Salivary mucin MUC5B inhibits HIV-1 subtype C in an in vitro pseudoviral assay

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SA HIV Clinicians Society 2012
Mucus and Mucins

Slimy, highly viscous secretion coating the surface of epithelial tissues


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• **Protective mucus barrier**
  
  – Highly elastic and adhesive properties – role in aggregating and removing pathogenic micro-organisms from the oral cavity

  – Insolubility and unstirred nature of the mucus gel layer on gastric epithelium (Rees and Turnberg, 1982)

  – The mucus layer provides spatial separation of the colonic epithelium with intraluminal bacteria

  – **Protection greatly limits the viral infection (SIV) of cervicovaginal epithelial tissues** (Miller *et al*, 2005)
Residual virions from the inoculum trapped in cervical mucus.
**AIM**

- Investigating this mucus/viral interaction – strategy for preventing transmission of HIV-1?

- Mucus-based microbicide?
Mucin composition, structure and conformation

- High molecular weight glycoproteins

**Generic structure of a mucin monomer**

- **H2N**
- **COOH**
- **oligosaccharide**
- **cysteine residue**
- **repeat structure**

- Secreted *salivary mucins* - MUC5B (gel forming) and MUC7 (not gel forming)
Transmission of HIV-AIDS occurs rarely in oral fluid exchange


H. Habte et al (2006) in our laboratory:

• **Mechanism** – postulate broad interaction between the extensive glycosylation of the mucin with that of the viral capsid – aggregating and removing virus from the oral cavity – preventing viral interaction with susceptible cells

• **Further studies by J. Peacocke *et al* (2012):**

  – **HIV-negative** crude saliva inhibited HIV-1
    • Subtype C strain
    • 70% HIV-negative group
    • 75% HIV-positive group
  – **HIV-positive crude saliva** mucus *also inhibited* the virus
  – Purified HIV-negative *and HIV-positive mucins* inhibited the virus
    • Unlike the previous study
  – *No significant difference in inhibition with HIV-status*
Neutralisation of HIV-1 by Crude Saliva

Crude saliva IC$_{50}$ – 17.34ug/ml protein conc.  IC$_{50}$ - 22.54ug/ml protein conc.

Pseudovirus - CAP45, subtype C, KZN
Representative samples

Dose-response nature of inhibition of HIV-1 pseudovirus

IC$_{50}$ is the concentration of the inhibitor at which 50% of the response is observed – calculated using a curve fit (GraphPad Prism)
Neutralisation of Subtype C HIV-1 Pseudovirus by Crude Saliva and its Purified Mucins

Subtype C viruses DU422.1 and CAP45, KZN

Protein concentration, ug/ml

Crude saliva      MUC5B      MUC7

-- beyond limit of detection
Salivary mucus and mucin samples tested against subtype C pseudovirus

MUC5B appears to have greater neutralising activity of HIV-1 than MUC7 (and crude saliva)
CONCLUSION

• **Salivary MUC5B neutralises** HIV-1 pseudoviruses CAP45 (KZN) and DU422 (Durban) of **subtype C** when purified from HIV-negative and HIV-positive individuals.

• **The neutralisation activity of MUC5B IC\(_{50}\) 25.52µg/ml appears greater than MUC7 IC\(_{50}\) 277.82µg/ml**

• Neutralising activity irrespective of subtype (A, Q168a.2 from Kenya)
THANKS

• Head of research, supervisor Prof Mall, HOD Prof Kahn, and our laboratory staff

• Dr Paul Roux from the HIV-clinic, Groote Schuur Hospital, for recruitment of donors

• Collaboration with Dr Jeff Dorfman and PhD student Rajesh Jacob at the ICGEB – International Centre for Genetic Engineering and Biotechnology

• Surgery Department, UCT, NRF for funding

'If all these players do their part, we will move forward, as fast as science can take us, to discoveries that can help block the transmission of HIV. This goal is worth our greatest efforts; it could very well be the turning point that leads to the end of this disease’

Melinda Gates
EVIDENCE

– MUC7 inhibitory potential against fungal and bacterial infection in the oral cavity? But not HIV-1?

– MUC5B is a larger and more extensively glycosylated and gel-forming molecule than MUC7 – refined approach to defining the mechanism of inhibition
  • Broad/physical interaction due to charge?
  • Specific binding between mucin and virus?

• Postulate altered glycosylation –
  – Further work using LC-MS to analyse glycosylation between mucins
  – Manipulation within neutralisation assay
Preliminary data – MUC5B neutralises Subtype C pseudoviruses CAP45 and DU422.1 as well as subtype A virus Q168a.2

<table>
<thead>
<tr>
<th>Neutralisation of virus</th>
<th>MUC5B</th>
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<tbody>
<tr>
<td><strong>CAP45</strong></td>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
<td><strong>Total</strong></td>
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<tr>
<td><strong>DU422</strong></td>
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<tr>
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<td><strong>Q168a.2</strong></td>
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<tr>
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</tr>
<tr>
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<tr>
<td>Neutralisation of virus</td>
<td>HIV status of donor</td>
</tr>
<tr>
<td>------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
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</table>

Preliminary data – MUC7 neutralises Subtype C pseudovirus DU422.1, potentially CAP45
Salivary mucus and mucin samples tested against subtype C pseudovirus

Greater number of neutralising samples for MUC5B than both crude saliva and MUC7
HIV-1 pseudovirus neutralisation assay

Genetically modified pseudovirus
- Multiple strain testing
- Any cloned env gene may be used
- Panels of env’s will be used

• All virus produced normally invade only once
  • “single cycle pseudo-virus”
    • (low risk of recombination to infectious virus)
  • Much safer
  • BSL2 lab

• Sensitive and technically easy assay readout
  • luminescence.

• Easy to reproduce
Step 1. make pseudovirus:

HIVΔ\textit{env} plasmid

transfect

Packaging cell line

Pseudovirus RNA

Make more pseudovirus RNA

All other viral proteins

Envelope producing plasmids

Envelope proteins

Pseudovirus ready for assay

Jeffrey Dorfman
ICGEB
Step 2. assay:

Pseudovirus env mutant panel

Add test sample:
-- dilution of serum
-- MUCIN

Blocks

No virus entry

Virus enters target cell

Does not block

Luciferase
(expression in target cell line driven by Tat protein) from the virus
Graph of Luciferase activity in HIV-Neutralisation assay

Crude concentration of material in lyophilised/dialyzed saliva, ug/ml

Luciferase, RLU

Crude Saliva HIV-Negative 116
Crude Saliva HIV-Positive 14
| Neutralisation of virus | HIV status of donor | CAP45 | | DU422 | | Q168a.2 |
|------------------------|---------------------|------|-------------------|-------------------|-------------------|
|                        | Negative            | 1    | 7                | 2                 | 3                 |
|                        | Positive             | 0    | 2                | 1                 | 2                 |
| Yes                    |                      |      |                  |                   |                   |
| Yes                    |                      |      |                  |                   |                   |
| No                     |                      |      |                  |                   |                   |
| No                     |                      |      |                  |                   |                   |
| Total                  |                      | 1    | 7                | 3                 | 5                 |

The table shows the HIV status of donors in relation to neutralisation of virus. The percentages reflect the success rate in neutralising the virus.