Transmission of HIV-2 compared with HIV-1 infection

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HIV-2: a naturally attenuated human retrovirus infection?

1983 - HIV-1 isolated
- Evolved from SIV_{cpz}
  - Group M cross-species transmission:
    - ~1930 +/- 15 years

1986 - HIV-2 isolated
- Evolved from SIV_{sm}
- Endemic amongst sooty mangabeys (Tou forest, Cote d’Ivoire)
  - Each subtype represents a distinct cross-species transmission:
    - Probably 8 separate entries into humans
    - ~1940 +/- 16 years (Lemey, PNAS 03)

Guinea Bissau suggested as epicentre of HIV-2 epidemic in West Africa
- Rapid spread began in 1960-70
- Coincides with war of independence (1963-74)

Epidemiological linkage with Portugal
- First HIV-2 cases in Europe recorded in Portuguese war veterans
- Lisbon has the largest number of HIV-2 cases in Europe
- HIV-2 found in Angola, Mozambique, Goa
- Also reported in India, Brazil, S. Korea, Japan

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Epidemiology of HIV infection in West Africa

- **Annual sentinel surveillance - HIV-1 seroprevalence now 2% in the Gambia and is probably rising, especially in young adults**
- **Stable seroprevalence of HIV-2 infection ~1% during 80s and 90s, more common in older age-groups**
- **HIV-2 seroprevalence at its peak (1980s - 90s)**
  - 8-10% Guinea-Bissau
  - >20% in adults > 40 yrs, Bissau
  - 1-2% The Gambia, Senegal
  - 28% Gambian sex workers (1991)
- **HIV-2 prevalence now reported to be declining throughout W. Africa**
  - Prevalence has fallen by half in Guinea-Bissau, replaced by HIV-1 in younger people
  - Also falling in Senegal, Burkina Faso

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Multiple cross-species transmissions (hunters/bushmeat/pets)

Human-human transmission

TMRCA HIV-2 B

TMRCA HIV-2 A

Migration exodus from GB

War of Independence in GB

1961-1974

Migration influx into GB

1975-1980

HIV-2 identified

1986

Civil war and political instability

1990s

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Dynamic migration rates in Guinea-Bissau: relationship to HIV-2 dissemination?

Work by Yannis Hodges and Philippe Lemey
Transmission of HIV-2

- Sexual transmission less efficient than for HIV-1 (Kanki, Lancet 1994): HIV-1 estimated to be 3.55x more transmissible than HIV-2 in Senegalese FSWs (Gilbert 03)
- Parenteral transmission (haemophiliacs/ IDUs)
- Vertical transmission reported, but infrequent: largest prospective study in the Gambia showed HIV-2 MTCT was 4% (O’Donovan, 2004)
- High prevalence of HIV-2 infection in Guinea-Bissau followed war of independence, associated with parenteral treatment for TB and trypanosomiasis, also female genital mutilation (Pepin, AIDS 06)
- Dual HIV-1/2 infection common in countries with both virus strains: may occur as simultaneous primary infection with both viruses (including vertical infection (van der Sande, 04)), or as HIV-1 super-infection of HIV-2-infected people
Why is HIV-2 transmission less efficient than for HIV-1?

- Most HIV-2-infected subjects have low plasma viral load; correlates with lower viral shedding in semen (Gottlieb, AIDS 2006) and female genital tract (Hawes, AIDS 2008) than for HIV-1.
- Vertical transmission closely related to maternal viral load (O'Donovan, AIDS 00).
- High frequency of HIV-2 infection in older women - cohort effect or increased susceptibility with age (Aaby, AIDS 1996)?
What might underly the reduced transmissibility of HIV-2?

- Overall, replicative capacity of HIV-2 in vitro is lower than HIV-1 (Arien, JVI 05)
- However, replication-competent virus can still be isolated from subjects with undetectable viral load (Blaak, Virology 06)
- HIV-2 does not result in productive infection of dendritic cells (mDCs or pDCs) (Duvall, JVI 07)
Protective immunity in HIV-2-exposed seronegatives?

- High serum HIV-2-specific IgA and potent HIV-2 neutralisation in partners of HIV-2-infected donors from Guinea-Bissau (Lizeng, JVI 2004)
- HIV-2-specific CTL (cross-reactive against HIV-1 epitopes and able to kill virus-infected cells) in exposed seronegative Gambian sex workers (Rowland-Jones, NM 1995)
- HIV-2-specific proliferative responses detected in EU donors (Andersson, CEI 2004)
- Is this similar to HIV-1-exposed uninfected donors or distinct?
Why study HIV-2 infection?

- ~1 million people infected in West Africa
- 15-20% HIV-2-infected people develop AIDS and could benefit from ART, but effective viral suppression with ART is much harder to achieve than for HIV-1 - intrinsic resistance to NNRTIs, fusion inhibitors and some protease inhibitors
- Does HIV-2 protect against HIV-1 infection? (Travers, Science 1995): protection not confirmed in three other studies - probably a risk factor in Caio (Schim van der Loeff, AIDS 2001)
- Majority of infected people have a normal lifespan and show no signs of immunodeficiency - natural human model of attenuated HIV infection
- Progressors (15-20%) clinically indistinguishable from people with HIV-1
- Responses to HIV-2 in non-progressors resemble those currently desired from an HIV-1 vaccine
How does HIV-2 infection differ from SIVsm in the natural host?

- Most SIVsm infected monkeys have a normal lifespan with no signs of immunodeficiency.
- Although the sequence of HIV-2 is very similar to SIVsm, there are significant differences from SIVsm model of naturally attenuated SIV infection in sooty mangabeys.
- HIV-2 infected LTNPs have low plasma viral load and strong immune responses.
- Immune activation is low in LTNPs but increased in progressors with HIV-2 infection to a level comparable with HIV-1 (Sousa, JI 02).
- SIVsm-infected monkeys have very high viral loads, absent immune activation and weak immune responses (Silvestri, 03).
MRC GUM clinic, Fajara:
~3000 HIV-1/-2/dual patients:
• ART available since Sept 2004 through the Global Fund

Caio: field station in Casheu region of Guinea Bissau (ART started in 2007)
• 4000 adults: 7.9% have HIV-2
• Community cohort followed since 1989
• NB Advantages of a community cohort for natural history studies
Caio is a small town in rural Guinea-Bissau, in the centre of the Cacheu region.

Main economic activities - subsistence: rice, cashew nuts, palm oil and palm wine (significant migration).

Most of the villagers are from the Manjako tribe and follow an animist belief system.

The adult population (~4,000 people) has been studied by MRC investigators since 1989, when 7.9% of adults were infected with HIV-2.

Most recent survey shows HIV-1 prevalence now 3.7% whilst HIV-2 has fallen to 4% (Carla van Tienen, J.AIDS 09).

HIV-2 prevalence is 5-fold higher in people > 45yrs.

All HIV-2, -1 and dual-infected adults enrolled into a case-control cohort with regular follow-up (currently > 400 HIV+ donors and controls).

How does the natural history of HIV-2 infection differ from HIV-1?

- Proviral load at different disease stages is similar to HIV-1, but plasma viral load is much lower (Berry, 1998, Popper, 2000)
  - Disease progression is predicted by plasma VL (Whittle, 1992, Berry 2003) and serum \( \beta_2m \) (Jaffar, 2005) but **NOT** by CD4 count/% (Jaffar, 2010)
  - Rates of progression similar between HIV-1 and HIV-2 for a given plasma VL (Gottlieb, 2002) (Hansmann, 2005)
- Progression to AIDS occurs in a minority, estimated at 20% in the Caio cohort (Jaffar, 2010)
  - Clinical features are indistinguishable from AIDS caused by HIV-1 (Martinez-Steele, 2006)
- **Age is NOT a risk factor for disease progression**
  - Life expectancy of older people (55-80 yrs) with HIV-2 same as uninfected population (Poulsen, 1997)
- Thus HIV-2 does not have a generally attenuated phenotype, but leads to a very high proportion of LTNPs
Survival with HIV-2 infection in the Caio cohort

- Overall mortality rate per 100 person years was 4.6 for the HIV-2+ subjects and 2.2 for the controls
- For subjects over 60 years old, survival is unaffected by HIV-2 status
- Survival shows strong inverse correlation with plasma viral load, no independent relationship with %CD4 cells
- Plasma viral load was stable in the non-progressors for over a decade

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Systemic immune activation is a feature of HIV-2 infection and is directly related to viral load

HIV-2 patients: N=107

<table>
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<tr>
<th>Activation markers</th>
<th>CD4+</th>
<th>CD8+</th>
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<td>HLA-DR+</td>
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</table>

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Leligdowicz et al, JID, 2010
Why is the course of HIV-2 disease attenuated?

- Viral Factors
- Host Genetic Factors
- Immune Factors
How different is HIV-2 from HIV-1?

- Homology with HIV-1 is 60% in Gag, Pol; 30-40% in Env
- 75-90% homology with SIVsm
- **Genomic structure differs from HIV-1 and SIVcpz in vpx gene** (Henderson et al., 1988)
- LTR: only 1 NFκB binding site (2 in HIV-1) (Markovitz et al., 1990)
- Nef deletions more common than HIV-1 (Switzer et al., 1998)
- **Co-receptor usage is broad** (broader than HIV-1) (Bron et al., 1997)
- **Cytopathic effect in vitro is comparable to HIV-1** (Schramm et al., 2000)
- Replicative fitness significantly lower than HIV-1 for many (but not all) HIV-2 isolates (Arjen et al., 2005): lower in patients with undetectable VL (Blaak et al., 2006)
- **Similar levels of integrated virus between HIV-1 and HIV-2** (MacNeil, JVI 07)

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Does HIV-2 capsid variation affect disease progression or susceptibility to TRIM5α?

- TRIM 5α is a member of a large family of intra-cellular proteins which target viruses for destruction shortly after entry into the cell by binding to capsid proteins.
- Differences in primate TRIM 5α account for the species specificity for SIV and HIV in humans and primate cells.
- HIV-2 is known to be more sensitive to human TRIM 5α than HIV-1.
- Amplification of gag p26 gene and sequence analysis from plasma virus stored from 2003 Caio samples (n = 69).
- Longitudinal analysis using samples from 1996 and 2006 (Clayton Onyango, Matt Cotten).
- Studies of TRIM 5α variation in the host and functional studies underway.

Cluster of family “idols” outside a mud-brick house in Caio.
The p26 ASA form associates with high viral load

P119 shown to affect susceptibility to TRIM5α: modelling of P119 to A or Q alters capsid structure. NB: SIVmac and SIVsmm have Q or A at this site (Song, JVI 07)

Highly significant correlation between presence of P at these sites and low viral load (C. Onyango et al, ms submitted)

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Matthew Cotten & Clayton Onyango (Vaccine 2010)

- HIV-2+ patients with PPP virus have significantly lower VL
- No phylogenetic clustering of gag or env with progression status (Grassley, JVI 98, Onyango, Vaccine 2010, Thushan de Silva, unpublished data)

- Capsid dimer formation energies are higher in non-PPP capsid variants
  - Increased capsid stability? Weaker TRIM binding?
  - Poor antigen processing/T cell responses? Virus-specific T-cell responses are higher in subjects with PPP virus

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Are there distinct strains of HIV-2 with different degrees of pathogenicity?

- Around 15-20% HIV-2-infected people develop high viral loads and a clinical course indistinguishable from HIV-1.
- Most *in vitro* virological studies of HIV-2 have used isolates taken from people with disease progression, so much less is known about the virus in non-progressors.
- If virulent strains of HIV-2 exist that are associated with high viral load and transmission then these would be likely to become dominant.
- In rare cases of vertical HIV-2 transmission in the Gambia, these were associated with progressive disease in the mothers but in only 1/4 HIV-2-infected children (*Ota* et al, *AIDS* 2000).
- How did the PPP form of HIV-2 arise?: SIVsmm has QPP or (rarely) APP.
Why is the course of HIV-2 disease attenuated?

Viral Factors + Immune Factors

Host Genetic Factors
Distinct HLA and KIR associations with HIV-2 susceptibility/disease outcome

- Studies performed in the Caio cohort (genetically homogeneous), 150 HIV-2 infected subjects with 328 HIV negative controls: (Louis-Marie Yindom, J.Virol. 2010)
- Sequence-based typing with Mary Carrington’s lab, NCI
- Strong association of HLA-B*08 with susceptibility to HIV-2 infection ($P = 0.003$, $OR = 2.20$, $CI = 1.31 - 3.70$)
- KIR2DL2 and KIR2DS2 + C1/x associated with protection against HIV-2 infection ($P = 0.04$, $OR = 0.66$, $P = 0.03$, $OR = 0.63$, $CI = 0.41 - 0.95$)
- HLA-B*15 is strongly associated with low absolute CD4 count ($P = 0.003$) and high mean log VL
Neutralising antibody and Natural Killer (NK) cell responses in HIV-2 infection


- Significant differences in NK activity (circulating numbers, cytotoxicity, cytokine and chemokine secretion) between HIV-1 and HIV-2 infected donors at normal CD4+ counts (CD4% above 28%)

- Most striking difference is in much higher production of MIP-1β by NK cells in HIV-2+ donors

- At lower CD4+ counts these differences are lost, i.e. HIV-2 progressors look like HIV-1 progressors (Nuvor et al, JVI 2006)
HIV-2+ donors have high titres of broadly neutralising antibodies

40 Caio plasma samples from 2006 tested for neutralisation of reference strains or recombinant Caio HIV-2 envelopes (Thushan de Silva)
HIV-2 neutralising antibody studies to date

- Broadly neutralising antibodies to primary virus strains are rare in HIV-1 infection: titres are usually low, rapid viral escape means nAbs don’t usually neutralise the currently circulating virus
- In contrast, high titres of neutralising autologous and heterologous nAb are found in most (60-70%) HIV-2-infected subjects
- No evidence that nAb play a major role in disease progression: no correlation of either presence or levels with disease outcome
- HIV-2 envelope glycoprotein structure may confer neutralization sensitive phenotype
- Some neutralisation-resistant HIV-2 envelopes exist - appear to be more common in advanced HIV-2 infection
  - Resistant and sensitive clones exist as variants of the quasispecies in some HIV-2+ donors with high viral load
HIV-2-specific CD4+ T cell function is preserved in non-progressors

Melody Duvall et al, JI 2006, EJI 2008

Subjects with CD4 >28%

Striped bars: HIV-1
Solid bars: HIV-2

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T-cell responses to the entire HIV-2 proteome using overlapping peptides

- “Consensus” HIV-2 sequence generated using viral genomes from The Gambia
  - HIV-2 D194, HIV-2 SBL/ISY, HIV-2 MCR35, HIV-2 MCN13
- Optimized 15-19 aa long peptides overlapping by 10 aa (18x10)
  - C-terminal modifications to enhance HLA class I binding
  - Total number of peptides: 425
- T-cell responses studied using IFNγ Elispot with 3-D matrix approach
- Studies in 69 subjects in the Caio cohort, comparing HIV-2+ donors with high (>1000 copies/ml) or undetectable (<100/ml) plasma viral load
In contrast to HIV-1 infection, HIV-2+ LTNPs have very strong T-cell responses.
HIV-2 epitopes cluster in a conserved region of p26

Peptide 46:
- MHR region of gag p24
- Patients with Peptide 046 specific responses have lower VL than those who do not \( (p=0.05) \) Leligdowicz et al, JCI, 2007
- Target for CD4+ responses (HLA-DR-restricted)
- Target for CD8+ responses (HLA-B14, B40-restricted)

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<th>Matrix Peptide #</th>
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<th>Peptide Sequence</th>
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HIV-2 gag contains a "protective" T-cell epitope

Patients with Peptide 046-specific responses have a lower VL than those who do not (p=0.05) Leligdowicz et al, JCI, 2007

Peptide 46 - MHR region of gag p26
Target for CD4+ responses, HLA-DR*1302-restricted
Also target for CD8+ responses, including B14 and B40-restricted
Characteristics of T-cell responses to the “protective” HIV-2 peptide p46

- T-cells target the Major Homology Region (MHR)
  - 20-αα region in capsid domain of Gag
  - Plays a critical role in particle assembly
  - Immunogenic: contains several T-cell epitopes in HIV-1
  - Proteasomal processing is more efficient from “PPP” capsid sequences
  - Highly conserved among most retroviruses
  - Good potential for resistance to escape mutation

- T-cells targeting this region show:
  - Unusually high avidity (several log orders greater than for most virus-specific T-cells)
  - Oligoclonal TCR usage (selection of Vβ17)
  - Early differentiation phenotype: proliferate well, not “exhausted” (despite long infection history)
  - Don’t select viral escape variants
HIV-2 pathogenesis: conclusions

- HIV-2 provides an important human model of control of a potentially pathogenic retrovirus.
- HIV-2 infection is not generally attenuated but yields a high proportion of LTNPs, whereas progressors develop AIDS in a similar way to people with HIV-1 infection.
- "Non-progression" is associated with good quality CD4+ T-cell help, high magnitude, polyfunctional CD8+ T-cell responses, and preserved innate responses.
- Control of viral load is strongly associated with T-cell responses to a single highly conserved region (MHR) in the capsid protein.
- T-cells responding to this region are of unusually high avidity with restricted T-cell receptor usage, and do not select escape variants.
- Is this the kind of immune response we need from an HIV-1 vaccine?
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