Immunity against rotavirus disease
How effective is rotavirus immunity?

- The natural place to begin to answer this question is to examine the protection associated with natural RV infections upon which all RV vaccine candidates evaluated in humans today are based.

- The general conclusions obtained after numerous studies is that immunity after natural RV infection depends on: (1) the time between exposures, (2) the properties of the RVs involved in those exposures, and (3) and the immune status of the human host at the times of each exposure.
**Best protection**

- Healthy children in a developed country (USA) who experienced their first RV infection after 4 months of age and reexposure was within 2 years to RVs belonging to the same serotype as the first (Bernstein et al, JID 164:277-83, 1991)

- In this case, 0/57 previously infected subjects experienced a symptomatic RV reinfection and only 2/57 had an asymptomatic reinfection

- In contrast, 11/85 previously uninfected subjects had symptomatic RV reinfections and 22/85 experienced asymptomatic reinfections

- Thus, protection after natural RV infection can be dramatic
Intermediate protection

- **Example 1:** Children in a less-developed country (Mexico) who were potentially exposed to all major G serotypes (G1-G4) of RV from the time of birth onward (Velaquez et al, NEJM 335:1022-8, 1996)
  
  Under these conditions, most subjects experienced at least 2 rotavirus infections over a 2-year period but efficacy was 77% against RV illness and nearly 100% against moderate-severe RV illness after a single RV infection.

- **Example 2:** Children who experienced neonatal infections (Australia, India) with strains that differed from circulating rotaviruses (Bishop et al, NEJM 309:72-6, 1983; Bhan et al, JID 168:282-7, 1993; Vethanayagam et al, JID 189:2282-9, 2004)
  
  Neonatal RV infections appeared to reduce the incidence of RV disease, particularly severe disease, but did not prevent RV infections.
Loss of protection

- Lack of reexposure to RV may allow full susceptibility to severe disease to develop with time (Nakajima et al, Lancet 357:1950, 2001)

- Severe RV disease is generally not found in adults, presumably due to protection induced by previous infections, but in this Japanese study, adults were reported to be hospitalized with RV diarrhea, presumably due to time-induced loss of immunity and/or exposure to RVs of different genetic make-up
In light of the imperfect protection provided by natural RV infection in most instances, a realistic expectation for a live RV vaccine is that it protect against severe disease during the first years of life.

Protection after vaccination could also wane with time in the absence of reinfection as may occur in hospitalized adults.

However, reinfection is probably a common occurrence during childhood and these reinfections are expected to both broaden and boost immunity.
# Increases in GMT of NA after CJN challenge of 16 adults

(Ward et al, JID 154:871-80, 1986)

<table>
<thead>
<tr>
<th>Viral Strain</th>
<th>Day 0</th>
<th>Day 28</th>
<th>Fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>CJN (G1P[8])</td>
<td>60.3</td>
<td>4,036</td>
<td>66.9</td>
</tr>
<tr>
<td>Wa (G1P[8])</td>
<td>51.8</td>
<td>1,483</td>
<td>28.6</td>
</tr>
<tr>
<td>DS-1 (G2P[4])</td>
<td>25.5</td>
<td>467</td>
<td>18.3</td>
</tr>
<tr>
<td>P  (G3P[8])</td>
<td>98.6</td>
<td>1,710</td>
<td>17.3</td>
</tr>
<tr>
<td>ST-3 (G4P[6])</td>
<td>13.4</td>
<td>286</td>
<td>21.3</td>
</tr>
</tbody>
</table>
What is responsible for protection after rotavirus infection?

Is it antibody?
Examples of reports where antibody titers have been correlated with protection after natural infection

- Specific levels of both serum IgG and IgA have been correlated with protection (O’Ryan et al, JID 169:504-11, 1994; Velazquez et al, JID 182:1602-9, 2000)

- Protection has also been correlated with the presence of serum RV IgG in a Third World nation (Clemens et al, JID 165:161-5, 1992)

- Likewise, levels of stool RV IgA have been correlated with protection (Coulson et al, JCM 1678-84, 1992; Matson et al, JID 167:577-83, 1993)
Titers of serotype-specific neutralizing antibody of $\geq 1:128$ were correlated with protection in a small study conducted in Japan (Chiba et al, Lancet 2:417-21, 1986)

This was not observed in a larger study in Bangladesh where titers of heterotypic neutralizing Abs correlated best with protection (Ward et al, JID 176:570-7, 1997)

Several reports suggest protection after natural infection is related to the serotype of the infecting strain while others suggest protection against a subsequent RV disease is independent of the serotype of RV that caused the first infection
Conclusions on RV antibody in protection after natural rotavirus infection

Total RV antibody titers in serum and stool specimens of naturally-infected subjects have typically correlated with protection.

In some, but not all studies, protection correlated with the serotype of the RV strain or levels of serotype-specific neutralizing Ab.

Note: Antibody (neutralizing or otherwise) was typically the only immune effector measured in these human studies.
Question: Do rotavirus antibody titers correlate with protection after immunization with live RV vaccines?

Answer: Generally not very well
Examples of poor correlations between post-vaccination RV Ab titers and protection

- In the initial large trial with tetravalent reassortant Rotashield vaccine in the USA, only 18-22% of subjects developed serum NA responses to G1-G4 RVs while >90% had detectable IgA responses. Overall protection was between these values (ca. 50% vs. all RV disease) and no Ab level correlated with immunity (Ward and Bernstein, Vaccine 13:1226-32, 1995)

- In the subsequent pivotal USA trial with Rotashield, the best correlate of protection was with heterotypic serum NAs (Ward et al, JID 176:570-7, 1997)

- NA responses to HRVs were generally higher after immunization with the pentavalent reassortant RotaTeq vaccine, but no correlate of protection was found, including stool RV IgA (Vesikari et al, Vaccine 24:4821-9, 2006)
The best correlation between RV Ab responses after vaccination and protection may have been with the monovalent Rotarix vaccine.

Subjects that did not develop serum RV IgA responses after two doses of vaccine have consistently been more likely to develop RV disease.


Protection against severe RV disease has, however, typically exceeded measurable serum RV IgA responses in vaccinees.
The enigma: Why did RRV and WC3 vaccines provide consistent protection only after they were developed into reassortants that contain VP7 or VP4 neutralization protein genes of human rotaviruses, yet there has been no serotype-specific correlates of immunity identified with the multivalent Rotashield and RotaTeq vaccines?
Possible reasons

- Serum Abs are not representative of intestinal antibody. However, RV IgA titers in stool and intestine appear to parallel those in the serum.
  (Hjelt et al, JPGN 4:60-6, 1985; Bernstein et al, JMV 28:90-5, 1989)

- Low levels of neutralizing antibodies cannot be accurately measured. This is true for intestinal Abs but seems less likely for the time-tested serum NA measurements.
Other possible reasons

- RV strains used for measuring NA titers are not matched to vaccine strains. This may play some role since vaccine strains are rarely used for measuring serotype-specific Ab responses and both VP4 and VP7 proteins of strains belonging to the same RV serotypes have been found to have differences in their neutralization epitopes.

- Multi-strain vaccines provide boosts in immunity not possible with single strain vaccines. Few Rotarix vaccinees with immune responses after the first dose respond again after the second dose while many subjects administered the quadrivalent precursor to RotaTeq experienced multiple boosts in immunity (Salinas et al, PIDJ 24:807-16, 2005; Ward et al, JID 189:2290-3, 2004)
Finally

- Something other than NA is generated after vaccination with reassortants containing human VP4 or VP7 genes that is responsible for protection, possibly T cells or non-neutralizing antibodies.

- Low levels of RV-specific CD4 and CD8 T cells have been found in blood of infected adults for short periods after RV infection but it has not been possible to correlate their levels with protection in human studies (Jaimes et al, JV 76:4741-9, 2002).

- This limitation with human studies stimulated the development of animal models with which to attempt to decipher the mechanisms of active immunity to rotavirus, e.g. the adult mouse model.
What has been learned about rotavirus immunity with the adult mouse model?
Importance of T cells in immunity

- CD8 T cells have been shown to be the first lymphocytes involved in resolution of a primary RV infection in mice (McNeal et al, Virol. 214:387-97, 1995; Franco and Greenberg, JV 69:7800-6, 1995)

- RV-specific CD8 T cells decline rapidly after infection and may play little role in prevention of reinfection (Franco et al, JV 71:4165-70, 1997)

- The role of CD4 T cells in protection after RV infection has been primarily associated with their helper function in B cell development and Ab production (VanCott et al, EJI 31:3380-7, 2001)
Importance of antibody in immunity in mice

- Protection against RV reinfection after a primary RV infection is greatly reduced in B cell ko mice (McNeal et al, Virol. 214:387-97, 1995; Franco and Greenberg, JV 69:7800-6, 1995)

- Infection of mice with a heterotypic RV can elicit nearly complete protection against RV reinfection but this protection is lost in J chain ko mice (Feng et al JID 175:330-41, 1997; VanCott et al, JV 804949-61, 2006)
Protection against EDIM (G3P[16]) shedding after D x RRV (G1P[3]) infection of wild type or J chain ko BALB/c mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Quantity RV shed</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>unimmunized</td>
<td>2,084 ng (Avg.)</td>
<td></td>
</tr>
<tr>
<td>immunized</td>
<td>0.2 ng</td>
<td>99.99 %</td>
</tr>
<tr>
<td>J chain ko mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>unimmunized</td>
<td>6,149 ng</td>
<td></td>
</tr>
<tr>
<td>immunized</td>
<td>4,261 ng</td>
<td>30.7 %</td>
</tr>
</tbody>
</table>
Type of antibody needed for protection in mice

- No detectable NA against EDIM was generated after D x RRV immunization (VanCott et al, JV 80:4949-61, 2006)

- Protection against EDIM reinfection was not associated with either serum or intestinal NA (Ward et al, Virol. 188:57-66, 1992)

- Protection after RV infection of mice correlated with serum and stool RV IgA levels (Feng et al, JV 68:7766-73, 1994; McNeal et al Virol. 204:642-50, 1994)

- A non-neutralizing anti-VP6 IgA mAb protected against VP6, possibly through intracellular neutralization (Burns et al, Science 272:104-7, 1996)
Summary

- RV Ab levels correlate with protection after natural RV infection but less well after live RV vaccination.

- Neutralizing antibodies probably have an important role in protection after live RV infection, if present, but other effectors, such as non-NAs and possibly T cells, appear to have important effector functions as well (immunological redundancy).

- Studies in adult mice indicate non-neutralizing, polymeric Ab is a major effector of protection after live RV infection.