Phenotypic methods for investigating the impact of variation on HBsAg antigenicity

Samreen Ijaz
Health Protection Agency
London, UK

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Impact of HBsAg mutants

- Use of both vaccine and antivirals selects for mutations which impact HBsAg antigenicity
- Raise concern over consequence of HBsAg mutants emerging in an era of increased levels of immunisation and use of antivirals
- What is the impact of HBsAg mutants on current public health and control policies
  - How common are these mutations in HBV infected populations?
  - Damaging in the antenatal setting?
  - Are these viruses being transmitted?
Prevalence of HBsAg mutants

- Studies limited by viral sequencing which is both laborious and expensive
- Studies indicate that it was not always possible to infer accurately changes in antigenicity from direct sequencing
- Backbone of the virus played an important role in determining the impact of amino acid changes
Luminex assay

- *Ex-vivo* phenotyping directly from patients’ sera
- HBsAg epitope mapping using monoclonal antibodies on the Luminex platform
- The advantage of Luminex technology lies in its sensitivity, high throughput and efficiency
Microsphere (100 distinct sets available)

Anti-HBs monoclonal antibody

HBsAg in plasma

Polyclonal anti-HBs labelled with RPE

Phycoerythrin is excited by laser and emits fluorescence which is quantified by Luminex

The immune-complex/microsphere is then excited by the laser. The bead specific emission is quantified by Luminex and the bead identified.

Anti-mouse IgG binds to the bead

Anti-HBs monoclonal antibody

HBsAg in plasma

Polyclonal anti-HBs labelled with RPE

Phycoerythrin is excited by laser and emits fluorescence which is quantified by Luminex
Mutant HBsAg in plasma
Polyclonal anti-HBs labelled with RPE

The immune-complex/microsphere is then excited by the laser. The bead specific emission is quantified by luminex and the bead identified.

Decrease in fluorescence emitted by the Phycoerythrin
Luminex assay

- Microspheres beads – each has a unique internal dye (100 sets)
- Each different bead type can be conjugated to an individual MAb
  - Several epitopes of the HBsAg can be investigated
- Panel of MAbs recognising discrete and overlapping epitopes on the HBV envelope
  - HBsAg mini loop
  - HBsAg first loop
  - HBsAg second loop
  - Pre S1
  - Pre S2
Epitope mapping – WT sample

Fluorescence

<table>
<thead>
<tr>
<th>MAb</th>
<th>P2D3</th>
<th>D2H5</th>
<th>H3F5</th>
<th>HB04</th>
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<td></td>
<td>25000</td>
<td>25000</td>
<td>20000</td>
<td>27000</td>
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</tbody>
</table>
Epitope mapping

G145R

P2D3  D2H5  H3F5  HB04

Fluorescence

P142S

P2D3  D2H5  H3F5  HB04

D144E, G145R

P2D3  D2H5  H3F5  HB04

P142L, G145R

P2D3  D2H5  H3F5  HB04

D144A

P2D3  D2H5  H3F5  HB04
Epitope mapping

P120Q, N131P

T126N, T143I

F134I, T140L

P120T, N131P, G145R

4aa ins between 118+119

T123A, M133I
Application of epitope mapping

Mother to Baby study

- UK has a selective HBV immunisation policy
- Only babies born to HBV-infected mums receive prophylaxis
  - HBeAg pos mums – vaccine and HBIg
  - Anti-HBe pos mum – vaccine only
  - Anti-HBe pos mum with a VL >1.0e+06IU/ml – vaccine and HBIg
- HPA follows up babies at 1 year (received vaccine and HBIg)
- Data shows that policy is effective in preventing transmission
- A small number of babies are HBV infected at 1 year
N=52 babies

21 aa changes between codons 120-150 on sequencing

31 WT HBsAg on sequencing

Epitope mapping

8 had WT HBsAg phenotype

29/31 WT phenotype

13 had altered HBsAg antigenicity
Additional analysis

- Testing of additional HBV makers in babies
- Determine HBV VL in mums
- Looking at records to check immunisation and also response to vaccine
- Aim to follow up babies and determine outcome of infection
Drug-driven HBsAg mutants

- Drug driven changes impacting on the overlapping HBsAg resulted in the alteration of HBsAg antigencity
  
  *Torresi J., Virology 2002, 293:305-313*

- SDM to introduce drug driven changes (codons 164, 195 & 196)

- Expressed proteins found to have reduced reactivity to vaccine induced antibody

- Drug-driven HBsAg mutant viruses result in successful infection in immunised chimps
  
  *Kamili S., Hepatology 2009, 49:1483-91*
Evidence of drug-driven HBsAg escape mutants

Expressed recombinant HBsAg bearing various combinations of amino acid substitutions associated with drug driven escape mutants

rtV173L/sE164D + rtM204V/sI195M

Total loss of one epitope induced by two mutations neither of which alone has a detectable effect

DRUG INDUCED MUTANTS BEHAVE LIKE VACCINE ESCAPE MUTANTS

Epitope mapping – drug-driven HBsAg mutants

- $s_{E164D, I195M}$
- $s_{I195M}$
- $s_{W196L}$
2008 longitudinal study undertaken in patients undergoing antiviral therapy


Investigating dynamics of antiviral resistance mutations whilst on therapy

- Lamivudine monotherapy
- Treatment change to lamivudine & adefovir combination therapy or adefovir alone

Developed method based on pyrosequencing to quantify specific mutations (polymerase at codons 180, 181, 204)
Patient 1 (genotype B)

Baseline
(100% WT rt204 / WT in surface)

Fluorescence

Baseline fluorescence levels for Patient 1 at baseline, showing 100% WT rt204 and WT in surface.

(95% rtM204I / sW196S)

Fluorescence

Fluorescence levels at 95% rtM204I and sW196S.

(94% rtM204I / sW196S)

Fluorescence

Fluorescence levels at 94% rtM204I and sW196S.

(100% rtM204I / sW196S)

Fluorescence

Fluorescence levels at 100% rtM204I and sW196S.
Patient 2 (genotype E)

Baseline
(100% WT rt204 / WT in surface)

Fluorescence

(39% rtM204V / sl195M)

Fluorescence

(97% rtM204V / sl195M)

Fluorescence

(100% rtM204V / sl195M)

Fluorescence
Patient 3  
(genotype A)

Baseline  
(100% WT rt204 / WT in surface)

Fluorescence

P2D3  D2H5  H3F5  HB04

(58% rtV173L&M204V / sE164D&I195M)

Fluorescence

P2D3  D2H5  H3F5  HB04

(95% rtV173L&M204V / sE164D&I195M)

Fluorescence

P2D3  D2H5  H3F5  HB04

(97% rtV173L/M204V / sE164E/D&I195I/M)

Fluorescence

P2D3  D2H5  H3F5  HB04
Epitope mapping – drug-driven HBsAg mutants

• No indication of epitope loss

• Panel of MAbs may not be appropriate for such analysis
  • P2D3, H3F5 and D2H5 have been used in other studies where epitope loss has been seen

• s/pol studies describing epitope loss have been undertaken using recombinant HBsAg
  • May not truly reflect native HBsAg conformation
  • Investigation into these viruses need to be in their ‘natural backbone’
HBsAg is a complex protein
Summary

- Epitope mapping using the luminex provides a rapid system for phenotyping HBsAg
- Allows for large population based studies to be undertaken which will improve our understanding of the prevalence of these mutants
- Data suggests that drug-driven HBsAg mutants do not impact on HBsAg antigenicity
- Data shows the importance of studying viruses in their natural backbone
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