New developments in rabies vaccines

Noël TORDO

working together to stop the ongoing tragedy of rabies!
Tools for prevention/therapy

Pasteur’s vaccine

*rabid rabbit spinal cord* -> *dessiccated*

**human vaccines** (prevention + therapy)

- sheep brain (Semple)
- suckling mouse brain (Fuenzalida)
- cell culture: safe + efficient (expensive ?)

**animal vaccines** (prevention)

- nervous tissue (injection)
- cell culture (injection)
- attenuated/recombinant (oral, wildlife)

*No efficient antiviral*

*Not recommended by WHO*
WHO Recommendations: Post-Exposure Prophylaxis

- **Category I**
  - no treatment
  - touch, feed an animal
  - licks on intact skin

- **Category II**
  - clean wound (soap)
  - vaccine (cell culture)
  - minor scratches
  - abrasions without blood
  - nibbling of uncovered skin

- **Category III**
  - clean wound (soap)
  - vaccine (cell culture)
  - HRIG (20IU/kg)
  - ERIG (40IU/kg)
  - transdermal bite(s)
  - scratches
  - exposures to bats
  - licks on broken skin /mucous membranes
# Rabies vaccines evolution: improved efficacy & safety

- **WHO pre-qualified vaccines for IntraMuscular (IM)** 1 dose = 2.5IU, 1-0.5 mL
- **WHO recommended vaccines for IntraDermal (ID)** 1 dose = 0.1 mL

<table>
<thead>
<tr>
<th>Nerve Tissue Vaccines</th>
<th>Semple</th>
<th>Sheep, Goat or Rabbit brain</th>
<th>Local Producers (LP)</th>
<th>Fuenzalida</th>
<th>Suckling mouse brain</th>
<th>LP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonated Eggs</td>
<td>Duck Embryo</td>
<td>PDEV: Purified Duck Embryo vaccine</td>
<td>Vaxirab™ (Zydus Cadila, India)</td>
<td>Primary Animal Cells</td>
<td>PHKC: Primary Hamster Kidney Cell</td>
<td>LP China</td>
</tr>
<tr>
<td>Cell Culture Vaccines</td>
<td>Primary Animal Cells</td>
<td>PCEC: Purified Chicken Embryo Cell</td>
<td>Rabipur™ / RabAvert™ (Novartis, India / Germany)</td>
<td>Human Diploïd Cell Line</td>
<td>HDCV: Human Diploïd Cell Vaccine</td>
<td>Imovax Rabies, Sanofi Pasteur</td>
</tr>
<tr>
<td></td>
<td>Continuous Cell Line</td>
<td>PVRV: Purified Vero Cell Rabies Vaccine</td>
<td>Rabivax™ (SII, India)</td>
<td></td>
<td>CPRV: Chromatographically Purified PVRV</td>
<td>Verorab™, Sanofi Pasteur</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PVRV serum free</td>
<td>Other Chinese et Indian LP</td>
<td>Speeda™ (Liaoning Chengda, China), Rabirix™/Indirab™, (Bharat Biotech, India)</td>
<td></td>
<td>Butantan, Brazil</td>
</tr>
</tbody>
</table>
WHO Schedules: Post-Exposure Prophylaxis

Cat III: RIG

- Zaghreb
  - J0, J3, J7, J14, J21, J28, J90
  - 5 visits
  - 5 IM doses

- Essen
  - J0, J3, J7, J14, J21, J28, J90
  - 3 visits
  - 4 IM doses

ID Thai Red Cross

- 4 visits
- 4 ID doses

WHO position paper, Wkly Epidemiol Rec 2010;85:309-20
Improving Post-Exposure Prophylaxis (PEP)

**US Advisory Committee on Immunization Practices**

### Cat III: RIG

- **IM Acip**
- **4 visits**
- **4 IM doses**

- **J0**
- **J3**
- **J7**
- **J14**
- **J21**
- **J28**
- **J90**
- **1 y**

**Thai Red Cross**

- **ID**
- **3 visits**
- **12 ID doses**

- **J0**
- **J3**
- **J7**
- **J14**
- **J21**
- **J28**
- **J90**
- **1 y**

**One week** schedule

- Reduce cost, limit shortage
- Increase compliance for complete PEP
- Decrease wastage of vaccine

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WHO Schedules: Pre-Exposure vaccination (PrEP)

- people at risk: veterinarians, farmers, laboratory workers...
- travellers/residents in dog rabies endemic countries

Upon exposure

• people at risk: veterinarians, farmers, laboratory workers…
• travellers/residents in dog rabies endemic countries

WHO encourages studies for immunization programmes of children/infants in dog rabies endemic countries
Animal vaccines (more brents)

Parenteral administration

- Target: domestic animals
- Inactivated (+ adjuvants), modified life (attenuated), recombinants
- Potency >1 IU / dose; annual boosters

Oral administration

- Target: stray / wild animals
- Administered as baits (oral immunization needs replicative antigen)
- Efficacy and safety (target & non-target species)
- Monitoring the impact of oral vaccination campaigns in the field
Animal vaccines for oral use

Modified life vaccines

- SAD lineage: ERA, SAD-Bern, SAD-B19, Vnukovo-32
  - Residual pathogenicity for adult rodents, skunks
- SAG1-SAG2 : R333D mutant obtained by selection with MAb
  - Residual pathogenicity for suckling mice

Recombinant vaccines

- Poxviruses : Vaccinia (VV), canarypox (ALVAC), MVA, Lumpy skin
  - VV efficient in fox, coyote, dog, raccoon; non efficient for skunks
  - ALVAC efficient for cats parenterally
- Adenovirus : CaV2, HuAd5
  - CaV2 efficient parenterally in mouse, dog, cat, swine, sheep
  - CaV2 efficient orally in mouse, dog, raccoon, skunk
- Herpes virus: pseudo-rabies : efficient orally in dogs
Diversity of the *Lyssavirus* genus

*Much more to expect*

**Phylogroup I**
- GT 1 - Rabies world
  - Carnivores (world)
- GT 7 - ABLV - Australia
  - Pteropus sp. + Saccolaimus sp.

**Phylogroup II**
- GT 2 - LAGOS BAT - Africa
  - Frugivorous + insectivorous
- GT 4 - DUVENHAGE - Africa
  - Insectivorous
- GT 5 - EBLV-1 - Europe
  - Eptesicus sp.
- GT 6 - EBLV-2 - Europe
  - Myotis sp.
- GT 3 - MOKOLA - Africa
  - Micro-mammals
- West Caucasian bat v. - Caucasus Miniopterus sp.
Current vaccines (genotype 1) protect against phylogroup 1

Malerczyk et al., Vaccine, 2009, 27: 5320-5

Current vaccine (genotype 1) do not protect against phylogroup II lyssaviruses + West Caucasian Bat (WCBV)

DNA vaccine, IM mouse

Virus challenge RFFIT

<table>
<thead>
<tr>
<th></th>
<th>RABV</th>
<th>LBV</th>
<th>MOKV</th>
<th>WCBV</th>
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</thead>
<tbody>
<tr>
<td>RABV</td>
<td>250</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
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<tr>
<td>LBV</td>
<td>93</td>
<td>3125</td>
<td>17</td>
<td>&lt;11</td>
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<tr>
<td>MOKV</td>
<td>&lt;11</td>
<td>431</td>
<td>989</td>
<td>&lt;11</td>
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<tr>
<td>WCBV</td>
<td>&lt;11</td>
<td>&lt;11</td>
<td>&lt;11</td>
<td>6390</td>
</tr>
</tbody>
</table>

Hanlon et al. 2005 Vir Res 111: 44-54

Inactivated virus, IM mouse

Bahloul et al. 1998 Vaccine 16: 417-25
Badrane et al, 2001, J. Virol. 75, 3268-76
Options to enlarge the vaccine spectrum

\textit{anti-rabies} -> \textit{anti-lyssavirus}

• Characterize « conserved » antigenic sites / epitopes
  ✓ requires more research

• Combine antigens / antigenic sites / epitopes on a same « carrier »

• Associate antigens / antigenic sites / epitopes in a same dose
  ✓ combined vaccine already exist :
    \begin{itemize}
    \item \textit{RABV + canine adenovirus / distemper / parvovirus}
    \end{itemize}
Increasing the vaccine spectrum: chimerical lyssavirus Glycoprotein G

(Bahloul et al. 1998 Vaccine 16: 417-25)
Neutralizing antibodies induced by pGPV, pGMok & pGMok-PV against the various lyssaviruses

Bahloul et al. 1998 Vaccine 16: 417-25
Desmezières et al, 1999 JGV: 2343-51
Increasing the vaccine spectrum: Vaccinia virus expressing 2 lyssavirus G proteins

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 21</th>
<th>% Surviving (no. of animals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VV-RG</td>
<td>&lt;1:00</td>
<td>1:7 (1-39:2-01)</td>
<td>2:45 (1:58-2:81)</td>
<td>90 (9/10)</td>
</tr>
<tr>
<td>VV-RGRG</td>
<td>&lt;1:00</td>
<td>1:7 (1-08-2-01)</td>
<td>2:01 (1:38-2:70)</td>
<td>100 (10/10)</td>
</tr>
<tr>
<td>VV-MG</td>
<td>&lt;1:00</td>
<td>&lt;1:00</td>
<td>&lt;1:00</td>
<td>100 (10/10)</td>
</tr>
<tr>
<td>VV-RGMG</td>
<td>&lt;1:00</td>
<td>1:63 (1-0-19)</td>
<td>2:48 (1:90-2-86)</td>
<td>80 (8/10)</td>
</tr>
<tr>
<td>VV-WG</td>
<td>&lt;1:00</td>
<td>&lt;1:00</td>
<td>&lt;1:00</td>
<td>0 (0/10)</td>
</tr>
<tr>
<td>VV-RGWG</td>
<td>&lt;1:00</td>
<td>1:7 (1-0-19)</td>
<td>2:10 (1:4-2-7)</td>
<td>20 (1/5)</td>
</tr>
<tr>
<td>Vacc Cop</td>
<td>&lt;1:00</td>
<td>&lt;1:00</td>
<td>&lt;1:00</td>
<td>0 (0/5)</td>
</tr>
<tr>
<td>MEM</td>
<td>&lt;1:00</td>
<td>&lt;1:00</td>
<td>&lt;1:00</td>
<td>0 (0/5)</td>
</tr>
</tbody>
</table>

VV recombinants expressing G genes from 2 ≠ lyssavirus protect mice against both homologous lyssavirus

Rabies virus as a vector: Shuffling / duplicating / adding genes along the genome

- HIV Gag inserted at the N/P junction
- IFNβ inserted at the G/L junction
  - divalent vaccine RABV / HIV
  - adjuvant effect / less pathogenic

\[ \text{Virology, 2008, 382: 226-38} \]

- Two G proteins
  - more immunogenic, larger spectrum
- Deletion of the P protein
  - reoriented immune response

\[ \text{Vaccine, 2008, 26: 6405-14} \]

- RABV G replaced by HIV Gag
  - single cycle RABV vector (more safe)

\[ \text{J. Virol, 2010, 84: 2820-31} \]

- Ebola GP inserted at the N/P junction
  - divalent vaccine RABV / EBOLA

\[ \text{J. Virol, 2011, in press} \]

All papers from the M. Schnell ’ lab
Rabies virus as a vector:
Shuffling / duplicating / adding genes along the genome

**GAS constructs**

- **1xGAS**
- **2xGAS**
- **3xGAS**

**G double mutants**: R333D + N194S
- Highly immunogenic by oral route
  - Good candidate for PrEP + PEP
- Non pathogenic for:
  - dog, skunk, raccoon, mongoose
- 3xGAS non pathogenic by IC
  - To immunocompromised mice
  - To suckling mice

**G simple mutant**: R333D
- Exchanging M <-> G positions
- IM injection then lethal challenge
  - Protects 100% mice / hamsters
- Co-infection with a lethal challenge
  - Better than inactivated vaccine

*Faber et al. Zoon Public Health, 2009, 56: 262-9*
*Faber et al. PNAS, 2009, 106: 11300-5*

*Wu et al. Vaccine, 2010, 29: 4195-201*
Conclusions and future challenges

Human vaccines

- Reduce the number of shots / visits (developing countries)
  - using highly attenuated (3xGAS-adenovirus)
  - priming children in regions at risk
- Homogenize ID procedures (based on potency rather than volume)
- Increase vaccine spectrum RABV -> Lyssavirus (conserved epitopes, combined antigens)

Animal vaccines

- Long lasting immunity in a single shot (cattle in the Amazonas)
- Shift on attenuated rabies vaccines (3 GAS ?)
  - understand the basis of their immunity
    - P is « controlling » the INF induction / signalisation - > deletion is not neutral
    - Gene transcription is regulated in cascade - > switching gene position is not neutral
- Combine rabies with other antigens - > targeting species
  - Dog: rabies, distemper, parvovirus, leishmaniosis, … dog contraception…
Acknowledgments

Adrian VOS, IDT - biologica
Michaël ATTLAN, Sanofi - Pasteur

Unit Antiviral Strategies:
C. Jallet, M. Chteoui
G. Castel, H. Badrane, C. Balhoul
Multivalent vaccinology: chimerical G protein carrying foreign epitopes/antigen

Bahloul et al. 1998 Vaccine 16: 417-25
Desmezières et al, 1999 JGV: 2343-51

antigen-epitope(s)
B (poliovirus) Tc (LCMV)