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Original Article

Effects of Maternal Immune System Status on Neonate's Immune System

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ABSTRACT

Background: This study evaluated the effects of the maternal immune system stimulation or suppression during the pregnancy on the development of the neonate's immune system.
Methods: A total of 20 female rats were divided into four groups. The groups were treated using *Leishmania major, Salmonella typhimurium*, Tacrolimus, and sterilized normal saline. The animals were mated after 3-time treatments. The neonate's humoral immune response, total body, thymus, liver, spleen weight, and histology were determined in this study.
Results: The spleen's mean weight of the two-month-old samples showed a significant reduction in the *Salmonella* group; in addition, the Tacrolimus group had a significant reduction in liver mean weight. The *Salmonella* and Tacrolimus groups showed a significant reduction ($P \leq 0.05$) in the anti-sheep red blood cells antibody titer.
Conclusion: Stimulation or suppression of the immune responses during the pregnancy has significant effects on the neonate's immune responses, spleen, liver, and thymus development.

Keywords: Immune response, Maternal, Neonate, Rat

Introduction

According to a previous common belief, a fetus has a pre-programmed nature that intakes its nutritional requirements from their maternal body. Genetics was the only basis for developments in this hypothesis (1). The recent researches declare the obscure topics. Nowadays, scientists approved the effects of the uterus microenvironments on cell proliferation, organ differentiation, and development of the various organs. The minor changes of the uterus niche could cause noticeable anatomical abnormalities, especially during the formation of the germ layer and organogenesis (1). The epigenetic forces and fetus developmental stage determine the type and level of the morphological defects (2). Altogether, the elements that form the uterus microenvironments would determine the healthiness of the fetus.

However, placenta and amniotic sac as barriers separating physically the mother and fetus have local and systemic associations in the decidua and intervillous space, respectively (3). The previous researchers observed the various elements, including hormones and cytokines in the uterus and the fetus setting (4). The maternal immune system is the main player of the uterus niche formation (5). Other evidence approved the noted objects, microbial infection, stress, and other environmental factors. Maternal immune response deviation could regulate the development and functional maturation of the fetal immune system (4, 6). Fetus development is highly dependent on both exogenous and endogenous signals, and the effects of any change concerning the gestational age can persist over the life course (7). Inappropriate activation of the maternal immune system may cause some developmental

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anomalies, autoimmune disease, allergies, and neurological disorders (8, 9). The maternal immune responses against various infectious agents during pregnancy are the causative agents of their neonate's neurological disorders, such as schizophrenia and autism, in humans and experimental rodent studies (10).

This study aimed at evaluating the effects of rats' maternal immune response stimulation or suppression on the development of their neonate's immune system. It is hypothesized that the exposure of the fetus to the different status of the maternal immune responses may influence the fetal immune system, which could persist in postnatal or adulthood. The pro-inflammatory and anti-inflammatory cytokines determined the T helper 1 (Th1) /T helper 2 (Th2) immune shifts (5). This experiment selected the infectious agents of *Salmonella typhimurium* and *Leishmania major* that deviate the immune responses to Th1 and Th 2, respectively (11, 12). The previous experiment considered the Tacrolimus a safe immune-suppressive drug during pregnancy (13). This immune suppressor drug used to produce the third state of the maternal immune system.

Methods

Animals

Male and female Wistar rats with the mean weight of 180±5 gr were housed separately until mating. All animals were kept under standard light conditions (12 h light/12 h dark with lights on between 6 a.m. and 6 p.m.). Food and water were available ad lib. All experimental protocols were approved by the research program animal care and the Ethics Committee of Shahid Chamran University of Ahvaz, Ahvaz, Iran (905824/28-10-2017).

Antigen

The isolate of *Salmonella typhimurium* (ATCC 14028) was prepared from the archive section of the microbiology department of veterinary medicine. Moreover, *Leishmania major* (ATCC 30012D) was prepared from the parasitological department of veterinary medicine.

Maternal immune manipulation

The rats were divided into 4 groups of *Leishmania*, *Salmonella*, Tacrolimus, and control (n=5). The *Leishmania* and *Salmonella* groups were treated 3 times at 2-week intervals with an intraperitoneal injection of about 3×10^8 CFU of killed *Leishmania major* and live *Salmonella typhimurium*, respectively. Tacrolimus (Cograft: Zahravi, Iran) (2mg/kg/day) was orally administrated to the Tacrolimus group in continuous 5 days. The control group was treated by the injection of sterilized normal saline. It is worth mentioning that one male rat was placed in each cage of the female rats for mating in one week.

Sampling

The newborns were divided according to maternal groups, and eight neonates were sacrificed in one- and two-month-old according to the animal's welfares. The neonate's total body, liver, thymus, and spleen weight were determined in this study. The prepared samples of the neonate's thymus and spleen were analyzed using histological methods. The samples were fixed in 10% formalin, and 5-6 µm sections were prepared using paraffin embedding techniques by a rotary microtome (RM2235, Leica Company from the USA) and stained with hematoxylin-eosin for the histological examination.

Regarding the histomorphometric study, the images were taken from sections using an Olympus optical microscope equipped with a Dino lit camera at a magnification of 4×, 10× and 40× at four random points, and Dino lit software was used for extracting the data. The number of the white and red pulps, as well as the cortex diameter of the thymus in 5 random high-power fields, were counted and averaged. The diameter of the Hassall's corpuscles at a magnification of 10× was measured with a Dino lit camera in each animal and 5 sections from one animal in each group. Moreover, the newborns were treated by intraperitoneal injection of 10% sheep red blood cells (SRBC) at 4 weeks of age. The neonate's humoral immune responses to maternal treated antigens and SRBC were evaluated at 5 weeks of age using the micro-agglutination test.

Statically analysis

The data were analyzed statistically in SPSS software (version 21) using Tukey's method with a one-way analysis of variance. A p-value less than 0.05 was considered statistically significant.

Results

The mean weight of the fetus's total body, spleen, thymus, and liver of the one- and two-month-old groups were shown in Tables 1 and 2, respectively. The significant elevations of the spleen and thymus weight were observed in the *Leishmania* group. Moreover, elevation and reduction of thymus and liver weight were

Table 1. The mean±SD weight of the total body, spleen, thymus, and liver of the one-month-old rats (n=8) whose mothers were treated with phosphate-buffered saline, inactivated *Salmonella* bacteria, killed *Leishmania* major, and Tacrolimus drug. The different lower-case superscripts in each column show a statically significant difference among the groups (P≤0.05).

Groups	Total Weight	Spleen Weight	Thymus Weight	Liver Weight
Control	72.6±6.7[a]	0.23±0.1[a]	0.13±0.01[a]	4.06±0.21[a]
Salmonella	79.6±9[a]	0.21±0.03[a]	0.22±0.04[b]	3.31±0.47[b]
Leishmania	79±10.4[a]	0.44±0.25[b]	0.23±0.02[b]	3.9±0.6[a]
Tacrolimus	71.4±6[a]	0.33±0.14[ab]	0.12±0.04[a]	3.98±0.47[a]

Table 2. The mean±SD weight of the total body, spleen, thymus, and liver of the two-month-old rats (n=8) whose mothers were treated with phosphate-buffered saline, inactivated *Salmonella* bacteria, killed *Leishmania* major, and Tacrolimus drug. The different lower-case superscripts in each column show a statically significant difference among the groups (P≤0.05).

Groups	Total Weight	Spleen Weight	Thymus Weight	Liver Weight
Control	149.23±11.7[a]	0.88±0.13[a]	0.28±0.05[a]	6.14±0.39[a]
Salmonella	152.24±21.4[a]	0.40±0.1[b]	0.30±0.06[a]	5.35±0.7[ab]
Leishmania	154.58±8.7[a]	0.88±0.3[a]	0.32±0.09[a]	6.53±0.36[ac]
Tacrolimus	139.3±10.2[a]	0.71±0.1[a]	0.28±0.04[a]	5.04±0.55[b]

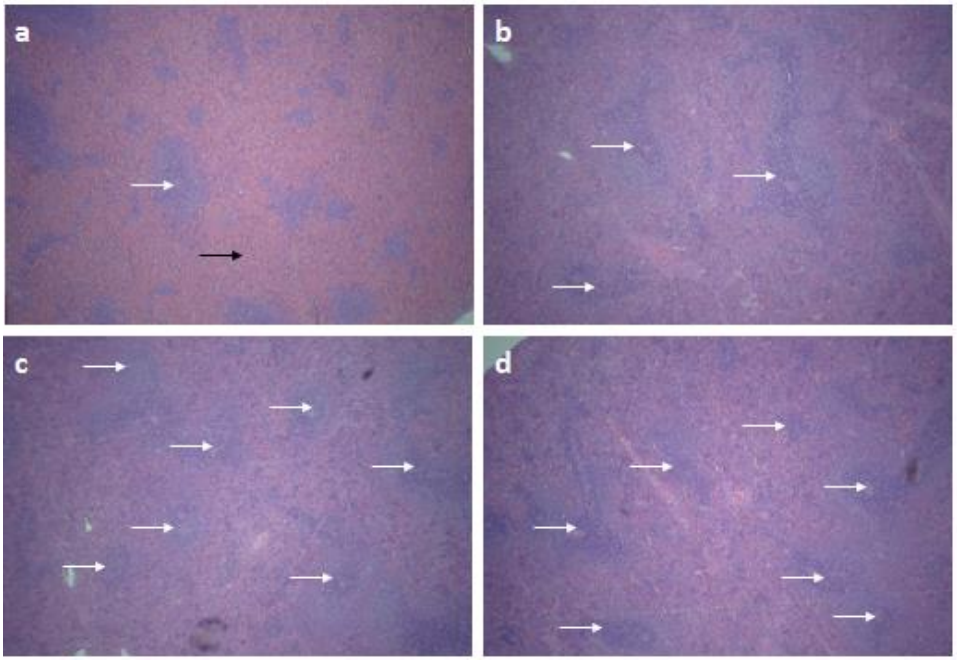

Figure 1. The histological examination of the spleen of the neonates whose mothers were treated with phosphate-buffered saline (a), Tacrolimus drug (b), inactivated *Salmonella* bacteria (c), and killed *Leishmania* (d) (hematoxylin-eosin, ×40). White and black arrows show the elevated number of the white pulps and the red pulp of the spleen, respectively.

detected, respectively, in one-month-old rats of the *Salmonella* group. Furthermore, the spleen's mean weight of the two-month-old samples showed a significant reduction (P≤0.05) in the *Salmonella* group; in addition, the Tacrolimus group had a significant reduction in liver mean weight.

The histological examination of the spleen samples (Figure 1) showed that the *Salmonella* group had a significant elevation on the white pulp's number, compared to the control group. Moreover, the *Leishmania* and Tacrolimus groups showed no significant difference despite the higher levels of the white pulp, compared to the control group (Figure 2).

The histological examination of the thymus samples (Figure 3) determined the significant elevation of the cortex diameter in *Salmonella* (P≤0.016), and *Leishmania* (P<0.018) groups, compared to the control group. Despite the higher size of the thymus cortex diameter than that in the control group, the Tacrolimus group showed no significant difference (Figure 4). The main feature of the thymus at 30-day samples was the very small size of the Hassall's corpuscles. Additionally, the medulla/cortex percentage was normal in all groups; however, the cortex diameter was expanded noticeably in the treated groups.

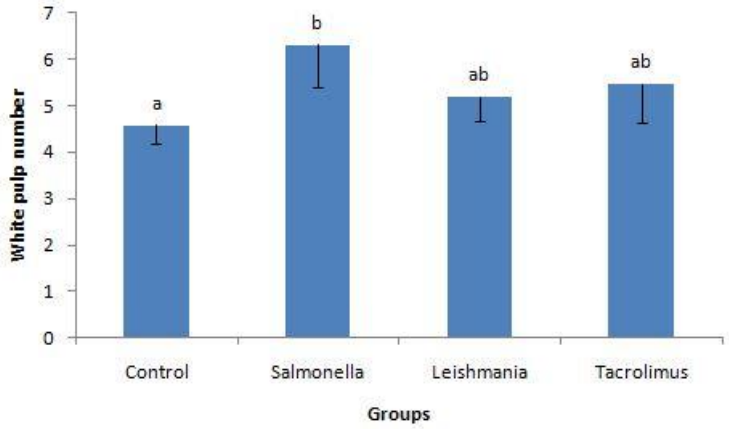

Figure 2. The number of spleen's white pulp of neonates whose mothers were treated with phosphate-buffered saline, inactivated *Salmonella* bacteria, killed *Leishmania*, and Tacrolimus drug. The different lower-case superscripts in each column show a statically significant difference among the groups (P≤0.05).

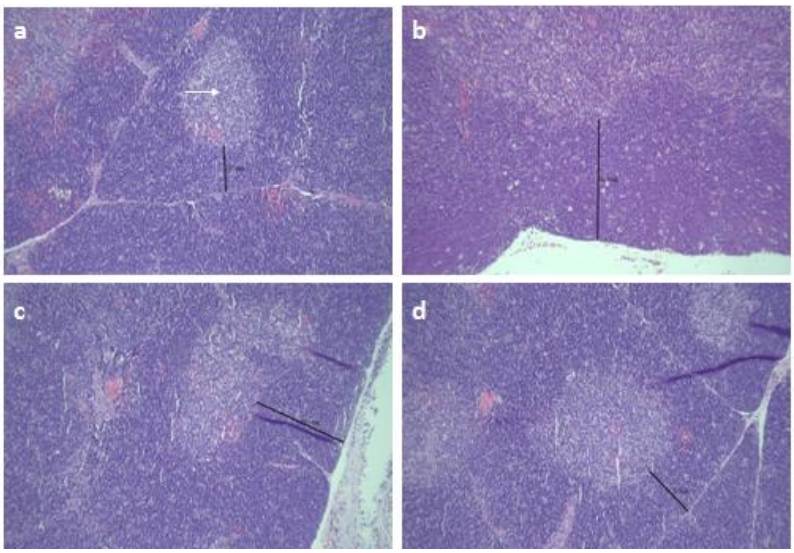

Figure 3. The histological examination of the thymus of the neonates whose mothers were treated with phosphate-buffered saline (a), inactivated *Salmonella* bacteria (b), killed *Leishmania* (c), and Tacrolimus drug (d) (hematoxylin-eosin, ×100). As it is shown, the *Salmonella* and *Leishmania* groups have higher cortex diameter (black arrow) than the other groups. The white arrows show the thymus medulla.

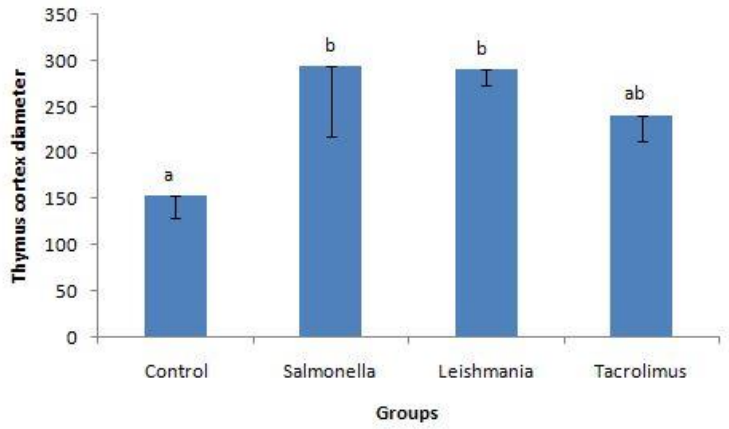

Figure 4. The thymus' s cortex diameter of the neonates whose mothers were treated with phosphate-buffered saline, inactivated *Salmonella* bacteria, killed *Leishmania*, and Tacrolimus drug. The different lower-case superscripts in each column show a statically significant difference among the groups (P≤0.05).

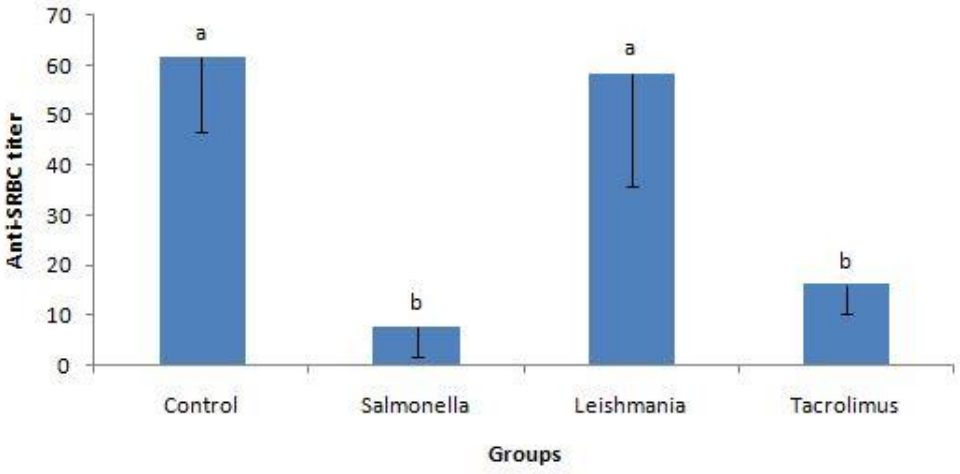

Figure 5. The humoral immune response against sheep red blood cells in neonates whose mothers were treated with phosphate-buffered saline, inactivated *Salmonella* bacteria, killed *Leishmania*, and Tacrolimus drug. The different lower-case superscripts in each column show a statically significant difference among the groups (P≤0.05).

The neonates of *Salmonella* and *Leishmania* groups had a mean antibody titer of 1024 and 512 against *S. Typhimurium* and *L. Major*, respectively. Moreover, the neonates of *Salmonella* and *Tacrolimus* groups had a significant reduction (P≤0.05) in the anti-SRBC antibody titer (Figure 5).

Discussion

This study investigated the effects of maternal immune system manipulation on offspring's immunity during pregnancy. There is some evidence about the effects of maternal exposure to infectious agents on the induction of fetus defects (14, 15). The fetal hypothalamic-pituitary-adrenal axis (16) and immunoregulatory mechanisms (17) are the most vulnerable parts of the uterus niche. Unlike the *Leishmania*, the *Salmonella* and Tacrolimus groups had suppressive thymus-dependent humoral immune responses. The anti-mouse TNF-α antibody injection to the pregnant mice caused a reduction in the antibody response to sheep red blood cells in pups which were in line with the results of this study (18).

In addition to the humoral immune responses, the *Leishmania* group had a normal white pulp number and regular weight of the spleen, thymus, and liver. In line with this result, Svensson et al. (19) and Fallon et al. (20) showed the normal pregnancies in the transgenic Th2 cytokine single-knockout mice. It appeared that the Th1 cytokines have a more important role in the uterus microenvironment than Th2 responses. All the neonates have normal weight during the current study period; however, the immune-related organs showed abnormal weights.

The transplacental transmission of the maternal immune cells, cytokines, microbial agents, or Tacrolimus was considered as the cause of the noted objects. The previous studies revealed similar results, and the activated immune cells could migrate into infants (21). The activation of the fetus Th1, Th17, and cytotoxic T cells were observed in immune stimulated pregnant dams (22). In addition, previous researches showed the transmission of the inflammatory cytokines (IL-1, IL-6, IL-12, TNF-α, GM-CSF) to the placenta (23).

The immune system recognizes microbes through pattern recognition receptors. The responsible toll-like receptors (TLR) stimulate cytokine production by competent immune cells (4). Different cytokine profiles could be created by each type of immune stimulators due to the varieties in TLR pathways. Some of the biological elements have higher adverse effects during pregnancy, such as the effects of lipopolysaccharide on the induction of hypertension and proteinuria (24). In addition to the noted indirect effects, the environmental organism may be directly transmitted to the uterus and fetus. Interestingly, the administrated labeled bacteria to the pregnant mice were detected in the offspring's gut (25). The immune manipulation during pregnancy changes the level of the fetus *bcl2*, *pkCa*, and *p53* gene expression (26). These proteins regulate the G1 cell cycle phase, DNA replication, and the repair process (27).

In the current study, the analyzed immune organs have abnormal growth, and the altered

development of the evaluated organs of the fetus may be originated from the stem cell expansion. Fetal stem cells are more sensitive to pro-inflammatory factors (28). Previously conducted studies revealed the effects of cytokines, glucocorticoid hormones, and stress on fetal hematopoietic stem cells and progenitor cell activation or apoptosis (29, 30).

The *Salmonella typhimurium* could stimulate the Th1 (11) and more production of the TNF-α than the other groups. The pro-inflammatory cytokines, including TNF-α and IL2 act as thymocyte growth factors (30). Consistent with these results, the treatment of pregnant rats using the *Salmonella* and *Leishmania* antigen resulted in the elevation of the offspring's thymus weight on one-month-old. Moreover, the neonates of the *Salmonella* group had a significantly higher number of spleen's white pulps. A previously performed study revealed the positive effects of the TNFα on the formation of the mice splenic B cell follicles, which was consistent with the results of this study (31). In addition, TNF-α signaling causes the spleen's white pulp defects (18); however, no teratogenic effects were noted in pulps of the exposed pregnant mice to the anti-mouse TNF-α antibody (32). Lymphotoxin signaling can attract hematopoietic cells (33), develop the spleen structure, and localize B and T cells in the spleen (34).

Both liver weight and humoral immune response to SRBS were fewer in *Salmonella* and Tacrolimus groups than the control group. The hepatocytes' development, hematopoietic migration, and maturation of the T cells are affected by prenatal stress (35, 36). Furthermore, the alterations of hepatic immune responses have a severe impact on innate immune response, T, and B cell function (22, 37). The effects of the Tacrolimus on the impairment of liver and humoral immune responses' development of the neonate are inconsistent with the previous reports for humans (13, 38). Due to the differences in the reproductive system, the structure of the placenta and development of the fetal immune system results cannot be expanded to the other species certainly. These issues should be investigated specifically in the human species in further studies.

Conclusion

Stimulation or suppression of the maternal immune responses during pregnancy has significant effects on the neonate's immune responses and development of spleen, liver, and thymus organs. The adverse effects may be resolved, remained, or appeared along with the growth of the neonates.

Acknowledgments

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Conflicts of interest

The author declares that s/he has no conflict of interest.

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