Short communication

Prenatal passive transfer of maternal immunity in Asian elephants (Elephas maximus)

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A R T I C L E   I N F O

Article history:
Received 21 January 2013
Received in revised form 5 March 2013
Accepted 19 March 2013

Keywords:
Asian elephant
Endotheliochorial
Passive immunity
Rabies
Tetanus

A B S T R A C T

Asian (Elephas maximus) and African (Loxodonta africana) elephants exhibit characteristics of endotheliochorial placentation, which is common in carnivore species and is associated with modest maternal to fetal transplacental antibody transfer. However, it remains unknown whether the bulk of passive immune transfer in elephants is achieved prenatally or postnatally through ingestion of colostrum, as has been documented for horses, a species whose medical knowledgebase is often extrapolated for elephants. To address this issue, we took advantage of the fact that many zoo elephants are immunized with tetanus toxoid and/or rabies vaccines as part of their routine health care, allowing a comparison of serum antibody levels against these antigens between dams and neonates. Serum samples were collected from 3 newborn Asian elephant calves at birth (before ingestion of colostrum); 2–4 days after birth; and 2–3 months of age. The findings indicate that the newborns had anti-tetanus toxoid and anti-rabies titer that were equivalent to or higher than the titers of their dams from birth to approximately 3 months of age, suggesting that the majority of maternal-to-fetal transfer is transplacental and higher than expected based on the architecture of the Asian elephant placenta.

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1. Introduction

The placentas of eutherian mammals are classified according to two major characteristics: (1) placental shape and contact sites between fetal and maternal tissue, and (2) the number of cellular layers that separate maternal and fetal circulation systems (Chucri et al., 2010; Moffett and Loke, 2006). Passive transfer of maternal antibodies to the fetus is determined by the structure of the fetomaternal barrier (Chucri et al., 2010; Moffett and Loke, 2006). Species such as horses, swine, and ruminants develop epitheliochorial or synepitheliochorial placentas, which generally impede passage of antibodies from maternal to fetal circulation systems (Tizard, 2001). In these animals, passive transfer of antibodies is dependent on the ingestion and absorption of colostrum. In contrast, endotheliochorial placentation, which occurs in dogs and cats, has been estimated to allow 5–10% of maternal transplacental antibody transfer (Chucri et al., 2010; Heddle and Rowley, 1975). Hemochorial placentation, exhibited by humans and rodents, allows significant transplacental antibody...
transferred (Chucr et al., 2010; Roopenian and Akilesh, 2007). In contrast to other hind-gut fermenters such as equines, both Asian and African elephants have been found to develop endotheliocorial placentas (Cooper et al., 1964; Perry, 1974). To the best of our knowledge, the nature and robustness of transplacental maternal antibody transfer in elephants has not been investigated. The importance of addressing this issue has arisen in recent years with the emergence of a newly recognized herpesvirus, known as elephant endotheliotropic herpesvirus (EEHV), that has been associated with the death of a significant number of captive juvenile Asian elephants (Richman and Hayward, 2011). Intriguingly, peak vulnerability to this virus appears to be between 1 and 3 years of age, which is approximately around the time when calves are weaned, leading to some speculation that susceptibility to lethal infections by EEHV may correlate to loss of protective immunity from their dams. Successful vaccines have been developed for other herpesviruses and should theoretically be possible for EEHV as well (Jarosinski et al., 2006; Smith and Khanna, 2010; Stegeman et al., 1994, 1995). To better understand the role of protective maternal immunity and how to best implement a vaccine strategy, additional information about the nature of passive immunity is needed. To address this issue, we took advantage of the fact that many captive elephants are routinely vaccinated against a variety of pathogens or toxins, including rabies and tetanus. The purpose of this study was to determine whether significant transplacental antibody transfer occurs in Asian elephants. The results will have significant impact in development of vaccine strategies for elephants, especially for future EEHV vaccines.

2. Materials and methods

2.1. Animals and sample acquisition

All elephants in the study were Asian elephants (E. maximus). The age, gender, and date of birth (DOB) for the elephants are summarized in Table 1. The Rabies (Imrab®®, Merial Inc. Athens, GA 30601) and Tetanus (Equiloid Innovator®, Ft. Dodge Animal Health, Ft. Dodge, IA, 50501) vaccines were administered intramuscularly (IM) to elephants 1–4 on the dates summarized in Table 1. Tetanus vaccine for elephants 5 and 6 was Tetanus Toxoid (Fort Dodge Animal Health, Ft. Dodge, IA, 50501) and was administered IM on dates shown in Table 1. During the study period, elephants 1–4 were located at the Houston zoo and elephants 5–6 at the Oklahoma City zoo. Serum was prepared from blood collected in Corvac serum separator tubes (Tyco Healthcare, Mansfield, MA 02048, USA). Serum was collected from elephants 2, 4, and 6 within 30 min after birth and before nursing and ingestion ofcolostrum from their dams (Day 0). Serum was collected opportunistically from elephants 2, 4 and 6 on days or weeks following their birth, as indicated in Fig. 1.

Rabies antibody titers. Anti-rabies virus titers were determined at Kansas State University as described previously (Isaza et al., 2006; Smith et al., 1973). Briefly, elephant sera were serially diluted and mixed with a standard amount of live rabies virus followed by addition of baby hamster kidney (BHK)-21 cells and then incubated on slides for an additional 24 h. Slides were fixed in acetone and Infectious foci detected with an anti-rabies FITC conjugate. The calculation of endpoint titer is made from the percent virus infected cells observed on the slide. Results are reported as RVNA (Rabies Virus Neutralizing Antibodies) potency in International Units/ml (IU/ml). The IU is calculated from the titer by comparing it against the titer of a standard reference serum. As described previously, RVNA titers > 0.5 IU/ml are indicative of a response to the rabies vaccine (Isaza et al., 2006).

Tetanus antibody titers. Anti-tetanus toxoid (ATA) titers were determined by ELISA, which was modified from a tetanus ELISA described previously (Lindsay et al., 2010). Briefly, ninety-six well polystyrene plates (Immuno 4 HBX, Thermo Electron Corp., Milford, MA) were coated with 1 μg of purified tetanus toxoid (List Biological Laboratories, USA) at a 20 μg/ml concentration in Carbonate Bicarbonate Buffer (pH 9.6). The plate was then incubated overnight at 4°C, washed with PBS/0.5% Tween, and blocked with 1% teleostean gelatin-PBS (Sigma Chemical Co., USA) for 1 h at 37°C. The plates were then blocked for 2 h at RT with blocking buffer (5% non-fat dry milk as in 2.5 g non-fat dry milk powder in 50 ml 0.01 PBS) and washed twice with PBS/Tween 0.05%. Elephant serum samples were serially diluted two-fold in blocking buffer. Tetanus toxoid-specific IgG was detected using a horse radish peroxidase (HRP)-conjugated rabbit anti-elephant-IgG. Rabbit antiserum to elephant immunoglobulin was prepared by immunizing rabbits with elephant IgG, which was purified as described previously (Kania et al., 1997). The IgG fraction from the rabbit antiserum was purified using Protein A/G affinity chromatography and then conjugated with HRP using a Pierce Conjugate purification kit (Thermo Scientific, Rockford, IL) according to the manufacturer's

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Table 1

Elephant demographics and relevant vaccination history.

<table>
<thead>
<tr>
<th>Dam/calf pair</th>
<th>Elephant</th>
<th>Gender</th>
<th>DOB*</th>
<th>Originb</th>
<th>Tetanusc</th>
<th>Rabiesd</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>F</td>
<td>10/11/90</td>
<td>CB</td>
<td>2/08, 4/09, 2/10</td>
<td>2/08, 4/09, 2/10</td>
</tr>
<tr>
<td>A</td>
<td>2</td>
<td>M</td>
<td>05/04/10</td>
<td>CB</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>F</td>
<td>Est 1981</td>
<td>WB</td>
<td>8/08, 4/09, 2/10</td>
<td>8/08, 4/09, 2/10</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>F</td>
<td>10/03/10</td>
<td>CB</td>
<td>4/11</td>
<td>4/11</td>
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<tr>
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<td>F</td>
<td>02/02/95</td>
<td>CB</td>
<td>11/08, 1/11</td>
<td>None</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>F</td>
<td>04/15/11</td>
<td>CB</td>
<td>12/12</td>
<td>12/12</td>
</tr>
</tbody>
</table>

* DOB, date of birth month/day/year for CB. Estimate for wild born WB.

b CB, captive born; WB, wild born.

c Dates of tetanus vaccination (month/year).

d Dates of rabies vaccination (month/year).
3. Results and discussion

The rabies antibody assay indicated that both elephant 1 and her calf, elephant 2 (elephant pair A), had detectable concentrations of RVNAs during the time period of the study (Fig. 1A). Interestingly, the newborn calf had RVNAs that were slightly higher at birth than his dam (Fig. 1A). The calves RVNAs increased approximately 2-fold at days 2 and 8 following birth, which was after he had ingested several feedings of colostrum. The calf maintained these RVNA titers out to almost 3 months after birth. The anti-tetanus assay also showed that elephant 2 had higher ATA titers at birth than his dam, and remained at this level until day 86 (Fig. 1B). Similar to elephants 1 and 2, elephant 3 and her calf, elephant 4 (elephant pair B) also had detectable concentrations of RVNAs (Fig. 1A). Like elephant 2, elephant 4 had detectable RVNAs at birth, although she did not have an appreciable increase in titers until day 8. At 2 months following birth, her RVNA titer appeared to be dropping from its peak at day 8. Elephant 4’s ATA titers were equal to her dam’s at birth until day 64 (Fig. 1B). Her Dam, elephant 3, continued to maintain post-partum ATAs of 400 for more than 3 months. Elephants 5 and 6 (dam and calf respectively; elephant pair C) had no known history of rabies virus vaccination and as expected, RVNA titers were 0.11, which are considered insignificant (Fig. 1A; note y-axis scale is different for this graph). Elephant 6 did have ATA titers of 200 at days 0 and 2, which were similar to those found in her dam, elephant 5 (Fig. 1B). No serum samples were available for elephant 6 after day 2.

This study indicates that elephants passively transfer significant amounts of immunoglobulin prenatally to their young. Evidence for this conclusion is based on consistent detection of RVNAs and ATAs in serum from newborn calves equal to or exceeding the levels of their dams before the ingestion of colostrum (Fig. 1). The contribution of colostrum in our study is less certain, as RVNA and ATA titers between days 3–8 in elephants 2 and 4 did not consistently increase. RVNA and ATA titers did not drop appreciably at 2–3 months in these elephants, which might be expected. The half-life of IgG in species such as mouse
(Vieira and Rajewsky, 1988), pig (Curtis and Bourne, 1973), horse (Wilson et al., 2001), and humans (Paul, 2013) has been estimated to range from 6 to 26 days and so we were surprised to observe that RVNA and ATA titers did not drop appreciably at 2–3 months in these elephants. One potential explanation for this is that elephants might express a neonatal receptor for IgG (FcRn) in their intestines like rodents, which would enable them to acquire IgG through milk for a sustained period of time. There are several other possibilities: elephant IgGs may have a longer half-life; the sensitivity of our assays is limited; and/or the lack of later time points prohibited our ability to observe declining titers. While the ATA titers are IgG specific, the role of other immunoglobulin isotypes in the rabies assays is possible, although the ATA and RVNA data are roughly comparable. Although endothelial trophoblast placentalation was expected to allow some prenatal antibody transfer, one surprising aspect of our data was the amount of prenatal immune transfer: antibody titers in the neonates were similar to or higher than the titers in the dams. Expression of FcRn receptors in elephant trophoblasts has not been reported to our knowledge. Whether FcRn-mediated transfer occurs at a sufficiently high level, or if alternative mechanisms have evolved in elephants to transport immunoglobulins across the placental barrier remains to be determined. In summary, this study demonstrated that the majority of passive transfer of maternal immunity occurs prenatally and that maternal antibody persists at levels comparable to the dam for up to 3 months of age. This information increases our understanding of the immunity of elephant neonates allowing us to better manage their care and to guide the design of optimal immunization protocols.

Acknowledgments

Financial support for these studies was provided by the Houston zoo. Sally Nofs was supported by National Institutes of Health training grant T32-Al-07471. We thank Erin Latimer of the National Herpesvirus laboratory for the generous gift of rabbit anti-elephant antibodies. We thank Dr. Margaret Conner of Baylor College of Medicine for assistance with the Tetanus Toxoid ELISA refinement. We would also like to thank the Houston Zoo, the Oklahoma City Zoo, and the St. Louis zoo for samples provided for this study. We especially appreciate the critical review of the study design and manuscript by Dr. Ian Tizard.

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