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Maternal immunomodulation of the offspring's immunological system

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ABSTRACT

The mother's and the offspring's immunological system are closely related thus one can influence the other. This hypothesis drove our aim to study the impact of the mother's immunological *status* over the immunological response of their offspring. For this, female mice tolerant or allergic to peanuts were exposed or not to a challenge diet containing peanuts during the gestation–lactation period (TEP/AEP; TNEP/ANEP, respectively). After weaning the offspring was submitted to the peanut allergy or peanut tolerization protocol and then challenged with a peanut diet. Our results showed that when the offspring is submitted to the allergy induction protocol, they behave differently depending on their mother's immunological status. Offspring born to TEP mothers produced the lowest antibody titters while those born to AEP mothers produced the highest antibody titters compared to mice born to TNEP and ANEP. On the other hand when the offspring was submitted to the tolerization protocol all groups presented low antibody titers with no significant difference between groups, independent of the mothers immunological status and/or contact with peanuts during the gestation–lactation period. The analysis of the histological profile of the offspring correlates well to the serological response. In other words, offspring born to TEP mothers and submitted to the allergy induction protocol presented a normal histological profile, while the offspring born to AEP mothers produced the worst gut inflammation. These results indicate that mothers, exposed to the antigen (by the oral route) during gestation, actively influence the immune response of their offspring. This work sheds some light on the importance of the immunomodulation induced by dietary antigens during gestation and their influence on the immunological response of their offspring. However, more work is needed to elucidate the molecular and cellular components of this regulatory phenomenon.

Abbreviations: AEP, allergic mothers exposed to peanuts during gestation; $Al(OH)_3$, aluminum hydroxide; ANEP, allergic mothers NOT exposed to peanuts during gestation; ANOVA, analysis of variance; BI, booster immunization; CD, challenge diet; eh/lpw, epithelial height per lamina propria width; EGF, epidermal growth factor; ELISA, enzyme-linked immunosorbent assay; H_2O_2, hydrogen peroxide; HE, hematoxylin–eosin; IEC/IEL, intestinal epithelial cells per intraepithelial leukocytes; IgG/IgE, immunoglobulin G or E; PBS, phosphate buffered saline; PBS-G, phosphate buffered saline gelatin; PPE, peanut protein extract; Pr, primary immunization; RT, room temperature; SC, subcutaneous; SD, standard deviation; SDS, sodium dodecyl sulfate; TEP, tolerant mothers exposed to peanuts during gestation; TGF, transforming growth factor; TNEP, tolerant mothers NOT exposed to peanuts during gestation; v/v, volume per volume; vh/vw, villi height per villi width; w/v, weight per volume.

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Introduction

The idea that maternal antibody transmission can influence the onset of immune mediate disease is not new. Studies of the immunologic relationship between fetus and mother have fascinated investigators for generations. Although this theme started with Paul Erlich in the early XX century with the publication of a series of experiments on the passive transfer of maternal antibody to the fetus and the newborn, this area of immunology was obscured by other important discoveries and forgotten for decades (Silverstein, 1996, 2000).

During the 1940s, at the *Jackson Laboratory*, an interest in the maternal–fetal relation reappears during the work on mouse genetics and mammary tumors. These studies were based on the survival of previous lactating mice submitted to foster mothers of a variety of strains, which were inoculated with different tumors after irradiation (Fekete and Little, 1942). At the end of the XX century the study of the maternal–fetal relation and pediatric immunology was again in evidence. Different authors demonstrated a bi-directional flux of substances and cells across the placenta (Lange et al., 1999; Lundin et al., 1999; Oldak, 1998; Prescott et al., 2000). In a study using a biofluorescence technique it was shown that the transfer of maternal leukocytes to the offspring occurs in approximately 50% of the cases (Zhou et al., 2000). In the other direction, Bianchi (1996) detected cells from a human fetus in the mother's peripheral blood 27 years after delivery. Despite all evidences, the importance of this bi-directional flux is still controversial (Lemke and Lange, 1999).

Although it is known that maternal cells that pass through the placenta are more easily incorporated in the blood circulation than cells that pass to the offspring through lactation, it is still not clear what are their roles over the offspring's immune system (Shimamura et al., 1994). In mammals, maternally derived antibodies provide immune protection to their offspring. Since these antibodies belong to the IgG class and result mainly from thymus-dependent immune responses that have undergone immune maturation, they represent the highest quality of the collective maternal immunological experience to the microbial environment. Another phenomenon associated with maternal antibodies concern's their influence in shaping the immune repertoire and priming the neonatal immune response (Lemke and Lange, 1999).

In fact, repertoire manipulations in the neonatal period have a strong influence on the adult repertoire (Kearney and Vakil, 1986). The mother's immune system is closely related to the offspring's and may influence, by epigenetic mechanisms, the incidence, for example, of autoimmune diseases and possibly much more can be explained based on immunological maternal–fetal relationship. For example, Greeley et al. (2002) provide compelling evidence for crucial pathogenic role of maternal antibodies in non-obese diabetic mice. As many other autoimmune diseases type 1 insulin-dependent diabetes is probably caused by environmental factors operating in a genetically susceptible host initiating the destructive immune reaction. These unknown environmental factors may operate over different periods in development, playing a particularly substantial role in adults.

Based on the fact that there are so many evidences in the literature of the influences on the offspring's immune system during gestation our group started a study on the influence of food antigens during embryogenesis. We wanted to understand the role of the mothers' immune system on their offspring's immune response. Thus some of our questions were: Is the mother's immunological status able to influence enough the immunoreactivity pattern of the offspring, transforming genetically low responder in high responder animals? Can an organism "learn" how to be allergic or tolerant to environmental molecules through the maternal/fetal relations? To try to address these questions we used a model developed in our lab for the induction of peanut oral tolerance and/or allergy (Paschoal et al., 2009; Teixeira et al., 2008, 2009).

Materials and methods

Animals

Male and female inbred C57BL/6 adult (8–12 weeks) mice, bred at the Animal Facility of the Federal Fluminense University (Niterói, RJ, Brazil) were maintained with free access to food and acidified water. The number of animals per group varied from 4 to 7 and this work was approved by the Ethical Committee of the School of Medicine at this University.

Peanut protein extract (PPE)

Peanut protein extract was prepared as previously described. In short, peanuts were minced in an electrical coffee grinder, sieved and added to an extraction buffer (sodium borate, 0.0125 M, SDS 1%, mercaptoethanol 1% – pH 10) maintaining a 1:10 (w/v) ratio (Teixeira et al., 2008). This mixture was then placed on a rocker for 30 min at room temperature, centrifuged at 600 × *g*, for 30 min and the supernatant was collected and kept frozen at −20 °C until use. The protein concentration was determined according to Lowry et al. (1951).

Determination of food ingestion

Diet was composed of either peanuts or commercial chow and offered at different periods of the experimental protocol. These components were weighed and distributed in the animal cage each morning. After 24 h of consumption the remaining food was collected, weighed and replaced with fresh food. The mean consumption of each group was evaluated dividing the difference between the offered and remaining food by the sum of the body mass of the mice in the group. The values were expressed as milligram of food per gram of total body mass per day (mg/(gbm day)).

$$\text{Consumption}\ \left(\frac{\text{mg}}{\text{gbw}}\right) = \frac{\text{offered}\ \ (\text{g}) - \text{collected}\ \ (\text{g})}{\text{total body weight}}$$

Induction of allergy to peanuts (immunization protocol)

Mice were immunized twice with 100 μg PPE sc. Primary immunization was performed with 1 mg $Al(OH)_3$ and booster immunization without the adjuvant after a 21-day interval. Control animals were sham immunized with physiologic saline plus 1 mg $Al(OH)_3$, and booster immunization without adjuvant.

Induction of oral tolerance to peanuts (tolerization protocol)

To induce oral tolerance to peanuts, animals were fed *ad libitum* peanuts *in natura* for 7–10 days in their cage prior to the immunization protocol with PPE.

Challenge diet (CD)

The challenge diet was composed exclusively of peanut seeds *in natura* offered *ad libitum* for a 30-day period and was introduced 1 week after booster immunization to induce the development of antigen specific gut inflammation.