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RESEARCH ARTICLE

Parental experience of a risky environment leads to improved offspring growth rate

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ABSTRACT

Parasites (or diseases) are a major selective force for the evolution of life history traits and parasite–host evolution. Mothers can show a variety of responses to parasites during pregnancy, with different consequences for them or their offspring. However, whether information in the maternal environment before pregnancy can cause a change in the phenotype of the offspring is unknown. To avoid the confounding effect of pathogens and to reduce the risk of a direct effect of maternal immune system activation, we injected female laboratory mice with lipopolysaccharides (LPS) before they mated. In order to provide constant information on the potential infectious risk of the environment, females were mated with males that were also exposed to LPS before mating. Offspring from immune-challenged parents were larger and grew at a faster rate than offspring from control parents (injected with PBS). Additionally, offspring from immune-challenged parents that suffered the most from inflammation grew at a faster rate than offspring from low suffering parents. Producing heavier offspring that will reach sexual maturity earlier is likely to have fitness benefits for parents and offspring through improved reproductive success.

KEY WORDS: Fetal programming, Inflammation, Maternal effect, Rodent, Thrifty gene hypothesis

INTRODUCTION

Parental effects occur when the phenotype or the environment experienced by one or both parents influences the phenotype of the offspring independently of the effects of direct genetic transmission (Marshall and Uller, 2007). The idea that the maternal environment can influence the phenotype of offspring has attracted particular attention in human studies. 'Fetal-or prenatal programming' refers to the long-term impact on offspring health that results from maternal exposure to disease, stress or malnutrition during gestation (Barker, 1998). These maternal effects in humans were typically viewed as being detrimental as a poor fetal environment is often associated with metabolic disorders such as obesity and type-2 diabetes (Fowden et al., 2006). It has also been suggested that the association between a poor maternal environment and the enhanced tendency of offspring to collect food and deposit fat could have conferred a selective advantage during human evolution. This hypothesis has been named the 'thrifty gene hypothesis' and postulates that poor maternal environment might be a cue used by fetuses to assess the conditions they will likely experience after birth. The thrifty gene hypothesis therefore predicts that during periods of food abundance, offspring will tend to acquire and store reserves that might become essential for survival during periods of food shortage. Modern, western societies no longer experience periods of famine, making the thrifty gene maladaptive nowadays. Evolutionary ecologists have also stressed the potential adaptive nature of parental effects in free-ranging animals, as the environment experienced by the parents can be used by offspring to adopt the phenotype that maximizes fitness under the prevailing conditions (Mousseau and Fox, 1998; Marshall and Uller, 2007; Allen et al., 2008). Maternal effects have been demonstrated in a wide range of taxa and traits (Räsänen and Kruuk, 2007): from bryozoan mothers that produce larger offspring when competition increases (Allen et al., 2008), to lizards that decide to leave their natal site depending on the availability of food experienced by their mother (Massot and Clobert, 1995), or to sticklebacks that produce offspring with tighter shoaling behaviour when exposed to predators (Giesing et al., 2011).

A special environmental characteristic that can have profound effects on both parental and offspring phenotype is the risk of contracting infectious diseases. Pathogens have direct fitness effects on parents, and environments with a high parasitic burden can also affect offspring development and growth. Life history theory predicts that living in a risky environment should select for faster development and growth because any potential benefit of delaying growth and maturity is largely outweighed by the increased likelihood of contracting infectious diseases. According to this scenario, parents experiencing the symptoms of an infectious disease should produce offspring with improved growth rate and earlier maturity. This prediction is therefore similar to the thrifty gene hypothesis, even though the triggering signal here is not a nutritional stress experienced by the mother but an infectious insult.

In the present study, we investigated the effect of parental exposure to an inflammatory challenge on offspring phenotype in laboratory mice. We wished to avoid the potential confounding effect that arises from using living pathogens and to this purpose we used lipopolysaccharides (LPS) from the bacterium *Escherichia coli*. LPS is a component of the cell wall of gram-negative bacteria that constitutes a pathogen-associated molecular pattern (PAMP) recognized by receptors of the innate immune system (in the case of LPS, the toll-like receptor 4). PAMP recognition therefore initiates an immune response without the concomitant negative effect of parasite replication. In addition, mice were LPS challenged before mating, which further reduces the risk of a direct effect of immune activation on offspring phenotype. Our experimental design therefore allowed us to disentangle the information on how risky the environment was from a direct effect of LPS challenge on offspring phenotype as offspring priming could only occur after the mothers were likely to have recovered from the immune insult. We also investigated the potential immune effectors (IL-6 and IL-10) that might trigger the adaptive change in phenotype. We chose to

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Table 1. Changes in body mass as a function of treatment and sex during the course of the experiment

Source of variation	d.f.	*F*	*P*
Time	1,616	87.02	<0.0001
Squared time	1,616	96.04	<0.0001
Treatment	1,115	6.51	0.012
Sex	1,115	520.56	<0.0001
Time × sex	1,616	5.74	0.017
Squared time × sex	1,616	4.09	0.044
Time × treatment	1,616	11.02	0.001
Squared time × treatment	1,616	22.80	<0.0001
Sex × treatment	1,115	0.78	0.378
Time × sex × treatment	1,616	8.64	0.003
Squared time × sex × treatment	1,616	9.86	0.002

Individual identity was included as a random factor.
Treatment was lipopolysaccharide (LPS) versus phosphate-buffered saline (PBS).

measure these two cytokines because IL-6 is a pro-inflammatory cytokine that promotes inflammatory reactions (Stenvinkel et al., 2005) and is a good predictor of the strength of the inflammatory response (Oberholzer et al., 2005), and IL-10 is an anti-inflammatory cytokine involved in the resolution of inflammation and is an essential regulatory effector preventing immunopathology (Couper et al., 2008).

RESULTS

Effect of LPS challenge on parents

LPS-injected mice lost significantly more body mass than control mice during the days that followed the inflammatory challenge and recovered by the end of the experimental period. Males tended to lose more body mass and to recover more slowly than females from the LPS challenge. This resulted in a statistically significant three-way interaction between treatment, sex and squared time (Table 1, Fig. 1).

Plasmatic concentration of IL-6 and IL-10 measured 3 h post-challenge were not correlated with initial body mass (IL-6: $F_{1,46}$=0.96, P=0.332; IL-10: $F_{1,46}$=1.42, P=0.239). However, IL-6 was negatively correlated to body mass at 48 h post-challenge ($F_{1,46}$=5.55, P=0.023; Fig. 2), whereas IL-10 was still not a good predictor of body mass ($F_{1,46}$=1.32, P=0.256). The negative slope between IL-6 and body mass at 48 h post-challenge was similar in males and females as shown by a non-significant IL-6 × sex interaction ($F_{1,45}$=1.11, P=0.298). Visual inspection of the data revealed one outlier with an abnormally low level of plasmatic IL-6. Removing this outlier, however, did not change the results (IL-6, $F_{1,45}$=5.64, P=0.022).

Reproductive consequences of LPS challenge

The onset of reproduction did not differ across treatments [4.6±0.6, 3.8±0.4 and 4.9±0.4 days for high-sufferer (HS), low-sufferer (LS) and phosphate-buffered saline (PBS) groups, respectively; $F_{2,35}$=0.86, P=0.431]. Similarly, cytokine production did not affect the timing of reproduction in HS and LS groups (treatment, $F_{1,18}$=0.77, P=0.39; IL-6 male, $F_{1,18}$=0.80, P=0.383; IL-6 female, $F_{1,18}$=0.09, P=0.771; IL-10 male, $F_{1,18}$=3.29, P=0.086; IL-10 female, $F_{1,18}$=0.58, P=0.455). Removing the IL-6 outlier did not change the results (not shown).

Treatment did not affect litter size at birth (12.7±0.8, 12.7±0.7 and 13.3±0.4 pups for HS, LS and PBS groups, respectively; $F_{2,35}$=0.13, P=0.878). There was very little offspring mortality, and consequently treatment did not affect litter size at weaning (12.7±0.9, 12.6±0.9 and 13.3±0.7 pups for HS, LS and PBS groups, respectively; $F_{2,35}$=0.15, P=0.862). Similarly, none of the cytokines was a good predictor of litter size at birth or at weaning (Table 2). Removing the IL6 outlier did not change the results.

Litter mass increased with squared time, showing the typical accelerating function (squared time, $F_{1,74}$=67.27, P<0.0001), but was not affected by treatment ($F_{2,35}$=1.07, P=0.355). Cytokine production did not affect litter mass (Table 3). Removing the IL6 outlier did not change the results.

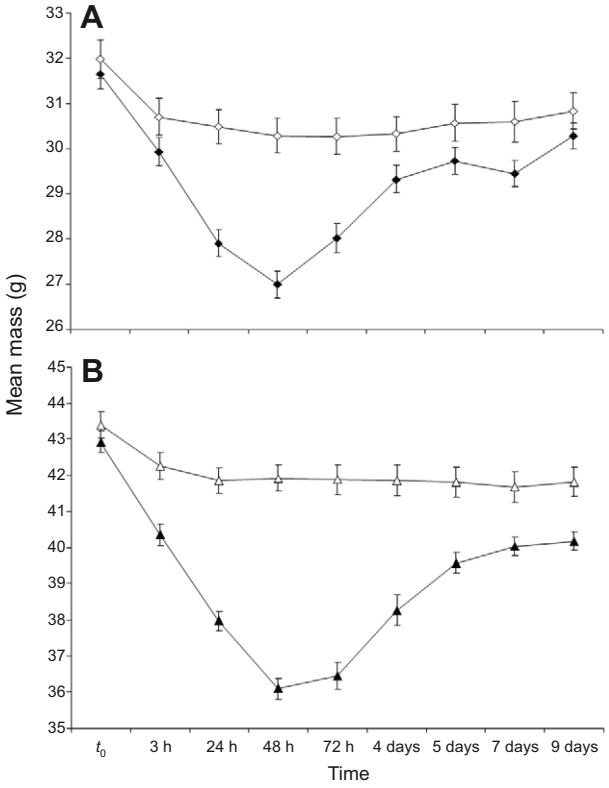

Fig. 1. Mean (±s.e.m.) mass variation in adult mice following immune challenge. Data are from female (A) and male (B) mice subsequent to an immune challenge with lipopolysaccharide (LPS; filled symbols) or saline solution (open symbols). t_0 represents the day before the immune challenge.

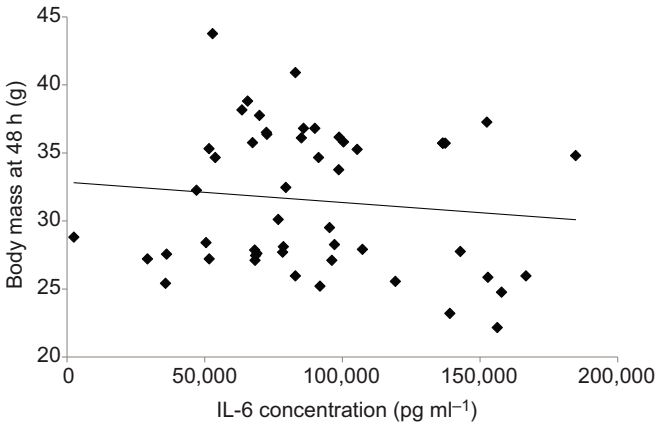

Fig. 2. Relationship between IL-6 concentration and mass loss in adult mice, 48 h after immune challenge with LPS. Data from both high sufferer (HS) and lower sufferer (LS) groups are shown.

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Table 2. Effect of treatment, and male and female IL-6 and IL-10 on the onset of reproduction

Source of variation	d.f.	*F*	*P*
Treatment	1,18	0.09	0.768
Female IL6	1,18	0.41	0.528
Female IL10	1,18	0.25	0.627
Male IL6	1,18	0.56	0.464
Male IL10	1,18	0.96	0.339

Effect of parental treatment on offspring growth

We randomly selected four offspring per litter (two males and two females) to investigate the effect of parental treatment on post-weaning growth rate (day 35 to day 79). At day 35, offspring from LPS-injected parents were heavier compared with PBS control parents (26.1±0.4, 26.5±0.4 and 24.4±0.6 g for HS, LS and PBS groups, respectively; treatment, $F_{2,26.5}$=4.89, P=0.016; sex, $F_{1,91.5}$=362.31, P<0.0001). During the period between the ages of 35 and 79 days, offspring from LPS-injected parents also kept growing at a faster rate than offspring of PBS parents as shown by a statistically significant time × treatment interaction (Table 4, Fig. 3).

Body mass at 35 days did not differ between offspring from LPS-injected parents in the HS and LS groups and was not explained by cytokine production (Table 5). Removing the outlier IL-6 point did not change the results. However, offspring from HS LPS-injected parents grew at a faster rate than offspring from LS LPS-injected parents (Table 6, Fig. 3), even though offspring growth rate was not correlated with cytokine levels (Table 6).

DISCUSSION

We examined how parental exposure to infection affects offspring phenotype in mice. The results support our predictions; parents that suffered the most from inflammation before pregnancy produced bigger offspring with accelerated growth.

The immune challenge had no effect on female reproductive output. Most studies on free-ranging populations have shown that females increased or decreased investment in reproduction (litter size, parental care, timing of birth, etc.) when infected by parasites [insects (Adamo, 1999), birds (Gallizzi et al., 2008), lizards (Sorci et al., 1996)]. Studies looking at the reproductive response of wild-type mice infected by a parasite observed clear trends (Willis and Poulin, 1999; Telfer et al., 2005; Schwanz, 2008). For example, wild-derived female mice parasitized with an intestinal nematode (*Heligmosomoides polygyrus*) produced 2.8 more pups per litter than unparasitized females (Kristan, 2004). However, in laboratory mice, parasitic infection generally leads to little or no change in reproduction (Kristan, 2002; Curno et al., 2011). It has been suggested that laboratory mice cannot increase their litter size in response to an immune challenge (or when infected by a parasite) because selection of increased litter size in captive mice colonies has reached its maximum (Kristan, 2004).

Table 3. Effect of treatment, and male and female IL-6 and IL-10 on litter mass measured at day 4, 14 and 21

Source of variation	d.f.	*F*	*P*
Time	1,46	0.11	0.740
Squared time	1,46	35.03	<0.0001
Treatment	1,18	0.88	0.361
Female IL6	1,18	0.97	0.339
Female IL10	1,18	0.04	0.847
Male IL6	1,18	0.13	0.726
Male IL10	1,18	0.24	0.633

Litter identity was included as a random factor.

Table 4. Effect of parental treatment and offspring sex on body mass between the ages of 35 and 79 days

Source of variation	d.f.	*F*	*P*
Time	1,372	140.99	<0.0001
Squared time	1,372	35.93	<0.0001
Treatment	2,64.2	2.92	0.061
Sex	1,395	2.33	0.128
Time × treatment	1,372	6.34	0.002
Time × sex	1,372	15.49	<0.0001
Squared time × sex	1,372	11.60	0.001

Litter identity and individual identity nested within the litter were included as random factors.

We found that offspring from immune-challenged parents grew at a faster rate after weaning than offspring from control parents. In addition, offspring from immune-challenged parents that suffered the most from inflammation grew at a faster rate after weaning than offspring from low suffering parents. The effect of maternal infection on offspring growth rate or body mass has been previously observed in studies where dams were infected by a parasite during pregnancy (Kristan, 2002; Schwanz, 2008), but it has never been observed in response to an infectious environment before females even mated. However, a few recent studies have demonstrated accelerated growth rates in offspring in response to predation risks perceived by mothers (Cheng and Martin, 2012; Coslovsky and Richner, 2012; Keiser and Mondor, 2013). For example, in an experimental study, Coslovsky and Richner exposed female great tits to predator models (Coslovsky and Richner, 2011). They produced offspring that were lighter and had faster wing growth compared with those from the control females, enabling them to

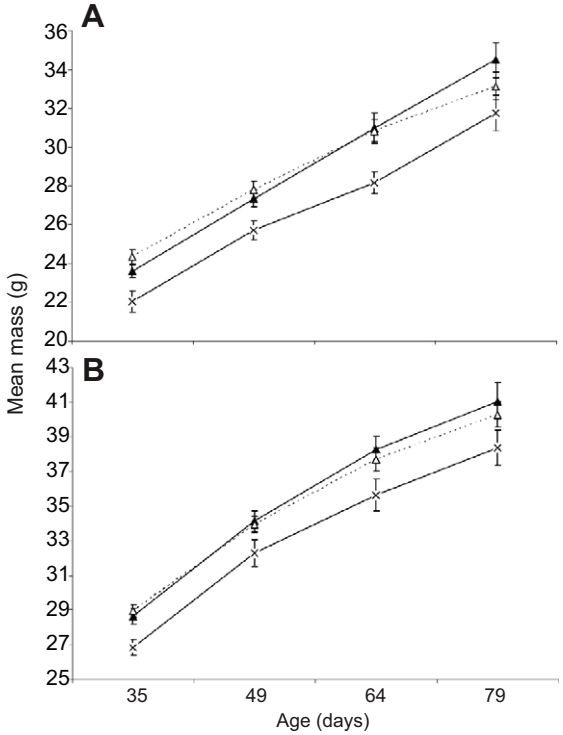

Fig. 3. Mean (±s.e.m.) mass increase in offspring from 35 to 79 days. Data are from female (A) and male (B) offspring of parents that were in the HS (filled symbols), LS (open symbols) or control (injected with PBS, crosses) groups.

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Table 5. Effect of parental treatment, parental cytokine production and offspring sex on offspring body mass at day 35

Source of variation	d.f.	*F*	*P*
Treatment	1,16.5	1.65	0.217
Sex	1,68.3	275.62	<0.0001
Paternal IL6	1,16.7	0.05	0.824
Paternal IL10	1,16.4	1.95	0.181
Maternal IL6	1,16.4	0.85	0.369
Maternal IL10	1,16.9	0.21	0.652

better escape nest predation. The accelerated growth rate observed here in offspring from LPS-treated mothers can be considered as an adaptive parental effect as offspring will reach sexual maturity and start reproducing early in an environment where life expectancy is reduced by infection risk (Sorci and Clobert, 1995).

Offspring from LPS-treated parents were heavier than control ones at the age of 35 days and grew at a faster rate during the 35–79 day period. Producing heavier offspring may have fitness benefits for parents and offspring through improved reproductive success. In laboratory and wild-type mice, greater mass in females usually leads to greater fecundity (Bünger et al., 2005; Schwanz, 2008), whereas heavier adult males are more likely to win male–male competition and maintain territories (Dewsbury, 1979). Therefore, offspring from immune-challenged parents may express higher reproductive performances that compensate for the negative effects of the bad quality of their natal environment.

Mechanisms involved in parental effects may be more or less direct and complex. Here, we can exclude a direct quantitative effect of the amount of milk provided by LPS-treated females during lactation because pup growth rate did not differ between treatments during that period. Females that were exposed to inflammation before reproducing might have transmitted compounds to their offspring during pregnancy or/and during lactation (through colostrum or milk). One mechanism by which females can alter offspring phenotype is by producing stress-induced hormones (Spencer and Robinson, 1992). Several studies have shown that immune activation may affect corticosteroid production (Rivier et al., 1989; Klein and Nelson, 1999; Owen-Ashley et al., 2006; Adelman and Martin, 2009). Maternal corticosterone levels during development have been shown to affect offspring behaviour, morphology, growth, dispersal, sex and survival (Glickman et al., 1987; De Fraipont et al., 2000; Groothuis et al., 2005; Love et al., 2005).

Paternal effects are less likely to have directly influenced offspring phenotype than maternal effects because males contributed less to the environment of fetuses and pups. Males were placed with the females for a short period of time (2 weeks) during conception and did not have access to the litters, excluding a direct effect through paternal care. However, we cannot fully exclude indirect paternal effects on offspring development via male-induced maternal effects (Curley et al., 2011). For instance, Drickamer et al. (Drickamer et al., 2000; Drickamer et al., 2003) showed that female house mice adjusted maternal care to the quality of the males they chose. In the present study, pairs were randomly settled within groups, but females might have assessed male reaction to LPS exposure through phenotypic traits, and adjusted maternal investment accordingly. Environmentally induced epigenetic changes can be inherited via female and male gametes in mammals (Daxinger and Whitelaw, 2012; Soubry et al., 2014). Recent studies suggest that paternal exposure to toxins, drugs or an inadequate diet can damage the paternal genome and lead to variations in offspring development (Anway and Skinner, 2008; Ng et al., 2010). However, in most studies, fathers are exposed to toxins during their own neonatal development or for a very long period (e.g. chronic diet). In our experiment, males were briefly exposed to LPS before mating. Even if this brief exposure to LPS led to epigenetic modifications, to be transmitted to offspring these modifications have to escape major phases of DNA epigenetic reprogramming (demethylation and remethylation) in the zygote, shortly after fertilization and during embryogenesis (Curley et al., 2011). Finally, LPS might have damaged sperm (Belloni et al., 2014) and the phenotype of offspring sired by LPS-injected fathers may be affected by poor sperm quality. However, this should have produced a negative effect on offspring growth rate, whereas we found that offspring from LPS-treated parents grew at a faster rate. Therefore, even if we cannot rule out the possibility that fathers contributed to the observed effects on offspring phenotype, they are more likely to be due to maternal effects. In addition, even though the immune challenge on males might have induced the observed pattern, we believe that this does not change the take-home message of the study that the perception of an infectious environment leads to an improved growth rate of the offspring. Further experiments with LPS challenge applied only to females should accurately disentangle maternal from paternal effects. Investment in current reproduction such as production of a heavier litter must incur a cost for females, otherwise mothers from the control group would have produced large offspring as well. Production of larger pups can sometimes lead to a reduction in future fecundity or survival of mothers (Oksanen et al., 2002; Bleu et al., 2012). Overall, we suggest that our results can be interpreted as an adaptive parental strategy, preparing offspring for a pathogen-rich environment by influencing their growth and size. Such changes are adaptive for the offspring, as they will potentially reach sexual maturity rapidly, reproduce early and have higher mating success in an environment in which the threat of infection is high and the survival rate low.

Table 6. Effect of parental treatment, parental cytokine production and offspring sex on offspring growth rate from 35 to 79 days

Source of variation	d.f.	*F*	*P*
Time	1,277	105.18	<0.0001
Squared time	1,277	28.47	<0.0001
Treatment	1,30.5	1.95	0.173
Sex	1,294	1.29	0.257
Paternal IL6	1,17.3	0.18	0.674
Paternal IL10	1,17	0.07	0.789
Maternal IL6	1,17	0.20	0.658
Maternal IL10	1,17.6	0.20	0.659
Time × treatment	1,277	11.24	0.001
Time × sex	1,277	9.87	0.002
Squared time × sex	1,277	7.18	0.008

Litter identity and individual identity nested within litter were included as random factors.

MATERIALS AND METHODS

Animals and housing

This experiment was conducted in compliance with, and received the agreement of, the Animal Care and Ethical Committee of the Université de Bourgogne, Dijon (protocol B1510). Male and female mice (outbred Swiss CD-1 strain *Mus musculus*), 6 weeks old, were purchased from Janvier (Laval, France). Mean body mass of females and males was 32±0.3 and 43±0.3 g, respectively (N=54 for each sex). Females were housed in groups of four whereas males were housed individually to avoid stress and injuries resulting from territorial aggression (Arndt et al., 2009). Social status in male groups has also been shown to alter cytokine production (Bartolomucci et al., 2001). Mice were provided with food and water *ad libitum*.

Temperature was maintained at 22±1°C, humidity was 60±10% and lighting was maintained on a 12 h cycle.

Experimental treatment

Mice were acclimated to laboratory conditions for 4 weeks before the start of the experiment. One-hundred and eight individuals (54 males and 54 females) were intraperitoneally injected with a solution of LPS (serotype 055:B5, Sigma) from *E. coli* at a concentration of 16.67 mg kg^{-1} in 100 µl of PBS. Control mice (N=54, 27 of each sex) were injected with the same volume (100 µl) of PBS. Blood samples (100–150 µl) obtained by retro-orbital puncture, under isoflurane anaesthesia, were collected the day before the immune challenge (t_0) and 3 h after LPS injection ($t_{+3\,h}$) to match the peak of cytokine production (Tateda et al., 1996). Blood was quickly centrifuged (4000 r.p.m., 15 min, 4°C) and the plasma was stored at −80°C until biological assay. Mice were weighed (accuracy ±0.1 g) at t_0, $t_{+3\,h}$ and then at day 1, 2, 3, 4, 5, 7 and 9 post-injection.

Reproduction

Mating pairs were formed within the LPS/PBS treatment 2 weeks after the immune challenge, once the direct debilitating effect of LPS was over and mice had fully recovered. Because we wished to provide parents with consistent information on the environmental quality they experienced, we decided to mate LPS females with LPS males and PBS females with PBS males. Within the LPS group, pairs were further stratified according to the effect that the injection had on changes in body mass. We used changes in body mass as a proxy of the intensity of the sickness behaviour syndrome induced by the LPS challenge. LPS is known to produce a number of physiological and behavioural modifications in the hours/days that follow the challenge. These alterations include a rise in body temperature and resting metabolic rate, a decrease in activity and food intake, and a loss of body mass (Inui, 2001; Johnson, 2002). Mating pairs of mice were stratified according to body mass loss, such that the 13 females and 13 males that lost the least body mass following the immune challenge were paired (LS group, mean percentage mass loss at 2 days post-injection: 11.5±0.4%). The same pairing method was applied to the 13 males and 13 females that lost the most mass following the immune challenge (HS group, mean percentage mass loss at 2 days post-injection: 19.1±0.2%) and to 13 males and 13 females that were injected with PBS (control group, mean percentage mass loss at 2 days post-injection: 3.6±1.1%). At the time of pair formation, cytokine concentrations were not known; however, *a posteriori* analysis confirmed that HS mice produced more IL-6 than LS mice ($F_{1,49}$=4.77, P=0.034; HS, 101,975.45±8046.67; LS, 78,855.34±6926.48 pg ml^{-1}), whereas IL-10 levels did not differ between HS and LS groups ($F_{1,49}$=0.02, P=0.891; HS, 234.70±61.95; LS, 192.12±41.60 pg ml^{-1}). Mice were kept in pairs for 2 weeks, and checked daily for the presence of a vaginal plug to determine the onset of reproduction. Pairs were then separated and females were checked twice a day near the end of gestation to determine date of birth (day 0) and number of pups alive at birth. Litter size and litter mass were measured at day 4, 14 and 21 (weaning). At weaning, four offspring per litter (two females and two males) were randomly chosen to assess growth rate after weaning. Offspring females were housed in groups of four (two females per litter) and males in groups of two (male siblings are not aggressive if they are group housed from birth) (Bartolomucci et al., 2002). Offspring mice were given an individual mark by ear-notching. Body mass was measured every 2 weeks to determine growth rate. We were particularly interested in the effect of parental treatment on post-weaning growth rate (day 35 to 79). The period of 30–90 days after birth (±10 days), from puberty to adulthood (Wang and vom Saal, 2000) is commonly used when looking at post-weaning growth rate in rodents (Spencer et al., 1992; Kristan, 2004; Schwanz, 2008).

Cytokine quantification

Circulating IL-6 and IL-10 were quantified by flow cytometry (Demas et al., 2011) using the Cytometric Bead Array (CBA) Mouse Cytokine Flex Set kit (BD Biosciences, San Diego, CA, USA) according to the manufacturer's instructions and following previous work (Prunet et al., 2006). IL-6 and IL-10 kits consist of beads of 7.5 µm diameter, with excitation and emission wavelengths at 488 nm and above 600 nm (FL3), respectively. Each particle is coupled to antibodies binding a specific cytokine and represents a discrete population, unique in its FL3 intensity. The captured cytokines are detected via a direct immunoassay using a specific antibody coupled to phycoerythrin (PE) emitting at 585 nm (FL2). Data were acquired with FlowMax software (Partec, Munster, Germany) on a GALAXY flow cytometer (Partec) equipped with a laser emitting at 488 nm, and analyzed using BD CBA software (BD Biosciences). Forward versus side scatter gating was employed to exclude any sample particle other than the 7.5 µm beads. Data were displayed as two-colour dot plots [FL2 (PE): band pass 580±10 nm versus FL3 (beads): long pass 665 nm] so that the discrete FL3 microparticle dye intensities were distributed along the y-axis. Ten-point standard curves ranging from 20 to 5000 pg ml^{-1} were obtained by serial dilution of the reconstituted lyophilized standard. Cytokine concentrations were determined from these standard curves. If a sample had a cytokine concentration below the detection limit for the assay (1.4 pg ml^{-1} for IL-6 and 9.6 pg ml^{-1} for IL-10), we used these values instead of zeros in the statistical analyses.

Statistical analyses

The effect of the LPS challenge on the change in body mass was assessed using a mixed model (normal error distribution) with time, sex, treatment and the interactions included as fixed factors and individual identity as random factor. As the time effect may be non-linear, we also added 'squared time' as a covariate. The onset of reproduction (number of days until the vaginal plug was noticed) and the number of offspring per litter were analysed using a Poisson distribution of errors. Litter mass was analysed using a mixed model (normal error distribution) with time, squared time and treatment included as fixed factors and litter identity as a random variable. These analyses were repeated focusing on LPS-injected animals from the HS and LS groups, which allowed inclusion of parental cytokines (log-transformed) in the models as fixed factors. Offspring growth rate during the period 35–79 days was analysed using a mixed model (normal error distribution) with time, squared time, parental treatment, offspring sex and the interactions between them entered as fixed factors, and litter identity and individual identity nested within litter as random factors. This model also was repeated by focusing on LPS-injected parents from the HS and LS groups to take into account parental cytokines. Degrees of freedom were adjusted using the Satterthwaite method, and models were reduced by dropping non-significant interactions (P>0.05).

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Competing interests

The authors declare no competing financial interests.

Author contributions

All authors played a role in data collection and analysis. A.A.B., G.S. and B.F. designed the study and prepared the manuscript. R.G., K.R. and J.B. provided input regarding the content of the manuscript.

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