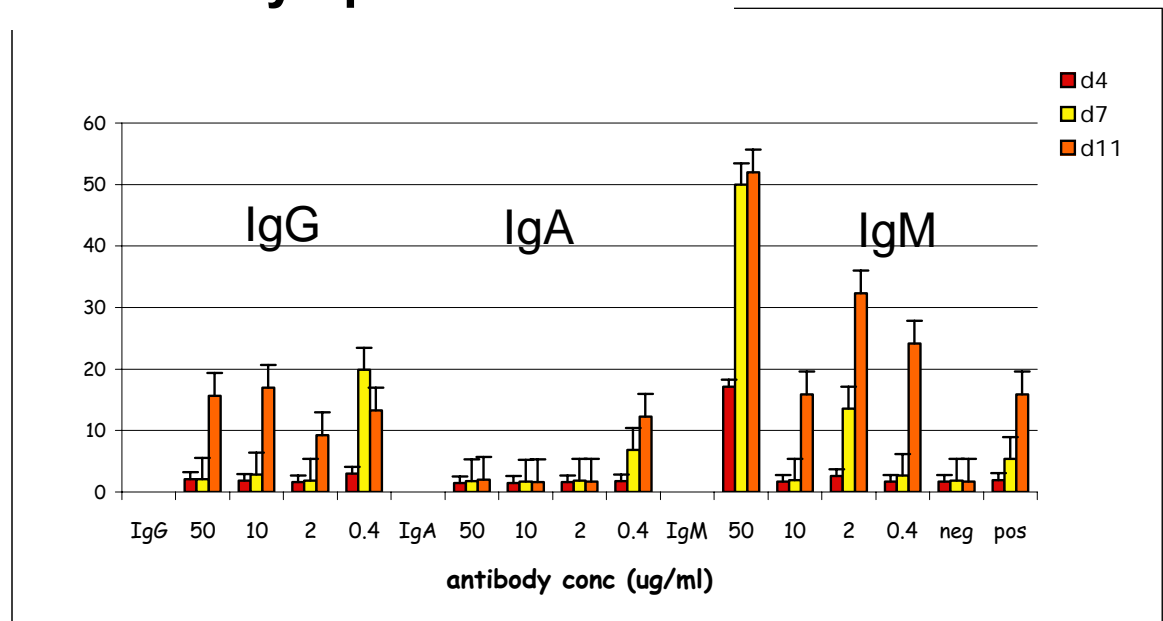
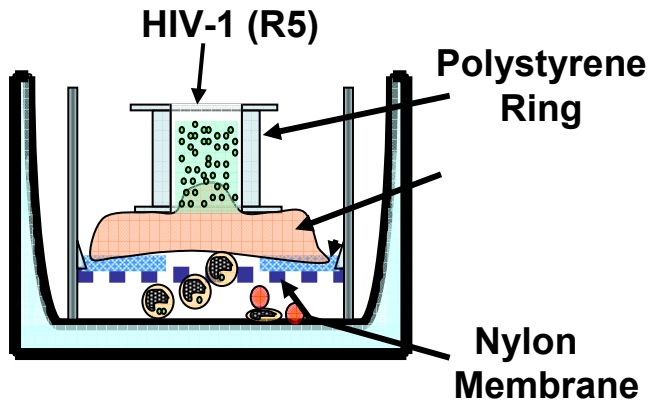
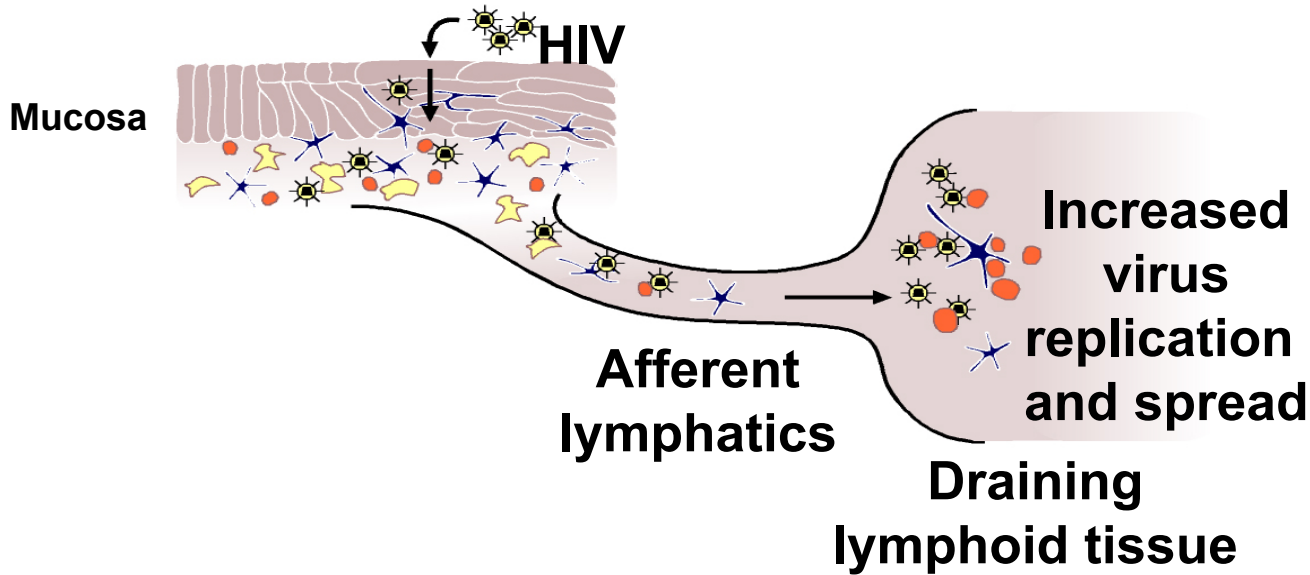
Mucosal explants (polarized) - transmission



Mucosal explants - replication and neutralization of 'acute' env / HIV



2F5 IgA provides the most potent inhibition of HIV-1 dissemination from cervical by migratory DCs



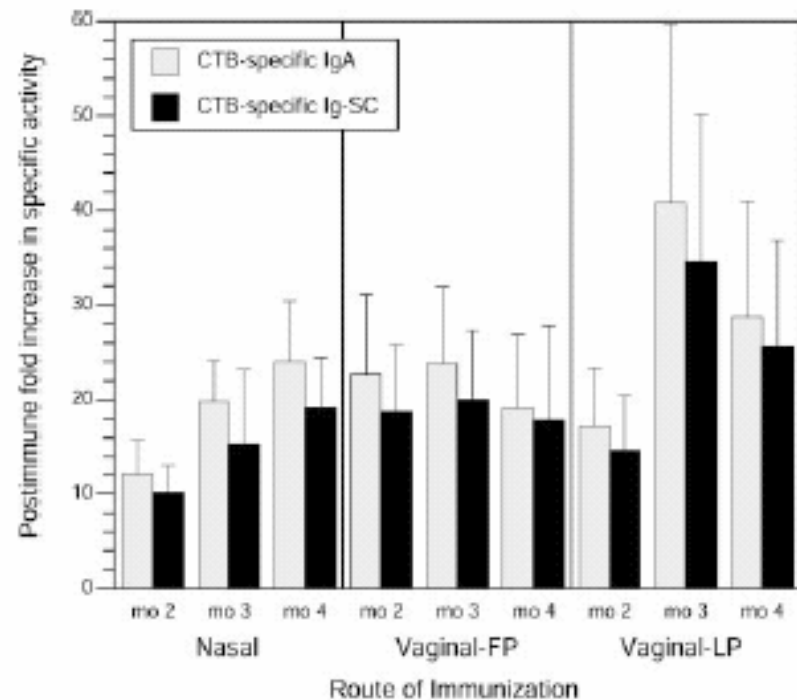
Can protective effector immune responses be maintained at the portal of entry?

- Inefficient targeting of mucosal antigen presenting cells (APCs).
- Rapid leakage from the administered sites.
- Degradation of antigens by enzymes in mucosal secretions
- Differences in immune response during the menstrual cycle.

However, appropriate cervicovaginal delivery of antigens can induce better local IgA responses than either rectal or nasal delivery

Vaginal immunization can induce localized and systemic immune responses

Dominant isotypes	IgG >< IgA
Hormonal regulation	+++
Inductive site	- to +
Effector site	++
Contribution from the circulation	+++ (~ 50%)



- *Vaginal immunization induces Cholera vaccine-specific cervical IgA Ab responses after vaginal immunization during the midfollicular menstrual cycle phase.*

- *Kozłowski et al Journal of Immunology, 2002, 169: 566–574.*

- *The magnitudes of these responses are 20-fold for IgA antitoxin and 7.1-fold for IgG antitoxin*

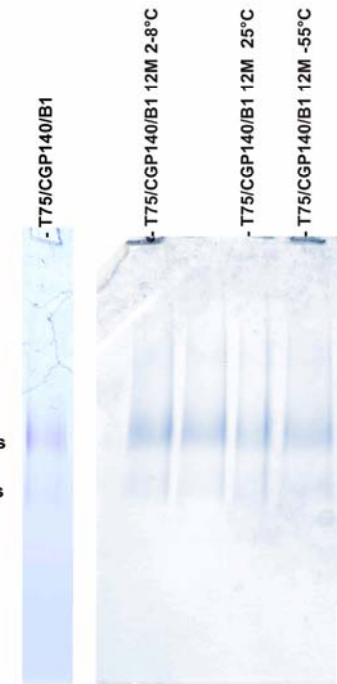
- *Wassen et al, Clinical Vaccine Immunology 2006, 13, 202-207*

GMP product

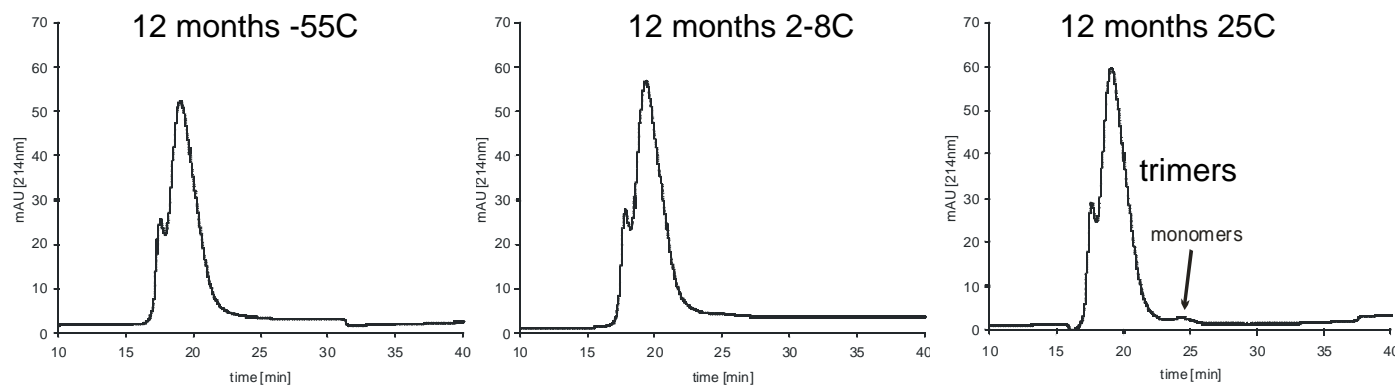
Stability data of GP140 final product after 12 months

Test	Day 0	12 Months		
		$\leq -55\text{ }^{\circ}\text{C}$	$2-8\text{ }^{\circ}\text{C}$	$25 \pm 2\text{ }^{\circ}\text{C}$
OD ($\mu\text{g/ml}$)	530	528	522	569
SEC (% fragments and monomer)	< 5	< 5	< 5	< 5
SDS-PAGE	pass	pass	pass	pass
Native PAGE	pass	pass	pass	pass
WB	pass	pass	pass	pass
Osmolality (mOsmol/kg)	313	311	313	320
pH	7.4	7.3	7.3	7.3
Visual appearance	colourless, clear	colourless, clear	colourless, clear	colourless, clear

Native PAGE
stained with Coomassie



Elution profiles of size exclusion chromatography



Antigen very stable, unlikely to require refrigeration

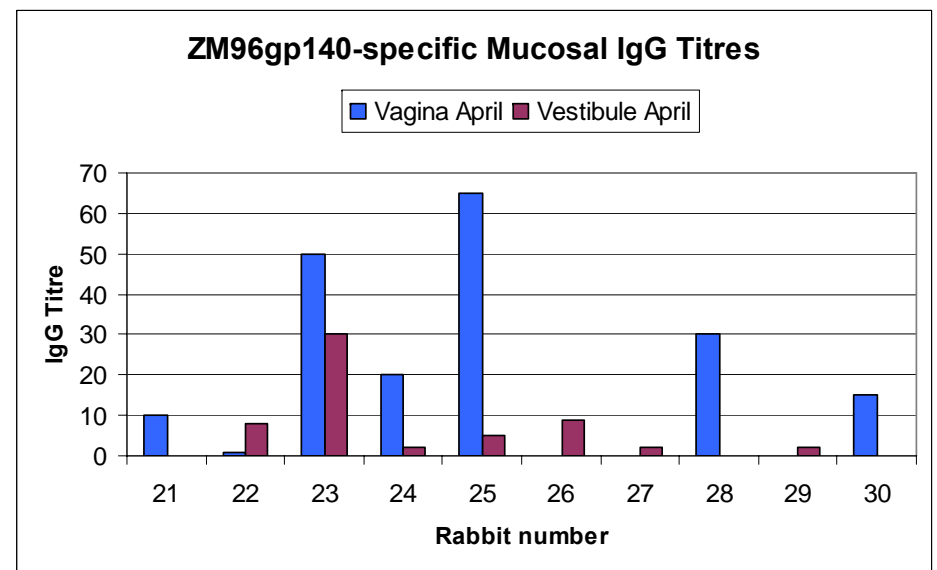
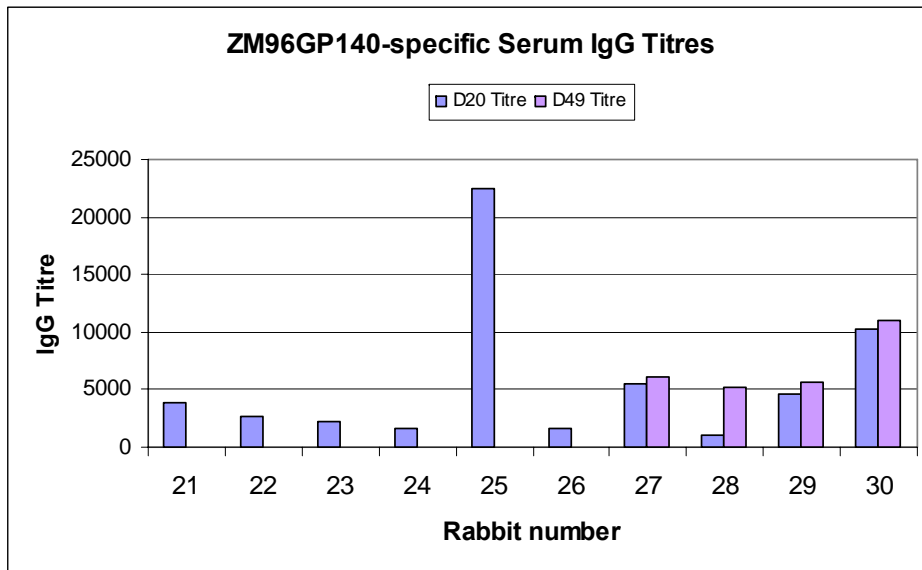
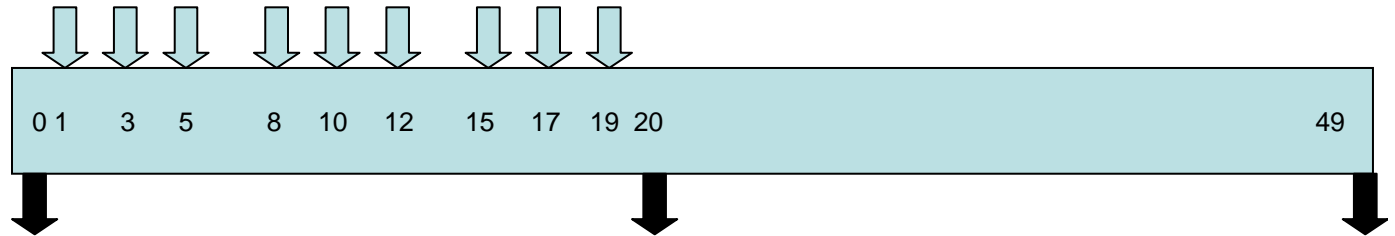
Grand Challenges
in Global Health



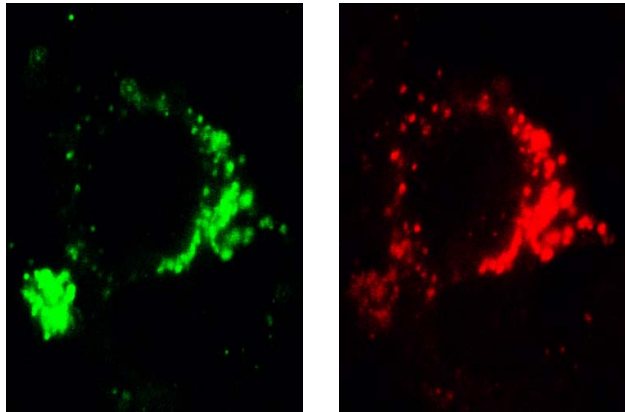
Can vaginal immunization induce specific immune responses?



Intravaginal application of
100 μ g (620 μ l) ZM96 gp140 in gel formulation

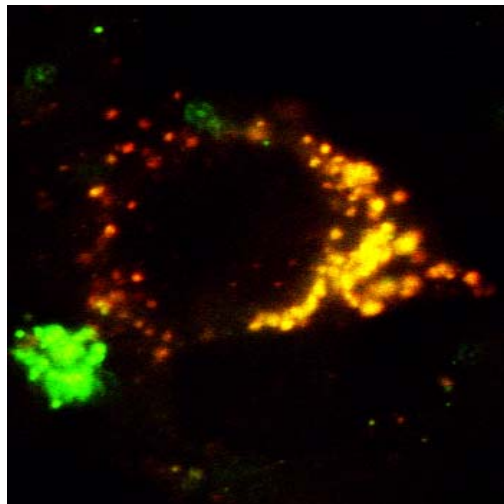


Nanoparticles are internalised by DC and incorporated into endolysosomes

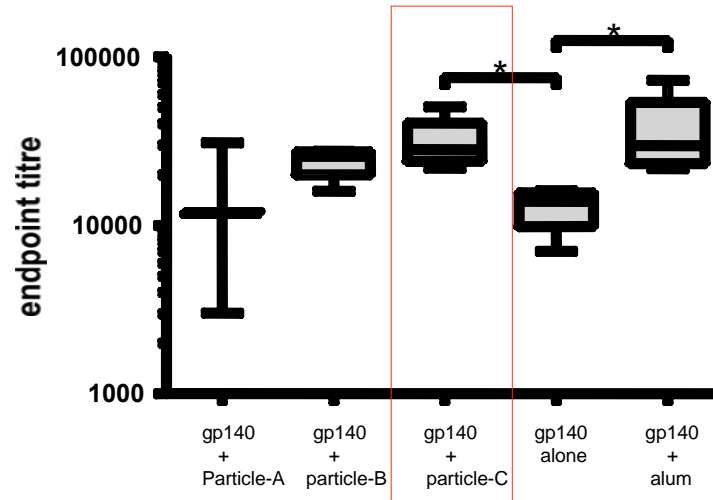


particles

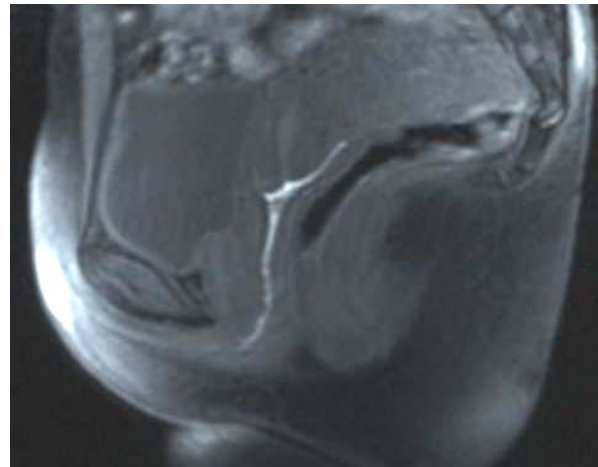
lysosomes



Yellow: particles within lysosomes



Loxley et al P13-44



Lacey et al

AIDS Vaccine 07, Seattle, August 20-23, 2007



**Global HIV Vaccine
Enterprise**

**EMERGING ISSUES in
mucosal and innate immunity to HIV**
**(Summary of the recommendations from an Enterprise
Working Group)**
Thursday 13.30 Grand Ballroom

Promoting innovation and collaboration
to speed the search for an HIV vaccine