Mammalian Biology 78 (2013) 361–364

Contents lists available at SciVerse ScienceDirect

Mammalian Biology

journal homepage: www.elsevier.com/locate/mambio

Short Communication

Evidence of cross-transfer of maternal antibodies through allosuckling in a mammal: Potential importance for behavioral ecology

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ARTICLE INFO

Article history:
Received 24 September 2012
Accepted 23 November 2012
Available online 24 December 2012

Keywords:
Meriones unguiculatus
Mongolian gerbil
Ecological immunology
Host–parasite interactions
Transfer of maternal antibodies
Transgenerational vaccination design

ABSTRACT

The transfer of maternal antibodies is a critical mechanism for the early life survival of vertebrate newborns. In mammals, passive transfer of immune compounds can occur prenatally through the placenta and postnatally through the consumption of colostrum and milk. In social mammals, it has been hypothesized that allosuckling may be a way for pups to broaden and strengthen their passive access to antibodies after birth, but empirical evidence for this mechanism is still lacking. In order to investigate the potential for the occurrence of a cross-transfer of antibodies between pups exposed to several females, we bred in a common environment groups of two females Mongolian gerbils (*Meriones unguiculatus*), each previously injected with a different vaccine. Here we report the dynamics of passively acquired specific antibodies in the serum of newborns, showing that pups acquired antibodies from both females of a group. Our result provides the first experimental evidence of a cross-transfer between litters of passively acquired antibodies. We discuss how such evidence opens perspectives for exploring the potential importance of horizontal transfer of immunity in natural host–parasite systems and how this could be used as a tool to answer important behavioral ecology questions.

Environmental conditions encountered by a newborn during early life are important for its fitness later in life (Lindström 1999). Parasites, in particular, represent a major pressure on the population dynamics and evolution of their host species (Grenfell and Dobson 1995; Tompkins et al. 2002). The effect of parasites may potentially be even stronger in young vertebrates whose immune response is not completely functional at birth (Frank 2002). In vertebrates, mothers have the ability to provide the newborn with a protection critical for early life survival (Boulinier and Staszewski 2008; Grindstaff et al. 2003) by transferring some specific molecules produced as part of the acquired immune response, the antibodies (also termed immunoglobulins [Ig]; Brambell 1970). In mammals, this transfer occurs through the placenta before birth and/or via the colostrum and the milk after birth (Baintner 2007). The amount of Igs transferred through the placenta is determined by the anatomical structure of the placenta (Chucri et al. 2010), and in particular by variations in the number of tissue layers between maternal and fetal bloods. Differences in Ig subtypes are also important to consider as only immunoglobulin G (IgG) can reach the newborns bloodstream (Pastoret 1998). Similarly, postnatal absorption of antibodies by newborns also reveals variations across mammalian species. For instance, in artiodactyls, no placental transfer of Igs is possible and colostrum absorption is critical for early life survival (Halliday 1978). In contrast, placental transfer seems to be the dominant route for Igs transfer in primates while Igs in the colostrum and the milk may mostly play a role in the local protection of the gut (Sadeharju et al. 2007). In rodents, both prenatal and postnatal absorption of antibodies appear to be important factors to ensure the survival of the newborn (Gustafsson et al. 1994).

How newborns gain access to colostrum and milk may thus be important in terms of passive transfer of immunity in many mammalian species. An interesting way for newborns to get milk is through allosuckling, when young individuals feed from a different female than their biological mother. Allonursing is indeed widely reported in mammals (Packer et al. 1992; Roulin 2002) and offspring could gain important immunological benefits by acquiring antibodies from various lactating females (immunological function of allosuckling hypothesis; Roulin and Heeb 1999). The potential for an immunological function of allosuckling has been suggested through the efficient transfer of antibodies from a foster mother to her non-biological offspring (e.g. Gustafsson et al. 1994; Halliday 1955). However, adoption in the laboratory does not reflect conditions encountered normally in social groups. In the wild, mothers and newborns are exposed to biological and foster mothers/pups

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http://dx.doi.org/10.1016/j.mambio.2012.11.004

at the same time. Mothers may thus choose which pups to suckle and pups may in turn choose from which females to get milk.

In order to investigate the potential for the occurrence of a cross-transfer of antibodies, we focused on a social mammal, the Mongolian gerbil (*Meriones unguiculatus*). In the wild, this species usually forms groups of one breeding pair associated with a number of subordinates. In the lab however, they can breed successfully in groups, with several females giving birth together (French 1994). Two groups of 12 "specific pathogen free" females (obtained from Janvier, Le Genet-St-Isle, France) were vaccinated twice at 8 and 12 weeks of age respectively against influenza (0.1 mL, intramuscular injection; Gripovac, Merial, France) or *Borrelia burgdorferi* (0.1 mL, subcutaneous injection; Merilym, Merial, France). Antibodies specific of *Borrelia* (females vaccinated against influenza) or specific of influenza (females vaccinated against *Borrelia*) remained at undetectable levels throughout the study.

After the booster vaccination, each female was housed during 2 weeks with a male. To prevent direct initial contact and limit aggression, we split the cages with transparent Plexiglas walls (drilled with holes of approximately 1 cm). We then switched individuals between each side of the separation everyday during a week to get them used to each other. At the end of the week, the separation was opened and contact between the male and the female was rendered possible. When aggressive interactions among adults were nevertheless recorded, individuals were immediately separated. The same procedure was repeated to match females by pairs based on their vaccination status (one female vaccinated against influenza and the other against *Borrelia*). The females were subsequently housed together without the males. Pups were thus born in an environment mimicking a natural social system and expected to gain access to the milk of both females and to display detectable levels for both antigens.

At birth, newborns were marked subcutaneously with an individual combination of tattoo ink dots. At 3 weeks of age, before the first marking faded, an additional numbered ear tag (Monel 1005-1, National Band & Tag Co., Newport, KY, USA) was used to ensure a long lasting individual marking of individuals. The same ear tags were also used for the marking of females throughout the experiment. To assess passive antibody acquisition, blood was regularly obtained from the newborns during the rearing period. At day 1 (i.e., within 24 h after birth, as cages were checked every day) and day 8, a blood sample (20–30 μL) was obtained by cardiac puncture using an insulin syringe with 30 G needle. After eyes had opened, blood sampling consisted of a puncture each week (starting day 19, and up to day 48) in the retro-orbital venal sinus with a heparinized capillary tube (75 μL), alternatively from the left and right eye. Blood was then stored in dry tubes and centrifugated within an hour. The plasma was collected and kept frozen at −20 °C pending analyses. This sampling protocol was chosen to follow the newborns for a sufficiently long period after weaning (between 21 and 30 day; Norris and Adams 1972).

Antibody levels in newborns were measured using specific commercial Enzyme Linked Immuno-Sorbent Assays (ELISA). An indirect ELISA kit was used for influenza (ID Screen Antibody Influenza A Competition, ID Vet, Montpellier, France). The percentage of inhibition (PI) relative to a negative control was used as a measure of antibody level. High PI values indicate high plasma concentrations of specific antibodies against influenza. A direct ELISA kit was used for *Borrelia* analyses (Borrelia IgG + VlsE ELISA, IBL International GMBH, Hamburg, Germany). As this kit is designed for humans, we replaced the secondary antibody by a peroxidase conjugated rabbit anti-gerbil IgG (Immunology Consultants Laboratory, Portland, OR, USA). Optical density (OD) was used as a measure of antibody levels in the newborns and high OD values reflected high serum levels of *Borrelia* specific antibodies. Analyses were run once for each sample due to the limited amount of serum available and were limited to the 4 pups that reached adulthood as complete dynamics over the rearing period were required to assess the dynamics of the passive transfer of antibodies.

The present study complies with the guidelines set in the Guide for the care and use of laboratory animals (National Research Council 2011) and the protocol has been approved by the Animal Care and Use Committee – Languedoc Roussillon (project number CEEA-LR-1003). In addition, because of the repeated sampling of newborns required by the protocol, we chose not to include any control group in order to minimize the number of animals used.

Despite preventive measures to limit agonistic interactions, females showed high levels of aggressiveness toward males. In addition, high rates of cannibalism of females on pups occurred rapidly after birth. The latter made it impossible to keep track of the exact number of births over the 12 female pairs set up. For only one pair of females, seven pups were found alive at day 1. Three of these newborns died during their first week, but the two females were able to raise the four remaining pups to adulthood. At birth, three of those four pups had high anti-*Borrelia* antibody levels while the remaining one had a much lower level (Fig. 1). As IgG against *Borrelia* can be transmitted through the placenta in rodents (Morshed et al. 1993), this result suggests that those three pups were born to the *Borrelia* vaccinated mother. The female vaccinated against influenza likely gave birth to the remaining one. Anti-*Borrelia* antibody levels showed a steep increase during the first week of age which can be explained by the acquisition of maternal antibodies from the milk. All individuals reached very high levels by 8 days of age. The antibody levels remained relatively stable between 8 and 19 days of age, which is expected as newborns fed on milk during this period. Afterwards, antibody levels decreased rapidly and reached non-detectable levels by 40 days of age. The dynamics was similar for anti-influenza antibodies throughout the rearing period of newborns (Fig. 2). However, at birth, all individuals had no detectable antibodies indicating that antibodies against the nucleoprotein of influenza viruses may not be transmitted through the placenta.

All newborns displayed at some point during the rearing period detectable antibody levels specifically directed against both *Borrelia* and influenza. Although there was no control group of offspring raised by unvaccinated mothers, the decay of antibody levels following weaning is not consistent with a stimulation of the immune system of the newborns. It rather fits the dynamics expected if the newborns passively acquired antibodies from both females. This result demonstrates the efficiency of allosuckling as a source of antibodies in newborns and provides evidence for a potential immunological function of allosuckling. The transmission of antibodies from a mother to foster offspring has been described before but this is the first time that cross-transfer of antibodies has been reported in a social context, when females can choose which young to suckle and when newborns can choose from which female to suck.

A number of challenges related to the breeding of gerbils have reduced the sample size in the present study. A way to reduce aggressiveness problems could be to rear non-sexually mature individuals in groups of one male and two females. Because the social group would not have been modified recently before the females give birth, lower levels of infanticide could be expected (e.g. Elwood 1991). Another possibility is to pair females only after the pups were born. In this latter case, the identity of the mother of the pups would be known and one could thus avoid sampling newborns in the very few days following birth. However, such a design would limit the access of pups to the colostrum of non-biological mothers and in turn may modify the dynamics of acquisition of passive antibodies by the newborns.

The rapid decay of antibody levels starting after 20 days of age is associated with weaning, which usually occurs between 21 and