Herpes simplex viral vaccine development

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Herpesvirus structure
Epidemiology of HSV 1 and 2

- Worldwide: HSV1 seroprevalence 55% (USA) to >90% (Africa); HSV2 <10% (Japan) to 65%

- In Australia: 80% seropositive for HSV1, 12% for HSV2 (↑ in indigenous, urban; Torres strait:50%)

- In developed nations, marked increase in HSV1 genital herpes in adolescents, young women

- Prior HSV2 (and HSV1 GH) infection increase risk of HIV acquisition 3-7 fold
Cycle of HSV infection

Primary herpes
- Skin
- Axon
- Neuron
- Dorsal root ganglion
- Spinal cord
- Latency

Recurrent herpes
- Anterograde transport
Immunopathogenesis of HSV infection and disease

- HSV1 and 2 closely related, many cross reactive and crossprotective antigens (to antibody and T cell)

- Immune control of HSV1/2 initial and recurrent infection at levels of both DRG and mucosa:
  - Innate; dendritic cells, interferon, NK cells, macrophages
  - Adaptive: neutralizing antibodies, CD4 and CD8 lymphocytes

- Genital HSV1 recurs infrequently

- Asymptomatic shedding of HSV1 and 2 at oral and genital mucosa respectively, HSV2 can be very frequent (12 hrly): responsible for most transmission
Components of innate immune system

A. Elements of the innate immune system and chemical/physical barriers

- Mucous membrane and secreted enzymes
- Ciliated epithelium
- Stomach acids, soluble peptides and mucus secreted by epithelial cells
- Mucus, peptides and stratified squamous epithelium
- Skin and secreted enzymes
- Resident and circulating innate immune cells

Innate

- Granulocyte (neutrophil, eosinophil, basophil)
- Mast cell
- Monocyte
- Dendritic cell
- Macrophage
- Natural killer cell

Chemokines
Cytokines
APC express receptors that recognize PAMPs (pathogen associated molecular patterns)
Role of immune cells and keratinocytes in lesions of recurrent Herpes simplex
Symptomatic vs asymptomatic Genital Herpes

- True asymptomatic shedding: 20%
- Symptomatic genital herpes: 20%
- Unrecognised HSV disease: 60%
Three ages of Immunization

• Paediatric
• Adolescence: Human Papillomavirus
  Herpes simplex virus*
  Epstein Barr virus*
  Hepatitis B virus
• Adult: Influenza virus
  Pneumococcus
  Zoster
AIMS of an HSV vaccine

• Reduce/eliminate viral replication in mucosa

• Prevent entry into nerves
  \[\rightarrow \downarrow \text{disease} \]
  asymptomatic shedding
  - preventing infection (sterilizing immunity) too difficult?

• Now need vaccine for both genital HSV1 and 2
Challenges for developing Vaccines for Genital Herpes

- Latent HSV infection in neurones
  - normally express no MHCI

- HSV has mechanisms of evading the immune response

- Finding the best immunodominant protein stimulators/targets (large virus, ~80 proteins)
  - Which are the most important immune mechanisms (cells)
    - How can they be best stimulated

- Delivery: DNA, recombinant virus, r-proteins

- Animal models
History of HSV vaccines

• Anderson and Burnet 1948: killed egg grown HSV1 for primary oral herpes in infants

• Many candidates since, including live attenuated (Roizman), killed, DNA extracted (Skinner), subunit: all failed

• 1999: Chiron vaccine recombinant HSV glycoproteins D and B: high neutralizing antibody titres but no efficacy against HSV2 acquisition

• GSK gD/dMPL: Simplirix trial 2002; Herpevac trial 2012
HSV2 gD/dMPL vaccine - the first (partially) successful vaccine for genital herpes

- Antigen: recombinant HSV2 glycoprotein D
- Adjuvant: ASO4 - Alum and monophosphoryl lipid A (DMPL)
  - Induces Th1 response (IFNg) in humans
- Simplirix trial: multicentre, RDBC
  - Consort design: immunize partners of subjects with GHD

Stanberry, Spruance, Cunningham et al NEJM 2002
Adjuvants

- Adjuvants are:
  - carriers (e.g., aluminium, emulsion) or
  - immunostimulatory molecules able to Modulate the immune response by
    - Activating dendritic cells (replace endogenous pathogen stimuli or PAMPs)
    - Stimulating the appropriate immune pathway → different patterns of cytokine production
Adjuvant Systems

- Combinations of:
  - Classical adjuvants: aluminum salts, emulsion, liposomes
  - Immunostimulants: MPL, QS21, (CpG), tocopherol

![dMPL](image1)

![QS21](image2)

![CpG](image3)

 tocopherol
HSV2 gD vaccine prevents disease in female seronegative subjects

**Study 1**

**Men**
- Placebo: 99%
- Vaccine: 98%
- VE = -11% (p = 0.81)

**Women**
- Placebo: 99%
- Vaccine: 99%
- VE = 73% (p = 0.01)

**Study 2**

**Men**
- Placebo: 97%
- Vaccine: 94%
- VE = 32% (p = 0.47)

**Women**
- Placebo: 98%
- Vaccine: 95%
- VE = 74% (p = 0.02)
Lessons from the GSK GD-dMPL vaccine trial

• Why only efficacious in females?
  - gender specific general immunity?
  or
  - genital tract immunobiology

• What is the critical protective modality?
  Th1 cytokines induced by MPL
Herpevac trial 2012

• Random recruitment design
• HSV seronegative women, 18-30 years
• HSV1 predominant cause of genital herpes disease (GHD) in controls
• 58% efficacy against HSV1 GHD
• No efficacy against HSV2 GHD
A  HSV-1 or HSV-2
Cumulative incidence of HSV-1 or HSV-2 Genital Disease (%)

Efficacy of HSV vaccine, 20%; 95% CI, 29 to 50
Control vaccine
HSV vaccine
Month after Dose 1

B  HSV-1 Only
Cumulative incidence of HSV-1 Genital Disease (%)

Efficacy of HSV vaccine, 58%; 95% CI, 12 to 80
Control vaccine
HSV vaccine
Month after Dose 1

C  HSV-2 Only
Cumulative incidence of HSV-2 Genital Disease (%)

Efficacy of HSV vaccine, 38%; 95% CI, 16 to 59
Control vaccine
HSV vaccine
Month after Dose 1
Why such differences between the Herpevac and Simplirix Trials

• Cohorts:
  - Simplirix: women with partners with GHD = Mucosal priming vs none with Herpevac

• Why efficacy with HSV1 and not HSV2:
  ?Easier to obtain immune control, much cross protection/immunogenicity
  - via antibody and T cells
HSV vaccines: current issues

- Priority: in developed vs developing world
- Need vaccine for HSV1 and 2?
- What immune modalities required to stimulate: Neut antibody, CD4/CD4 lymphocytes, Dendritic cells, other aspects of innate immunity
- Need better animal models to help decide
- Need human CD8 lymphocyte adjuvant
- What are best candidates: specific live attenuated vaccines, viral vector, recombinant proteins
Current classification of vaccines

- Live attenuated (mumps, measles)
- Inactivated or killed (HAV)
- Subunit (influenza)
- Polysaccharide conjugated (H, Influenza, Pneumococcal)
- Virus Like Particles
- DNA
- Live vectors
- Subunit or recombinant antigens (including peptides) with Adjuvant
<table>
<thead>
<tr>
<th>Live, attenuated</th>
<th>Killed/inactivated</th>
</tr>
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<tbody>
<tr>
<td>Eg OPV, MMR, VZV, some influenza, BCG</td>
<td>Eg IPV, HAV, whole-cell pertussis</td>
</tr>
<tr>
<td>Mimic the natural infection, may retain immune evasion factors</td>
<td>Usually require adjuvants</td>
</tr>
<tr>
<td>Strong priming (1–2 doses)</td>
<td>Multiple doses needed for priming</td>
</tr>
<tr>
<td>Long-term persistence of immunity</td>
<td>Booster may be needed for long-term immunity</td>
</tr>
<tr>
<td>May induce mild disease symptoms</td>
<td>No disease symptoms</td>
</tr>
<tr>
<td>Rare reversion to virulence</td>
<td>No reactivation, non-infectious</td>
</tr>
<tr>
<td>Potential for immunological interference with other live vaccines</td>
<td>Low risk of immunological interference</td>
</tr>
<tr>
<td>Less stable over time</td>
<td>More stable over time</td>
</tr>
<tr>
<td>Poor resistance to cold chain deviation</td>
<td>Better resistance to cold chain deviation</td>
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<tr>
<td>Affected by administration of blood/blood-derived products or maternal antibodies in infants</td>
<td>Generally not affected by administration of blood/blood products</td>
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Westmead Millennium Institute

Langerhans cell
Apoptosis

HSV-infected apoptotic DCs
Phagocytosis by "bystander" Langerhans cells

Phagocytosis by "bystander" dermal DCs

Maturation

Migration via lymphatics

Direct Ag presentation
OR
Ag transfer to other DCs?

Lymph node

Cytokines (and other danger signals)

Keratinocytes

HSV
New approaches: Synthetic peptide antigens

Conformational (non-linear) epitopes

Effective structure of epitopes

Recombinant peptide including both conformational and linear epitopes

Primary structure

Linear epitopes
HSV Vaccine Candidates
HSV1/2 protein targets for CD4 and CD8 lymphocytes

Infected Keratinocyte

CD4
- gD
- gB
- gC
- gH
tegument

BLOOD
(HSV restimulated type common, specific)

CD8
IE/E proteins
HSV-2 gD (gD2) Sequence

MGRLTSVGTAALKVAVGLRVVCAKYALADPSLMADPNRFGRKNLPVL

DQLTDPGVKVYHIQPSLEDPFQPPSIPITVYYAVLERACRSHELAPS

EAPQIVRGADEARKHTYNLTIAWYRMGDNCAPITVMETECYYNKSLG

VCPIRTPRWSYYDSFSAVSEDNLGFLMHAPAFETAGTYLRLVKINDWTE

ITQFILEHRASCWITALPPLRIPPAACLTSKAYQQGTVDSIGMLPRFTP

ENQRTVALSEAKWHPKAPPYTSSTLPPELSDTNTAQPELVEDPED

SALLEDPAGTVSSQIPPNWHISIQDVAPHAPPAAPANPGLIIGALAGST

LAALVIGGIAFWVRRRSVA
HSV2 Peptide-TLR2 stimulant vaccine

Glycoprotein D of HSV-2 (gD2) for CD4 T cell

Kim, M et al., J Immunol. 181, 2008
• gD2 lipopeptide stimulates NK cells as well as Dendritic cells via TLR2 which they both express

• How do NK and CD4 T cells interact with each other? Cytokines vs direct contact
NK cell-CD4 lymphocyte conjugates induced by lipopeptide
Conclusions

• Triple DC, NK and CD4 T cell interaction is required for maximal stimulation of PBMC by lipopeptide.

• Act in pairs: DC-NK, DC-CD4, NK-DC and as a trio
  - interacting via ‘immunologic’ synapses and cytokines

• Must consider NK cells as well as DCs as targets for adjuvants
Vaccines for Genital (and neonatal) Herpes: Conclusions

• Better knowledge of mechanisms of immune control and antigen presentation will allow most appropriate selection of adjuvants

• Like HIV, HSV vaccine should be aimed at all immune modalities - innate and adaptive: myeloid & plasmacytoid DCs & NK cells, CD4 and CD8 lymphocytes, neutralizing antibodies

• Need HSV1/2 cross reactive vaccine
  - Increasing incidence of HSV-1 genital herpes in adolescence

• Many vaccine candidates, including specific live attenuated, DNA, killed and recombinant protein and viral vector vaccines will be trialled in the future

• Is there a place for peptide-adjuvant vaccines - Alone, in combination or just to elucidate appropriate antigens and adjuvants?
Key Remaining Vaccine challenges

Pathogens

• Highly variable pathogens that evade the immune system
  • HCV, HIV, TB, Herpesviruses
• Pathogens requiring multistage immune responses
  • Malaria
• Safer immunogens are potentially weak
  • Highly purified or recombinant proteins/peptides
  • Polysaccharides
• Need for rapid immunity
• Need for cross protection against antigenic variants
  • Pandemic influenza subtypes
Classes of Licensed Adjuvants

Only a few adjuvants are used in registered commercial vaccines for human use:

• Aluminum salts: the most widely used adjuvant
• Emulsions: MF59 (Novartis)
• dMPL + Aluminum : AS04 (GSK Bio)
Why we need NEW adjuvants

• To better target immune responses (humoral and cellular, Th1, CD8)

• To induce higher and longer-term persistence of protection

• To bypass weakened immunity:
  • Immunosenescence
  • Immunosuppression

• To reduce the amount of antigen needed (antigen-sparing effect eg pandemic influenza)
  • Need for the right adjuvant(s) with the right antigen(s) to protect against disease in the right target population (ie tailoring)
Identifying and producing vaccine antigens (2)

- Recombinant protein Ag
- DNA
- Live vaccine vector

A. Pathogen → Clone gene encoding antigen → Insert gene into expression system (e.g., yeast, insect cells) → Protein expression → Purification of recombinant protein encoding antigen → Administration as vaccine

B. DNA plasmid → Protein expressed within host cell

C. Live vaccine vector → Protein expressed in host
Early detection and response to pathogens

A. Description of PAMP sensors

B. Description of PAMP effectors, eg receptor binding and intracellular signal transduction
Detection of pathogens by innate immune cells

APCs translate and drives information to the adaptive immune system
Figure 2.10: Vaccine administration and immune response process.

1. Inflammation induction by vaccine.
2. Antigen presentation to lymphocytes.
3. Recruitment of immune cells through blood vessels.

Confidential information
Vaccine composition

Correct target / stimulator: viral protein or gene

- Correct immune mechanism: adjuvants, cytokines
Results of Simplirix trial 2002
(GSK gD2 dMPL vaccine)

In HSV1-2- females

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<thead>
<tr>
<th>Vaccine efficacy</th>
<th>STUDY 1</th>
<th>STUDY 2</th>
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<tr>
<td>Disease</td>
<td>73% (p=0.01)</td>
<td>74% (p=0.02)</td>
</tr>
<tr>
<td>Infection</td>
<td>48% (p=0.06)</td>
<td>39% (p=0.07)</td>
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Males
HSV1+2- females ] no efficacy

Mild moderate local reactogenicity
Simplirux trial of GSKgD/dMPL vaccine, 2002: Conundrums

Why only efficacious in women
- gender specific general immunity?
  Or
  - genital tract immunobiology

❖ Why no enhanced effect in HSV1+2- females (constantly re-immunised by HSV1?)

❖ Is there really protection against infection? p=0.06

❖ What was the critical protective modality
  - ? no CTLs
  - Th1 cytokines induced by dMPL not MF59 (?IFNγ)
Herpes Simplex Vaccines

Improvements

1) To recombinant protein vaccine
   • Broader range of targets/stimulators (eg ICP27)
   • Induce T-lymphocyte cytotoxicity (adjuvant like QS21)

2) Alternate strategies
   • DNA vaccines
   • Recombinant viruses: vaccinia, avipox
     - incorporate viral antigens, cytokines
   • Mucosal vaccines
Neutralizing anti-gD and interferons inhibit axonal transmission of HSV and spread in ECs (Mikloska et al JVI 1999,2001)
Role of DCs
Characteristics of split and subunit protein/peptide antigens

<table>
<thead>
<tr>
<th>Eg Split – influenza, Subunit – Pertussis, HBV, HPV</th>
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<tr>
<td>Highly focused, specific response</td>
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<tr>
<td>Reduced immunogenicity and potential for escape mutants</td>
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<tr>
<td>Non-infectious, Low reactogenicity, acceptable tolerability</td>
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<tr>
<td>No or limited availability of innate defensive triggers</td>
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<tr>
<td>Adjuvants needed to compensate for lower immunogenicity</td>
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<tr>
<td>Synthetic production may be possible, facilitating supply</td>
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