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Maternal antibodies reduce costs of an immune response during development

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SUMMARY

Young vertebrates are dependent primarily on innate immunity and maternally derived antibodies for immune defense. This reliance on innate immunity and the associated inflammatory response often leads to reduced growth rates after antigenic challenge. However, if offspring have maternal antibodies that recognize an antigen, these antibodies should block stimulation of the inflammatory response and reduce growth suppression. To determine whether maternal and/or offspring antigen exposure affect antibody transmission and offspring growth, female Japanese quail (*Coturnix japonica*) and their newly hatched chicks were immunized. Mothers were immunized with lipopolysaccharide (LPS), killed avian reovirus vaccine (AR), or were given a control, phosphate-buffered saline, injection. Within each family, one-third of offspring were immunized with LPS, one-third were immunized with AR, and one-third were given the control treatment. Maternal immunization significantly affected the specific types of antibodies that were transmitted. In general, immunization depressed offspring growth. However, offspring immunized with the same antigen as their mother exhibited elevated growth in comparison to siblings immunized with a different antigen. This suggests that the growth suppressive effects of antigen exposure during development can be partially ameliorated by the presence of maternal antibodies, but in the absence of specific maternal antibodies, offspring are dependent on more costly innate immune defenses. Together, the results suggest that the local disease environment of mothers prior to reproduction significantly affects maternal antibody transmission and these maternal antibodies may allow offspring to partially maintain growth during infection in addition to providing passive humoral immune defense.

Key words: antibody transmission, maternal effects, growth, maternal antibodies, cost, immune response.

INTRODUCTION

Neonatal vertebrates have limited ability to synthesize antibodies endogenously (Brambell, 1970; Solomon, 1971; Lawrence et al., 1981). Instead, maternally derived antibodies provide the primary form of humoral (antibody-mediated) immune defense (Brambell, 1970; Grindstaff et al., 2003). The diversity and quantity of specific antibodies transmitted to offspring have been shown to reflect differences in the local disease environment experienced by females prior to reproduction (Lemke and Lange, 1999; Lundin et al., 1999; Gasparini et al., 2001). Collectively, these antibodies represent the cumulative antigen exposure of females over their lifetime (Lemke et al., 2003). Conversely, those females not exposed to particular pathogens prior to yolk deposition cannot transfer antibodies to those pathogens, leaving their offspring susceptible to more severe infections (Heller et al., 1990). Furthermore, the strength of the female antibody response may partially determine offspring susceptibility to disease. For example, offspring survival after challenge with *Escherichia coli* is positively correlated with the mother's antibody response during egg laying in the domestic hen (Heller et al., 1990). Offspring of hens with low antibody titers to *E. coli* are more likely to die after immunization with *E. coli* than offspring of hens with high antibody titers. Interestingly, maternal antibodies provide protection only for homologous strains of *E. coli*, not heterologous strains (Heller et al., 1990). Thus, maternal antibodies seem to provide very specific resistance against the pathogens encountered by mothers prior to reproduction.

As mothers and offspring are likely to be naturally infected with the same pathogens, the antibodies to endemic pathogens that mothers have in circulation, once transmitted to offspring, will also provide young with protection against endemic pathogens. Moreover, maternal antibodies may provide offspring with the benefits of immune defense without the growth suppressive costs of generating an endogenous immune response. Several previous studies have documented suppressive effects of natural infection or experimental immunization on the growth of young animals (Klasing et al., 1987; Fair et al., 1999; Soler et al., 2003; Brommer, 2004). Because differentiation of the specific immune response is largely determined by exposure to antigens, it is poorly developed in neonatal vertebrates with little previous antigenic exposure. Instead during development, the immune response is biased towards production of non-specific, innate immune responses rather than lymphocyte-mediated specific responses (Seto, 1981; Klasing and Leshchinsky, 1999). Although innate immune responses are more rapid than specific responses, they are associated with suppression of growth and reproduction through activation of the inflammatory response (Klasing, 1997). Growth suppression is primarily a result of the anorexia, fever, and changes in nutrient use induced during the response (Klasing and Leshchinsky, 1999).

Maternal antibodies should allow offspring to resist infection without invoking the physiological and growth-retarding expense of an innate immune response (Klasing et al., 1987; Heeb et al., 1998; Buechler et al., 2002; Kristan, 2002). Therefore, young with

high levels of maternally derived antibodies would be predicted to have both elevated resistance against endemic pathogens (Heller et al., 1990; Goddard et al., 1994) and higher growth rates than young with low maternal antibody levels or young without maternal antibodies for the antigens they encounter. This might be achieved either through the direct action of maternal antibodies (Heeb et al., 1998) or through a priming of the offspring's own antibody production (Gasparini et al., 2006; Grindstaff et al., 2006; Reid et al., 2006). In both cases, offspring could potentially reduce the energetically costly and growth-retarding action of the innate immune system (Klasing and Leschinsky, 1999).

In order to test whether the presence of specific maternal antibodies could ameliorate the growth suppressive effects of immunization during growth, I immunized adult female Japanese quail (*Coturnix japonica*) and then cross-fostered offspring across antigenic environments. I used three treatments (two antigens and one control), lipopolysaccharide (LPS) derived from *Salmonella typhimurium*, killed avian reovirus (AR) vaccine, and a control treatment of phosphate-buffered saline (PBS). Consequently, one group of offspring had experimentally induced maternal antibodies specific for the antigenic challenge they received, a second group of offspring possessed maternal antibodies specific for a different antigen than the one they were immunized with, and the third group of offspring did not have experimentally induced maternal antibodies.

LPS is the major component of the outer membranes of Gram-negative bacteria. It induces fever, inflammation, and behavioral changes (e.g. listlessness, anorexia) such that immunization entails energetic costs (Johnson et al., 1993; Koutsos and Klasing, 2001). However, LPS is non-replicating so that only those individuals that are immunized are affected. LPS also mimics the effects of antigenic exposure in the wild because it is derived from pathogens that induce illness in natural populations. More importantly, in birds it elicits an antibody response by the immunized individual and these antibodies are transmitted to egg yolks (Sunwoo et al., 1996). Avian reovirus is a viral infection that affects captive poultry, as well as wild populations of Galliformes (Magee et al., 1993; Jones, 2000). Infection induces arthritis-like symptoms in the joints and may lead to stunted growth and development (Read-Connole, 2000). I used a heat-killed avian reovirus vaccine to stimulate an antibody response without inducing pathological symptoms or transmission to non-immunized individuals. Use of the heat-killed virus does not adversely affect the transfer of antibodies to egg yolks (Jones, 2000).

I predicted that offspring with maternal antibodies specific for the immunization they received would maintain growth rates after immunization. By contrast, offspring immunized with a novel antigen (defined as one their mothers had not been exposed to) would have reduced growth. Offspring immunized with a novel antigen were expected to have reduced growth because of the growth suppressive effects associated with invoking an innate immune response (Roura et al., 1992; Dritz et al., 1996; Klasing and Leshchinsky, 1999). Offspring immunized with the same antigen as their mother would be predicted to possess maternally derived antigen specific antibodies and, thus would not need to rely on innate immunity. As control offspring were not immunized, their immune systems were not stimulated so growth should not have been affected.

MATERIALS AND METHODS

Study species

Adult Japanese quail (*Coturnix japonica* Temminck and Schlegel 1849) were obtained from a commercial breeder (Northwest Gamebirds, Kennewick, WA, USA) and were maintained on the same photoperiod (16 h:8 h L:D) and diet (quail layer crumbs; Wayne Animal Nutrition, Columbia City, IN, USA) for the duration of the experiment. Birds were housed in a quail battery breeder (Georgia Quail Farms, Savannah, GA, USA) with one breeding pair per cage. The mass of adult females was measured both at the beginning of the study and after the secondary immunization.

Maternal immunization

Pre-immunization blood samples (approximately 500 μl) were collected from the brachial vein to assess previous exposure to LPS and AR. None of the females had detectable levels of antibodies to LPS or AR prior to immunization. Females were then randomly assigned to one of three experimental groups: LPS immunized, avian reovirus immunized, or the control phosphate-buffered saline (PBS) group. LPS females were immunized intraperitoneally with LPS isolated from *Salmonella typhimurium* (Sigma L-7261, St Louis, MO, USA) at a concentration of 1.5 mg LPS kg^{-1} body mass suspended in 0.5 ml PBS (Sigma P-4417). This dose has been shown to elicit both an antibody response and mild sickness behaviors in Japanese quail (Koutsos and Klasing, 2001). Avian reovirus females were subcutaneously immunized with 0.05 ml inactivated AR vaccine (Lohmann Animal Health Intl. 1815, Dassel, MN, USA) according to the manufacturer's recommendations. Control (PBS) females were injected intraperitoneally with 0.5 ml of PBS to control for any effects of handling and immunization. All females were given a secondary immunization 10 days after the primary immunization, at the same concentration as the primary immunization to increase the magnitude of the antibody response. Blood samples (approximately 500 μl) were collected 10 days after the secondary immunization to quantify antibody responses to LPS and AR.

Egg and offspring measurements

Eggs were collected from females throughout the experiment. All eggs were measured (length, width and mass) at the time of collection. Every third egg laid by each female was frozen intact and reserved for antibody analyses. A subset of eggs laid at least 12 days after the maternal secondary immunization was incubated at 37.5°C in a commercial incubator (Stromberg's Chicks INC 1202; Pine River, MN, USA). Immediately prior to hatching, eggs were placed in individually marked cups to ensure that parentage of chicks could be determined accurately. Chicks were individually banded at hatching for identification. All quail chicks were measured (mass, tarsus and wing length) every other day from hatching to 2 weeks post-hatch, with the exception of day 6 post-hatch when blood samples were taken from the chicks and they were immunized. At the completion of the experiment, chicks were sexed based on plumage differences or internal anatomy for chicks with ambiguous plumage.

Offspring immunization

Offspring were randomly assigned to one of the three antigen treatments (LPS, AR, control) 6 days post-hatch. Offspring within a family were divided among treatments such that each female had at least one chick in each treatment group. Offspring were immunized with the same antigen doses as mothers, but were only immunized once. A blood sample (at least 50 μl) was collected from all chicks immediately prior to immunization to quantify maternal antibody transmission and a second blood sample was collected from a subset of chicks 5 days post-immunization to

assess changes in maternal antibody levels and any potential endogenous antibody production by chicks. Specific antibody responses to LPS and AR as well as total IgG concentrations were quantified in all blood samples with sufficient amounts of plasma.

Total IgG enzyme-linked immunosorbent assay

Total IgG concentrations were quantified using an enzyme-linked immunosorbent assay (ELISA) as described previously (Grindstaff et al., 2005). ELISA plates were coated with 100 μl of anti-chicken IgG (donkey anti-chicken IgY; Jackson ImmunoResearch Labs, product no. 703-005-155, West Grove, PA, USA) at a concentration of 3 μg ml^{-1} suspended in carbonate buffer (0.15 mol l^{-1}, pH 9.6). Plasma or egg yolk samples were diluted 1:20 000 in diluent (1% milk powder, PBS–Tween 20). Egg yolks were prepared for ELISA as described previously (Grindstaff et al., 2005). After washing, diluted samples were added to the plate in duplicate. At least two blank wells (containing diluent only) were included on each plate. The labeling antibody (AP-conjugated rabbit, anti-chicken IgG; Sigma, catalogue no. A-9171) was diluted 1:1000. Plates were read on a Bio-Rad (Hercules, CA, USA) Benchmark microplate reader (catalogue no. 170-6850). All antibody concentrations are reported as the slope of the substrate conversion [in $10^{-3}\times$ optical densities (OD); m_{od}] over time (m_{od} min^{-1}), with a steeper slope indicating a higher concentration of antibodies in the sample.

To compute antibody concentration, the mean of the duplicate values for each sample was calculated. The mean value of the blanks was subtracted from the measured antibody concentration to account for non-specific binding. On each plate, a serial dilution of a chicken-IgY standard (chicken IgY, Promega, catalogue no. G116A, Madison, WI, USA) was included for a standard curve (0.1, 0.05, 0.025, 0.0125, 0.00625 and 0.003125 μg ml^{-1}). The differences between the standard curves was used to account for between-plate variation.

LPS ELISA

Ninety-six well ELISA plates were coated with 100 μl of LPS at a concentration of 5 μg ml^{-1} suspended in carbonate buffer (0.15 mol l^{-1}, pH 9.6). Plates were then incubated overnight at 4°C. The next day the plates were blocked with 5% milk powder (Mix 'N Drink, Saco Foods Inc., Middleton, WI, USA) diluted in 0.01 mol l^{-1} PBS, pH 7.2 and Tween 20 for at least 2 h at room temperature. During the incubation, plasma samples from females and offspring were diluted 1:50 in diluent (1% powdered milk in PBS and Tween 20). For egg assays, yolk samples were diluted 1:10. After washing the plate, samples were added in duplicate. At least two blank wells were included on each plate that contained diluent only. After sample addition, the plates were again incubated overnight at 4°C. On the third day, 100 μl of the labeling antibody (AP-conjugated rabbit, anti-chicken IgG, Sigma catalogue no. A-9171) diluted 1:1000 were added to every well of the plates after washing. The plates were then incubated for 1 h at 37°C. After washing, 100 μl of substrate buffer were added to every well of the plates. The plates were then immediately transferred to a Bio-Rad Benchmark microplate reader (catalogue no. 170-6850). The plates were read at 30 s intervals for 14 min using a 405 nm wavelength filter. Antibody titers are the slope of the substrate conversion. Antibody titers were calculated in the same manner as described above for IgG concentrations.

AR ELISA

A commercial ELISA kit was used to quantify antibody responses to the AR vaccine (AffiniTech REO 1000, Madison, NJ, USA). Assay procedures followed the kit instructions, except that plasma samples were diluted 1:50 and yolk samples were diluted 1:10 in the sample diluent. To minimize interassay variability, the mean optical density for each sample was expressed as a percentage of its plate positive control optical density.

Statistical analyses

Before conducting analyses, normality of residuals and homogeneity of variance were checked. LPS and AR antibody titers were log transformed to achieve normality. Data were analyzed using mixed models (Proc Mixed, SAS version 9.1) in which female identity, day by female identity, and offspring treatment by female treatment nested within female identity were included as random factors. Denominator degrees of freedom were determined by the Satterthwaite method. Sample sizes represent 12 families with LPS-immunized mothers, nine families with AR-immunized mothers, and 11 families with control mothers. Analyses of chick growth were divided into early growth prior to offspring immunization (growth from hatching to day 4) and later growth after offspring immunization (growth from day 8 to day 14 post-hatch). There were no significant differences in body size among offspring in the three offspring treatment groups prior to immunization (all $P>0.4$). Therefore, in analyses of early growth, offspring treatment group was not included. Offspring sex did not contribute significantly to any of the models and was, therefore, excluded.

RESULTS

No effect of immunization on maternal mass or egg size

Maternal antigen treatment did not significantly affect female body mass ($F_{2,31}=0.1$, $P=0.91$) or any measure of egg size (length: $F_{2,31}=0.88$, $P=0.43$; width: $F_{2,31}=0.76$, $P=0.48$; mass: $F_{2,31}=0.80$, $P=0.46$).

Effect of maternal treatment on antibody levels in mothers

All females immunized with LPS or AR mounted an antibody response as a result of the challenge, and no control females produced detectable levels of LPS- or AR-specific antibodies. Consequently, antibody titers in maternal circulation varied by treatment group. As expected, LPS antibody levels were highest in the females immunized with LPS and not different from background in control females and females immunized with AR ($F_{2,30}=8.61$, $P=0.001$). Similarly, AR antibody levels were highest in females immunized with AR and not different from background in control females and females immunized with LPS ($F_{2,30}=73.09$, $P<0.0001$). Total IgG concentrations in maternal circulation were not significantly impacted by maternal treatment ($F_{2,30}=2.46$, $P=0.10$).

Maternal antibody transmission

Antibody levels in maternal circulation and in eggs were significantly positively correlated (IgG: $R^2=0.32$, $P=0.001$, $N=31$; LPS: $R^2=0.94$, $P<0.0001$, $N=31$; AR: $R^2=0.88$, $P<0.0001$, $N=31$). Similarly, antibody levels in maternal circulation and in offspring circulation on day 6 post-hatch were positively correlated [IgG: $R^2=0.44$, $P<0.0001$, $N=31$ (Fig. 1); LPS: $R^2=0.72$, $P<0.0001$, $N=31$; AR: $R^2=0.71$, $P<0.0001$, $N=31$], although antibody levels in chick circulation were lower than antibody levels in maternal circulation. There was no difference in maternally derived antibody levels between male and female chicks on day 6 (IgG: $F_{1,151}=0.09$, $P=0.77$; LPS: $F_{1,136}=0.46$, $P=0.50$; AR: $F_{1,137}=1.47$, $P=0.23$).

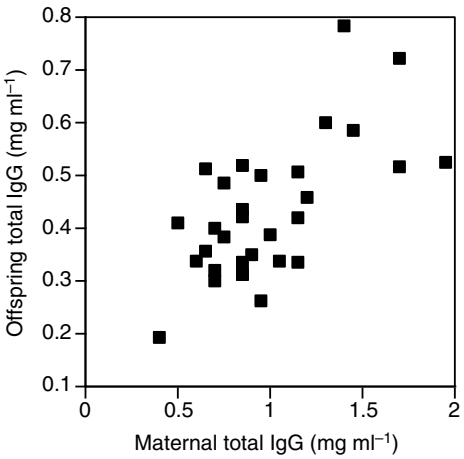

Fig. 1. Relationship between total IgG concentrations measured in maternal circulation and total IgG concentrations measured in offspring on day 6 post-hatch. Points represent brood means.

Effect of maternal treatment on day 6 antibody levels in chicks

Chicks whose mothers were immunized with LPS had higher levels of LPS antibodies than did chicks whose mothers were immunized with AR or who were not immunized ($F_{2,26}$=26.65, P<0.0001) (Table 1). Offspring whose mothers were immunized with AR had higher levels of AR-specific antibodies than did chicks whose mothers were immunized with LPS or who were not immunized ($F_{2,30.9}$=47.66, P<0.0001) (Table 1). However, offspring of immunized mothers did not have significantly higher total IgG concentrations than offspring of control mothers ($F_{2,30.5}$=2.82, P=0.075) (Table 1).

Antibody levels after chick immunization

On day 11, neither maternal nor chick treatment significantly affected total IgG levels in offspring circulation (maternal treatment: $F_{2,28.6}$=1.79, P=0.18; chick treatment: $F_{2,41.8}$=1.02, P=0.37; maternal treatment × chick treatment: $F_{4,40.2}$=0.75, P=0.56). LPS antibody titers in offspring circulation on day 11 were still affected by maternal treatment but were not affected by chick treatment (maternal treatment: $F_{2,32.8}$=31.65, P<0.0001; chick treatment: $F_{2,55.2}$=0.69, P=0.51; maternal treatment × chick treatment: $F_{4,54.6}$=0.23, P=0.92). Similarly, AR antibody titers were affected by maternal treatment, but not chick treatment (maternal treatment: $F_{2,28.2}$=26.23, P<0.0001; chick treatment: $F_{2,53.1}$=0.53, P=0.59; maternal treatment × chick treatment: $F_{4,51.4}$=0.12, P=0.97).

Changes in offspring antibody levels during the experiment

In general, between days 6 and 11 total IgG concentration and titers of LPS- and AR-specific antibodies declined in chick circulation [IgG day 6 mean=8.97±0.38 (± s.e.m.), day 11 mean=4.58±0.19; LPS day 6 mean=2.72±0.63, day 11 mean=0.80±0.15; AR day 6 mean=4.88±1.03, day 11 mean=1.79±0.40]. Within chicks, antibody levels on days 6 and 11 were significantly positively correlated (IgG: R^2=0.38, P<0.0001, N=111; LPS: R^2=0.74, P<0.0001, N=111; AR: R^2=0.94, P<0.0001, N=109).

Effect of maternal immunization on early offspring growth

Maternal antigen treatment did not significantly affect early mass gain or tarsus growth of offspring before immunization (tarsus: maternal treatment × age: $F_{4,60}$=1.22, P=0.31; mass: maternal treatment × age: $F_{4,60}$=0.59, P=0.67). However, offspring of control mothers had significantly reduced early wing growth in comparison to the offspring of either LPS- or AR-immunized mothers (maternal treatment × age: $F_{4,60}$=3.26, P=0.017).

Effect of maternal and offspring immunizations on later offspring growth

Maternal treatment did not influence tarsal growth rate (Table 2). However, tarsal growth was impacted by offspring treatment (Table 2). Control, non-immunized offspring had faster tarsal growth than LPS- or AR-immunized offspring. Mass gain was also not significantly impacted by maternal treatment, but was influenced by offspring treatment (Table 2). Again control offspring were significantly heavier than immunized offspring. Wing growth was influenced both by maternal treatment and by

Table 1. Effect of maternal antigen treatment on specific antibody titers and total IgG concentration in offspring circulation 6 days post-hatch

Maternal treatment	Total IgG concentration (mg ml^{-1})	Log-transformed LPS antibody titer	Log-transformed AR antibody titer
Control	0.36±0.04	–3.21±0.43	–1.26±0.29
LPS	0.49±0.04	0.83±0.40	–0.97±0.27
AR	0.45±0.04	–2.37±0.47	2.46±0.31

Values are means ± s.e.m.
AR, avian reovirus; LPS, lipopolysaccharide.

Table 2. Effects of maternal and offspring treatments (control, LPS or AR) and age on the tarsus growth, mass gain and wing growth of offspring from days 8 to 14 after offspring immunization on day 6

Dependent variable	Tarsus growth (mm)			Mass gain (g)			Wing growth (mm)		
Source	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*
Maternal treatment	2, 23.5	1.29	0.29	2, 29.4	1.89	0.17	2, 25.9	2.70	0.086
Offspring treatment	2, 41.3	5.90	0.0056	2, 42.3	6.32	0.0040	2, 45.5	3.26	0.047
Maternal treatment by offspring treatment	4, 40.1	1.38	0.26	4, 40.5	2.19	0.087	4, 43.8	2.15	0.091
Age	3, 74.2	2931	<0.0001	3, 72.6	1633	<0.0001	3, 73.4	5578	<0.0001
Age by maternal treatment	6, 74.2	1.34	0.25	6, 72.6	1.01	0.43	6, 73.4	2.57	0.026
Age by offspring treatment	6, 216	8.90	<0.0001	6, 213	1.16	0.33	6, 224	2.21	0.043
Age by maternal treatment by offspring treatment	12, 216	1.65	0.080	12, 213	1.04	0.42	12, 224	0.53	0.89

offspring treatment (Table 2). Offspring of control mothers had slower wing growth than offspring of LPS- or AR-immunized mothers. Conversely, non-immunized offspring exhibited faster wing growth than LPS- or AR-immunized offspring.

Effect of a novel immunization on growth rates

I had predicted that offspring with maternal antibodies specific for the immunization they received would have elevated growth in comparison to offspring immunized with a novel antigen. To more directly test this prediction, I categorized offspring on the basis of whether they had experimentally induced maternal antibodies specific for the immunization they received, experimentally induced maternal antibodies for a different antigen challenge than they received, or were not immunized (control group).

Tarsal growth rates were significantly impacted by immunization with a novel antigen (immunization × age: $F_{6,137}$=5.85, P<0.0001) (Fig. 2A). Control, non-immunized, offspring had the highest growth rates, offspring immunized with a novel antigen (one their mothers had not been exposed to) had the lowest growth rates, and offspring with specific maternal antibodies for the antigen challenge had intermediate growth rates. Mass gain was also impacted by immunization with a novel antigen (immunization: $F_{2,57.9}$=4.89, P=0.011) (Fig. 2B). Again control offspring exhibited the greatest mass gain, offspring immunized with a novel antigen gained the least mass, and offspring with specific maternal antibodies had intermediate mass gain. Wing growth was similarly affected by immunization (immunization: $F_{2,64}$=5.02, P=0.0094) (Fig. 2C). Control offspring and offspring immunized with the same antigen as their mothers exhibited equivalent wing growth, but offspring immunized with a novel antigen had significantly reduced growth.

DISCUSSION

Maternal immunization did not significantly affect body mass or egg size, but did stimulate the production of antibodies specific to the challenge. Therefore, the effects of maternal treatment on offspring growth and antibody titers are unlikely to be caused by indirect effects mediated through maternal condition or egg size. Total concentrations of IgG also did not significantly differ among maternal treatment groups. This indicates that the synthesis rate and transmission of total IgG was not significantly affected (Leslie and Clem, 1970) and the primary effect of maternal immunization was to elevate specific antibody titers. Indeed maternal immunization significantly altered antibody transmission to offspring as measured by antibody levels in offspring on day 6 post-hatch. By day 11 post-hatch, maternal immunization still had significant effects on offspring antibody measures. Conversely, chick treatment did not affect specific or total antibody levels. Because antibody levels declined between day 6 and day 11 post-hatch, it appears that endogenous antibody synthesis does not begin until some time after day 11 in precocial (well developed at hatch) quail.

In general, offspring immunization reduced growth rates. However, the presence of specific maternal antibodies partially ameliorated the growth suppressive effects of immunization. By contrast, possession of experimentally elevated maternal antibodies for a different antigen did not ameliorate growth suppression. Offspring that received the same antigenic challenge as their mother, or were in the control group, exhibited faster tarsal growth and greater wing growth and mass gain than offspring immunized with a novel antigen for which they did not have maternal antibodies. This effect occurred even though the antigens used in the study were non-replicating and the young had *ad libitum* access

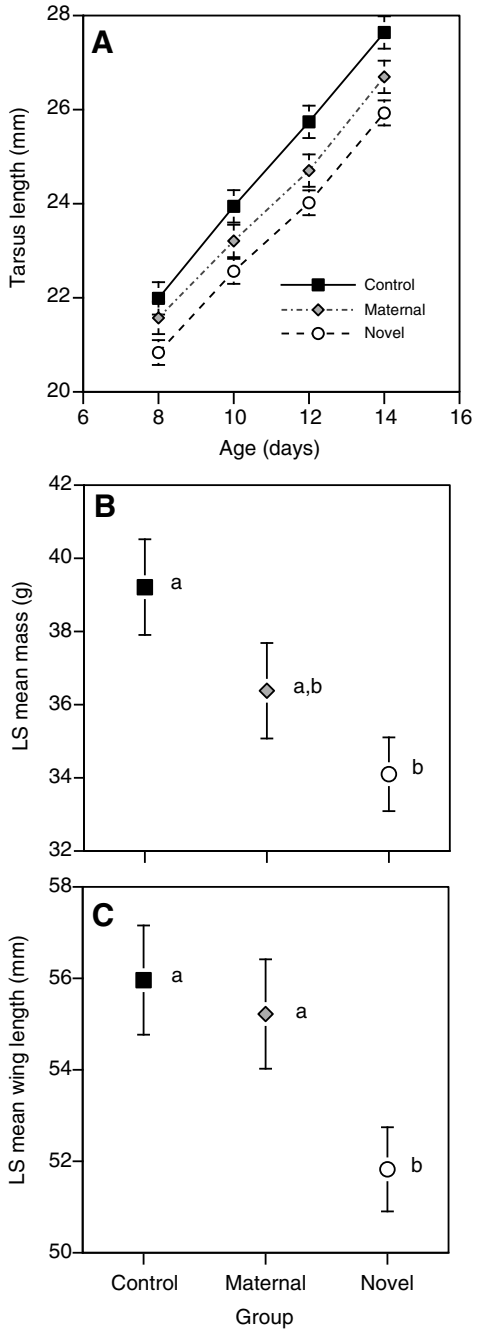

Fig. 2. (A) Effect of offspring immunization group on tarsus growth from day 8 to day 14, after immunization on day 6. Offspring in the control group were given an injection of phosphate-buffered saline, offspring in the maternal group were immunized with the same antigen as their mothers, and offspring in the novel group were immunized with an antigen to which their mothers had not been exposed. (B) Effect of offspring immunization group on mass gain from day 8 to 14, after immunization on day 6. Offspring in the control group were given an injection of phosphate-buffered saline, offspring in the maternal group were immunized with the same antigen as their mothers, and offspring in the novel group were immunized with an antigen to which their mothers had not been exposed. Groups with different letters are significantly different. (C) Effect of offspring immunization group on wing growth from day 8 to 14, after immunization on day 6. Offspring in the control group were given an injection of phosphate-buffered saline, offspring in the maternal group were immunized with the same antigen as their mothers, and offspring in the novel group were immunized with an antigen to which their mothers had not been exposed. Groups with different letters are significantly different.

to food. This provides further support for a trade-off between immunocompetence and growth (Soler et al., 2003; Brommer, 2004). In response to exposure to antigens, macrophages release inflammatory cytokines and provoke an inflammatory response. The production of these cytokines is also necessary to stimulate the adaptive immune response. However, this stimulation of the inflammatory response greatly increases the cost of antigenic exposure and may explain the reduction in growth or condition that is often observed after immunization with even fairly innocuous antigens (Siegel et al., 1982; Demas et al., 1997; Ots et al., 2001; Martin et al., 2003).

Maternal antibodies provide offspring, at least temporarily, with specific immunity to local diseases. When offspring encounter the same pathogens as their mothers, maternal antibodies provide protection without invoking the innate immune system of offspring (Brambell, 1970). Innate immunity is particularly expensive to growing young because the inflammatory response induces anorexia and diverts nutrients needed for growth to the acute phase response (Klasing, 1994; Klasing and Leshchinsky, 1999). Maternal antibodies allow offspring to maintain rapid growth when infected, by suppressing stimulation of the innate immune system (Klasing and Leshchinsky, 1999). Therefore, it is not simply the presence or absence of maternal IgG that may influence offspring growth (e.g. Gustafsson et al., 1994), but also the specific types of antibodies that are transferred in relation to the antigens that offspring are exposed to.

For example, LPS has been demonstrated to elicit a sustained inflammatory response in Japanese quail chicks as measured by interleukin-1 (IL-1) activity (Klasing and Leshchinsky, 1999). However, chicks of LPS-immunized mothers exhibited reduced levels of IL-1 activity presumably because maternal antibodies prevent LPS from binding macrophage LPS receptors to trigger the release of inflammatory cytokines (Klasing and Leshchinsky, 1999). This may reduce growth suppression in LPS-immunized chicks whose mothers were also immunized with LPS as observed here. Unfortunately, maternal antibodies do not completely eliminate the growth suppression associated with immunization. It remains to be determined whether this is a dose-dependent effect such that high levels of specific maternal antibodies are able to completely block stimulation of the innate response, whereas lower levels do not. Alternatively, maternal antibodies may be unable to completely block involvement of the offspring immune response, regardless of concentration. Antigenic exposure during development has an important educational role in the differentiation of the specific immune response and it may consequently be essential for the offspring immune system to be actively involved in responses to antigenic exposure, irrespective of potential effects on morphological growth.

Unexpectedly, offspring of control mothers had significantly reduced early wing growth in comparison to the offspring of immunized mothers. This may suggest that immunized mothers made a terminal investment in reproduction as a result of the immunization (Bonneaud et al., 2004). However, this seems unlikely given that offspring did not differ prior to immunization in any other size measure and immunized mothers did not lay larger eggs than control mothers. Instead, this may suggest that offspring of control mothers invest more in tarsal growth and mass gain, rather than wing growth.

Precocial young have been predicted to begin endogenous antibody production earlier after hatch relative to altricial (poorly developed at hatch) young because they hatch at a later developmental stage (Apanius, 1998; Klasing and Leshchinsky, 1999). However, I did not find any evidence in this study that Japanese quail chicks begin to produce antibodies within the first 11 days post-hatch. Even though offspring were given a fairly short period of time to respond to the immunization, one would expect that antibody titers should increase after immunization, if offspring are capable of mounting a specific response. Instead, the titers of maternally derived antibodies and total IgG concentrations declined in these quail chicks over the measured time period. Conversely, similar measurements in semi-precocial and altricial birds have revealed an increase in both specific and total antibody levels within the first 10 days to 2 weeks post-hatch (Gasparini et al., 2006; Grindstaff et al., 2006; Pihlaja et al., 2006). The lower relative proportion of yolk in altricial species than in precocial species suggests that altricial young receive lower levels of maternally derived antibodies (Klasing and Leshchinsky, 1999). Therefore, it may be important for altricial species to accelerate antibody production to minimize reliance on the more nutritionally expensive mechanisms of innate immunity.

Maternal antibodies have a critical role in providing offspring with humoral immune protection early in life (Grindstaff et al., 2003) and may also affect early endogenous antibody production by offspring (Gasparini et al., 2006; Grindstaff et al., 2006; Reid et al., 2006). One primary determinant of the diversity of antibodies transmitted to offspring is the maternal antigenic environment (this study) (Gasparini et al., 2001). Furthermore, these results suggest that if offspring are exposed to the same antigens as mothers during the period of maternal immune protection, maternal antibodies may also play an important role in reducing growth suppression after infection. Because offspring with initially high levels of maternal antibodies maintain detectable levels of antibodies in circulation longer than young with low initial antibody levels (J.L.G., unpublished) (Nicoara et al., 1999), the primary benefit of enhanced antibody transmission is likely to be an extension of the period of protection for offspring. This may allow offspring to complete the majority of the growth period before maternal protection is lost. In the wild, offspring of mothers with higher circulating levels of antibodies should maintain maternal protection for a greater proportion of the growth period than offspring of mothers with low antibody levels. Future research should assess the mechanisms through which maternal antibodies allow offspring to maintain growth after infection and the interactions between maternal antibodies and stimulation of the inflammatory response.

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