Rabies Vaccinology

Susan Moore, Rabies Laboratory/KSVDL/College of Veterinary Medicine/Kansas State University, Manhattan, KS 66502, USA
Overview

• Human and animal immune responses to rabies vaccines
• Responses which are key to mediating or correlating with Rabies protection
• Requirements necessary for validating in vitro assays as replacements for in vivo efficacy testing
WHO Immunologic basis for immunization: Module 17 Rabies

WHO Immunological Basis for Immunization Series

Module 17: Rabies
Update 2017

[Graph showing the progression of rabies virus concentration and antibody response over days postexposure, with phases labeled for vaccine-induced humoral immune response, CNS virus, Salivary glands virus, Passive immunity - HRIG, and Virus present at entry site, and spread and replication of virus in the absence of appropriate PEP.]
Human vaccine response to rabies vaccination - humoral

Human vaccine response to rabies vaccination - cellular

## Peak response

<table>
<thead>
<tr>
<th>Vaccine regimen</th>
<th>Year of testing</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-exposure</td>
<td>2010</td>
<td>69.1</td>
<td>91.2</td>
<td>34.0</td>
</tr>
<tr>
<td>Post-exposure</td>
<td>2014</td>
<td>57.1</td>
<td>97.4</td>
<td>24.7</td>
</tr>
<tr>
<td>Post-exposure</td>
<td>2012</td>
<td>81.2</td>
<td>92.5</td>
<td>64.0</td>
</tr>
</tbody>
</table>
### Peak response

<table>
<thead>
<tr>
<th>Study</th>
<th>Group</th>
<th># Subjects</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aoki et al</td>
<td>post IM</td>
<td>10</td>
<td>0.17 (0.10-0.42)</td>
<td>0.30 (0.13-0.42)</td>
<td>7.6 (2.3-13.0)</td>
<td>Comment in discussion that the peak for those receiving HRIG was at day 42, without HRIG peaked at day 14</td>
</tr>
<tr>
<td>Jones et al</td>
<td>post IM</td>
<td>118</td>
<td>nd</td>
<td>0.17 (0.16-0.19)</td>
<td>6.9 (5.8-8.1)</td>
<td>Comment in discussion that the peak for those receiving HRIG was at day 42, without HRIG peaked at day 14</td>
</tr>
<tr>
<td>Jones et al</td>
<td>post IM</td>
<td>124</td>
<td>nd</td>
<td>0.17 (0.16-0.19)</td>
<td>10.3 (8.8-12.1)</td>
<td>Comment in discussion that the peak for those receiving HRIG was at day 42, without HRIG peaked at day 14</td>
</tr>
<tr>
<td>Briggs et al</td>
<td>post ID/IM</td>
<td>154</td>
<td>nd</td>
<td>0.32 (&lt;0.05-19.1)</td>
<td>23.2 (0.4--1318.0)</td>
<td>Summary of 3 groups, 2 ID and 1 IM, the lowest responses are im the IM group</td>
</tr>
<tr>
<td>Warrell et al</td>
<td>post ID</td>
<td>227</td>
<td>nd</td>
<td>0.59 (0.02-8.39)</td>
<td>308.72 (5.5-3711.5)</td>
<td>Summary of 4 groups, 3 were ID/different # sites and 1 IM</td>
</tr>
</tbody>
</table>
The expected percentage of people to require a booster by WHO level is 10-30% after 1 year (Strady, 1998), and by ACIP level is 2-7% after 2 years (ACIP, 2008).
Animal vaccine response to rabies vaccine

- Dog and cat  
  - Aubert, 1992  
  - Lawson, 1972  
  - Bunn, 1984
- Hamster, mice
- Wildlife

Day 28  
Day of challenge
Results – Animal studies assay and species difference

Open circles: survived
Black triangles: succumbed

A) % Inhibition

B) RFFIT [IU/mL]

http://www.mdpi.com/journal/tropicalmed
Longevity of response

- Lawson and Crawley, 1972
  - Dogs vaccinated 5 years previously survived challenge
  - Cats vaccinated 4 years previously survived challenge

- Strady, et al. 1988
  - Pre-exposure series and booster at 1 year—human subjects
  - >95% maintained titers above 0.5 IU/mL for 10 years

- What level is significant? 4 weeks after vaccination? Day of challenge?

### Table 4: Summary of data on anamnestic response according to vaccine administration

<table>
<thead>
<tr>
<th>Initial vaccination</th>
<th>Route of administration</th>
<th>Booster schedule</th>
<th>Timing of booster</th>
<th>Results</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initially received 1, 2 or 3 doses of HDCV</td>
<td>ID or IM</td>
<td>1 dose of HDCV</td>
<td>6–24 months</td>
<td>All subjects in this study, regardless of whether they had received the vaccine ID or IM, had an anamnestic response when boosted</td>
<td>Turner GS et al.</td>
</tr>
<tr>
<td>Initially vaccinated with HDCV</td>
<td>ID</td>
<td>1 dose of HDCV</td>
<td>2 years</td>
<td>Anamnestic response occurred in all individuals</td>
<td>Horman JT et al.</td>
</tr>
<tr>
<td>Initially vaccinated with a 3-dose ID HDCV regimen</td>
<td>IV</td>
<td>with 1 booster dose</td>
<td>2–14 months</td>
<td>Anamnestic response occurred in all individuals</td>
<td>Gherardin AW et al.</td>
</tr>
<tr>
<td>Either by the 5-dose Eien regimen or the Thai Red Cross regimen with HDCV, PCEC, PVRV or PDEV</td>
<td>ID</td>
<td>boosted with 2 doses of PDEV</td>
<td>5–10 years</td>
<td>All patients developed an anamnestic response after boosters were administered. No significant difference in the antibody level in patients who had received vaccination 5–10 years earlier and those who had been vaccinated more than 10 years previously</td>
<td>Naraporn N et al.</td>
</tr>
<tr>
<td>Received primary PEP or P/E/P with HDCV or PVRV</td>
<td>ID</td>
<td>with 2 doses of PVRV</td>
<td>5–21 years</td>
<td>All patients vaccinated developed an immunological response with no significant difference in the level of titres whether patients received P/E/P or PEP, nor in the length of time since their initial vaccination was administered</td>
<td>Suwansrinon K et al.</td>
</tr>
</tbody>
</table>
• The most important role of rabies vaccination is the induction of a sustained antibody response with the help of CD4+ T cell activation.

• Protective mechanisms involved in the immune response to rabies infection indicate cooperative action of neutralizing antibody, cellular immune soluble factors as well as action by CD8+ T cells to play primary roles.

• “Early/high and late/low” responders to rabies vaccination have been noted with modern cell culture vaccines.
Correlates of Protection

- Rabies Virus Neutralizing Antibodies
- RFFIT and derivatives (e.g. FAVN, EP)
- Issues
  - Challenge strain
  - Virus dose
  - Standard reference serum
  - Tissue culture cells
  - Calculation (ED50, IU/mL)
- Rabies anti-glycoprotein antibodies
  - Measured by ELISA
- Other assays
Challenge studies: rabies antibody level in vaccinated animals and survival

• Animals with “detectable” RVNA at day of challenge survive?
  – Mostly…..
  – **Rare** reports of animals with levels above 0.5 IU/mL dying after challenge

• Animals with no detectable RVNA at day of challenge succumb?
  – Some do, some survive
  – Cellular immunity or undetectable antibody
Practical significance of rabies antibodies in cats and dogs.

Aubert MF1.

1Centre national d'études vétérinaires et alimentaires, Laboratoire d'études sur la rage et la pathologie des animaux sauvages, Malzéville, France.

Abstract

Doubt has sometimes been cast upon the protective effect of rabies antibodies in serum. Animals and humans suffering from fatal rabies often produce high antibody titres, while rabies cases are also observed in vaccinated animals. Cellular immunity is also largely involved in protection. Nevertheless, a large number of laboratory experiments and field observations clearly demonstrate that cats and dogs which develop antibodies after vaccination and before challenge have a very high probability of surviving any challenge, no matter how strong the dose and which virus strain was used. Rabies antibody titration can, therefore, afford a strong additional guarantee to the vaccination certificates accompanying domestic carnivores during transportation between countries. Quarantine rules should also be adapted to the epidemiological features in the exporting country, e.g. statistics of vaccination failure in cats and dogs and host-virus adaptation of the rabies strains circulating in these countries.

Therefore, based on a designated minimum level of neutralising antibodies, and could be proposed as an alternative to quarantine measures. The designated threshold could be based on the results presented in this study. The security of the protection constituted by this threshold would be increased by the extent to which it exceeds the level recognised as effective against experimental challenge in cats and dogs (0.1 IU/ml and 0.2 IU/ml, respectively, measured by RFFIT).
Human Minimum Acceptable RVNA level

• Based on early vaccine clinical trials
  – Measurement of RVNA by mouse neutralization test (MNT) or Rapid Fluorescent Focus Inhibition Test (RFFIT)
  – Determination of adequate vaccine response

• Two guidelines give recommendations:
  – World Health Organization (WHO) – 0.5 IU/mL
  – Advisory Committee on Immunization Practices (ACIP) – complete neutralization of rabies virus at a 1:5 serum dilution in the RFFIT (0.1 IU/mL)
  – These two levels are different
CFR 113.209 Immunogenicity

- Serology required on days 30, 90, 180, 270 & 365
- Species other than carnivores:
  - Challenge can be limited to:
    - 5 vaccinates with the lowest day 270 SN titers, plus
    - 5 vaccinates with the lowest day 365 SN titer, and
    - All vaccinates with SN titers <1:16 by RFFIT, and
    - 5 controls
- Valid test requires:
  - All vaccinates must survive
  - 80% of controls must die due to rabies (humane endpoints allowed)
Proposed *in vitro* assay replacement for NIH: Serology

- OIE – for veterinary vaccines allow for potency test by challenge or serological method.
- Krämer, et al., 2009
  - Modification of the RFFIT/FAVN following the European Pharmacopeia method for RIG potency for higher precision
Proposed *in vitro* assay replacement for NIH: Antigen Quantification

- Gilbert et al., 2013
  - Immuno-capture ELISA using one monoclonal to antigenic site III for both capture and detection
- Chabaud-Riou et al., 2017
  - Immuno-capture ELISA using two monoclonals: to antigenic site II for capture and to antigenic site III for detection.
Replacing the NIH test

- There are clear advantages in replacement of the NIH test for rabies vaccine (cost, time, scientific validity, and 3R’s)
- Validation of a new method is a concern – correlation to NIH is expected to be poor
- Reluctance to give up the NIH test
  - It has been the ‘gold-standard’ for over 60 years
  - Accepted globally
  - Risk aversion (sub potent lots)
  - Availability of new method reagents and consensus of method
- Better acceptance of a combination of serological and antigen quantification results for vaccine approval
Validation of assays

ICH Expert Working Group(Quality) ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology 2018


“Validation of an analytical method is the process by which it is established, by laboratory studies, the performance characteristics of the method meet the requirements for the intended analytical application.” – United States Pharmacopoeia General Chapter 1225.

Good fit?
Assay Validation – Parameters 
as appropriate to the method

- Specificity
- Linearity
- Range
- Accuracy
- Precision
  - Repeatability
  - Intermediate precision
- Detection limit
- Quantitation limit/Sensitivity
- Robustness
- Stability
- Dilution effects
Immunogenicity Assay Validation – specific considerations

• Vaccine assays = biomarker assays?
  – Issues arising from neutralizing and binding antibodies and their overlap
  – Issues relating to class or subclass of immunoglobulins produced.

• Reagent issues
  – Obtaining and preparing reagents is often underestimated
  – Obtaining relevant reference and QC sera can be challenging, including negative samples for matrix evaluation and spiked samples.

• Guidelines and acceptance criteria are aimed at LBA, not cell-based assays

• Focus on LOD not applicable for immunogenicity (vaccine response) where the number of responders at/above the protective level is needed.
Conclusions

• Rabies PEP and PrEP vaccination works because:
  – There is a long incubation period.
  – Vaccine response involves production of rabies virus neutralizing antibodies (RVNA) after activation of CD4+ T cells and B cells.

• Studies show a correlation between antibody level and survival with increasing probability of survival up to 0.5 IU/mL.

• Standardization and quality control of reagents and procedures is absolutely essential and must be evaluated before assigning the assay as fit for purpose.

• Whatever assay is selected, it should have a defined (by validation) relationship to protection and robustly meet the minimum requirements for the purpose and in the designated laboratory(ies).
Questions