Comparative Developmental Immunology and Implications for Testing and Data Interpretation

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This presentation does not represent the opinions and policies of Horizon Therapeutics. There are no conflicts of interest to disclose.
Objectives

- Understand the overarching differences and similarities in immune system development for species used in non-clinical safety studies.

- Understand the application of this knowledge to immune intervention in vivo animal studies.
Overview

- A healthy immune system is strongly conditioned in early life.
- Overall, immune system anatomy and function between mammals is highly similar.
- The primary difference relates to the timing of the developmental events.
- In non-clinical studies, the period of immune development in humans should be modeled in animals to enhance translatability and predictability (reliability).
Importance of Early Immune Development

- “The first 1,000 days of life is a period of growth and development in which the foundations of lifelong immune homeostasis and microbial colonization are established in humans.” van Bilsen et al, 2020

- No regulatory guidance on how to evaluate the risk/benefit of immune system interventions.
  - Must rely on costly clinical follow-up studies.
Children currently receive a notable amount of immune interventions.
  - Immunizations
  - T1D
    - Extensive number of clinical trials in children (infants to sexually mature) using immune interventions (often supplements) to preserve β-cell mass and delay T1D formation
  - In-born errors in immunity

Furthermore, immune interventions are under investigation to improve immune function or restore immune system balance in children.
  - e.g., testing of specific human milk oligosaccharides to decrease diarrhetic effects of rotavirus in suckling rats (Azagra-Boronat et al 2018)
Early Embryonic Development

- Relative to humans, the timing of early embryonic events are relatively similar across species.
  - Blastocyst formation
  - Implantation

### Table 1
Prenatal and Postnatal Milestones.

<table>
<thead>
<tr>
<th>Gestational period</th>
<th>Human</th>
<th>NHP</th>
<th>Dog</th>
<th>Minipig</th>
<th>Rabbit</th>
<th>Rat</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Early</td>
<td>GD 0-12</td>
<td>GD 0-50</td>
<td>GD 0-30</td>
<td>GD 0-37</td>
<td>GD 0-7</td>
<td>GD 0-6</td>
<td>GD 0-6</td>
</tr>
<tr>
<td>2. Mid</td>
<td>GW 13-28</td>
<td>GD 51-100</td>
<td>GD 31-43</td>
<td>GD 38-75</td>
<td>GD 7-20</td>
<td>GD 7-13</td>
<td>GD 7-13</td>
</tr>
<tr>
<td>3. Late</td>
<td>GW 29-40</td>
<td>GD 101-160/165</td>
<td>GD 44-65</td>
<td>GD 76-113</td>
<td>GD 20-29</td>
<td>GD 14-21</td>
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</tr>
</tbody>
</table>

- Blastocyst formation
- Implantation

- Primitive streak/primitive hematopoiesis
- Placental completion
  1. Inverted yolk sac placenta
  2. Chorioallantoic placenta
  - Diffuse epitheliocorial type
  - Discoid hemochorial type
  - Endotheliocorial type

Skaggs et al 2019
Postnatal Events

- Relative to humans, the **timing of the newborn period is similar across species**
- Differences between species **begin to emerge after the newborn period**

### Table 2
Postnatal key age classes.

<table>
<thead>
<tr>
<th>Species</th>
<th>Duration of gestation from fertilization - birth Birth</th>
<th>Newborn</th>
<th>Infant Weaning</th>
<th>Child/Juvenile</th>
<th>Adolescent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>252–280 days</td>
<td>0–28 days</td>
<td>1–23 months</td>
<td>2–12 years</td>
<td>12–16 years</td>
</tr>
<tr>
<td>NHP</td>
<td>155–165 days</td>
<td>0–15 days</td>
<td>0.5–6 months</td>
<td>0.5–3 years</td>
<td>3–4 years</td>
</tr>
<tr>
<td>Dog</td>
<td>65 ± 2 days</td>
<td>0–21 days</td>
<td>3–6 weeks</td>
<td>6–20 weeks</td>
<td>5–7 months</td>
</tr>
<tr>
<td>Minipig</td>
<td>112–115 days</td>
<td>0–15 days</td>
<td>2–4 weeks</td>
<td>4–14 weeks</td>
<td>4–6 months</td>
</tr>
<tr>
<td>Rabbit</td>
<td>31 ± 2 days</td>
<td>0–10 days</td>
<td>1.5–5 weeks</td>
<td>5–12 weeks</td>
<td>3–6 months</td>
</tr>
<tr>
<td>Rat</td>
<td>20–21 days</td>
<td>0–7/10 days</td>
<td>1/1.5–3 weeks</td>
<td>3–4.5/6 weeks</td>
<td>5/7–10/11 weeks</td>
</tr>
<tr>
<td>Mouse</td>
<td>20–21 days</td>
<td>0–7/10 days</td>
<td>1/1.5–3 weeks</td>
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Skaggs et al 2019
Timing of Immune System Development

- Relative to humans and NHP, rodents generally display a delay in immune system maturation.

- Examples:
  - Adult Levels of IgG
    - Rodents—young adult (PND42-49)
    - NHP—juvenile period (<1 year old)
  - First Detectable TDAR
    - Rodents—uring/after weaning
    - NHP—during lactation
The process of development, maturation, and regression of the thymus in adulthood, is generally similar across species.

Development of the thymus is delayed in rodents.

Example (blue arrows):
- First thymocytes
  Rodents: mid-gestation
  Humans: early gestation
Spleen Development

• Spleen is an excellent example of extended development in rodents.
  ‒ At birth, humans and NHP spleen are anatomically well developed.
  ‒ Rodents and rabbits achieve adult morphology closer to birth.

• This translates to functional effects as well.

• Example (blue arrows):
  ‒ First follicles detected
    Rodents: infancy
    Humans: mid-gestation

• Similar to the spleen, lymph nodes in humans and NHPs are morphologically well developed at birth, while other species obtain adult morphology in the post-natal period.

• Example (blue arrows):
  – Cortex development
    Rodents: newborn period
    Humans: mid-gestation
• Example of species-specific differences in immune function using a standard immunotoxicology assay (TDAR).

• Blue star represents the maximal TDAR response identified in the literature for:
  - Rodent—Young adult stage
  - Minipig—Young adult stage
  - Human—After parturition

### Critical Windows of Toxicity/Intervention

**Table 1: Prenatal and Postnatal Milestones.**

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</tr>
<tr>
<td>GD 4-6.5</td>
<td>GD 7-8</td>
<td>GD 9-10</td>
<td>GD 5-6</td>
<td>GD 5-6</td>
<td>GD 3.5-5.5</td>
<td>GD 3-6</td>
</tr>
<tr>
<td>GD 6-7</td>
<td>GD 8-10</td>
<td>GD 17-19</td>
<td>GD 11-13</td>
<td>GD 7-7.5</td>
<td>GD 5-6</td>
<td>GD 5</td>
</tr>
<tr>
<td>GD 13</td>
<td>GD 14</td>
<td>GD 16-21</td>
<td>GD 8-12</td>
<td>GD 7.5</td>
<td>GD 8.5</td>
<td>GD 6.5</td>
</tr>
</tbody>
</table>

**Critical windows for toxicity/intervention:**

1. Initiation hematopoiesis
   - GW 8-10
   - GD 14-21
   - GD 11 (in)
   - GD 7-9

2. Migration stem cells + expansion progenitor cells
   - GW 10-16
   - GD 21-25
   - GD 23-25
   - GD 9-16

3. Colonization bone marrow, thymus
   - GW 16-birth
   - GD 35-56
   - GD 13 to birth
   - GD 13-birth

4. Maturation of immune competence
   - Birth-year 1
   - 0-3 months
   - Birth to 8 weeks?
   - Birth-day 30

5. Establishment of immune memory (ends at sexual maturity)
   - Years 1-18
   - Years 1-3
   - Months 1-3mo
   - 20 weeks?
Conclusion

- A healthy immune system starts early in life.
- Immune interventions often occur in children.
- Immunotoxicology studies can be conducted in non-human species and translated to humans, given:
  - The endpoint or function that is being assessed is matched chronologically between humans and the test species
    - Notably, the prolonged maturation of rodents is taken into consideration.
  - Species exceptions are considered.

- There is not one perfect species—dependent on the process and endpoint

- When considering data, the context of the species in which the study was conducted should be carefully considered.