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ORIGINAL ARTICLE

Intrauterine sensitization of ovalbumin in the third trimester increases the risk of food allergy in progeny

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Abstract Intrauterine sensitization caused by food allergens plays an important role in the food allergy development in progeny. The aim of our study was to determine the critical period of intrauterine sensitization during pregnancy. Female mice were exposed to ovalbumin (OVA) during different trimesters of pregnancy. Lymphocytes from their offspring were isolated and cultured, and proliferation was evaluated by CCK-8 assay. The levels of IFN-γ and IL-4 in serum were measured using ELISA. In addition, the expressions of IFN-γ and IL-4 mRNAs and proteins were detected by real-time PCR and western blot. The mice were divided into the first trimester pregnancy (FTP1 and FTP2) group, the second trimester pregnancy (STP1 and STP2) group, and the third trimester pregnancy (TTP1 and TTP2) group based on the stages of pregnancy in which their mothers were exposed to OVA and their ages. The OVA-specific lymphocyte proliferation of the TTP1 group was statistically significantly greater that in the FTP1 and STP1 groups. The serum level of IFN-γ in the TTP1 group was significantly decreased, and the serum level of IL-4 in the TTP1 group was significantly increased compared with the levels in the FTP1 and STP1 groups. The mRNA and protein expression levels of IFN-γ in the TTP1 group were significantly decreased and the mRNA and protein expression levels of IL-4 in this group were significantly increased compared with the levels in

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the FTP1 and STP1 groups. Our results suggest that OVA-induced intrauterine sensitization in the third trimester may increase the risk of food allergy after birth.

1. Introduction

In recent decades, with economic and social development, the incidence of food allergy is rising and causing concerns worldwide. As a widespread health problem from the epidemiology perspective in developed countries, food allergy affects nearly 10 percent of children (Umetsu et al., 2015). Over the past 15 years, the incidence of food allergy has exhibited a rapid growth in the United States, Britain, China and other countries (Prescott et al., 2013; Bunyavanich et al., 2014). Food allergy often occurs in infants and young children within the first three years after birth, and children suffering from food allergy have a higher risk of other allergic diseases compared with non-allergic children, including atopic dermatitis, allergic respiratory diseases, and asthma (Branum and Lukacs, 2009; Peng et al., 2015). Therefore, children suffering from food allergy have an increased risk of developing other allergic diseases at the later stage of life. However, due to the complexity of food allergy pathogenesis, effective prevention and treatment measures are still lacking. The only effective treatment nowadays is to strictly avoid intake of certain food antigens to prevent its occurrence (Patel and Volcheck, 2015). However, this method does not completely cure food allergy and restricting food intake may lead to certain type of malnutrition in children. Therefore, the early prevention of food allergy is particularly important. It is critical to find the initial antigen sensitization period.

Generally speaking, allergen sensitization is the outcome of the allergen and the host. The antigen penetrates through the mucosal epithelial layer of the gastrointestinal tract, skin or respiratory tract and interacts with innate immune receptors, such as Toll-like and protease-activated receptors on epithelial cells, stimulating them to produce cytokines that drive T-helper 2-like adaptive immunity in allergy-prone individuals. While clinical practice finds that infants who were exclusively breastfed do not contact with antigens such as milk, they still will develop early gastrointestinal symptoms indicating milk allergy, which suggests that exposure to food antigens during pregnancy might be one of the mechanisms of early allergen sensitization (Atanaskovic-Markovic, 2014; Liu, 2013). At present, whether exposure to food antigens can cause intrauterine sensitization still remain to be debated. Iván et al. (2003) confirmed that, in mice and other animal models, maternal low-dose peanut exposure during pregnancy and lactation are more effective in alleviating offspring sensitization reactions compared with completely avoiding peanut intake. This study suggested that low-dose exposure of food antigens during pregnancy may facilitate intrauterine tolerance of the fetus and reduce the risk of allergic diseases after birth. However, increasingly more lines of evidence demonstrate that intrauterine sensitization caused by food antigens plays an important role in the allergy development of the progeny. Prokešová et al. (2008) showed that allergen-specific IgE was significantly increased in the blood of mothers who suffered from allergic diseases and fetal cord blood, and this increase predicted the increased risk that the offspring would suffer from allergic diseases in the future. Desroches (2010) found that pregnant women who were early exposed to food allergens had an increased incidence of fetal food allergy after birth, suggesting that these children had been primed in uterus during pregnancy.

Although there are many studies supporting intrauterine sensitization, the start time of intrauterine sensitization is unclear. Studies have shown that consumption of nuts in the first two months of pregnancy increased the risk of nut allergy in offspring (Hsu et al., 2012, 2013). The offspring of pregnant women who were exposed to birch pollen during the weeks 20–28 of pregnancy had a higher specific response to birch pollen (Duren-Schmidt et al., 1997). There was also a positive correlation between exposure to passive smoking in the third trimester of pregnancy and asthma and other allergic symptoms in offspring (Xepapadaki et al., 2009). The results of our previous study also suggested that pregnant mice exposed to OVA during the period of pregnancy could induce sensitization of fetal mice, and the critical period may be in the late of pregnancy (Hu et al., 2005). Therefore, we hypothesized that a certain stage of pregnancy is a critical period for intrauterine sensitization. Antigen exposure during this period could significantly increase the risk of food allergy in the progeny. This study aimed to determine the critical period of intrauterine sensitization by giving OVA to pregnant mice in the different stages of gestation. Our findings will help to improve the understanding of the underlying mechanisms of food allergy during early childhood.

2. Methods

2.1. Experimental animals

A total of 20 healthy BALB/c mice (male: 5, female: 15) were obtained from the Chongqing Medical University Experimental Animal Center. According to Keppel's method (Knippels and Spanhaak, 1998), the mice were provided with special purebred feed with successive generations. The third generation of six-to eight-week-old female mice, with an average body weight of 20 ± 2 g, were selected as experimental animals. The institutional guide to the care and use of experimental animals was followed.

Taking the presence of a vaginal plug as the first day of pregnancy, pregnant mice were randomly divided into the following groups: Group FTP (the first trimester of pregnancy), Group STP (the second trimester of pregnancy), Group TTP (the third trimester of pregnancy), and the corresponding Group C (control). All the young born to a mouse at one time were used as a sample, therefore the number of offspring samples in each group corresponded to the parental mice number in each group.

According to the stage of pregnancy in which mice were exposed to OVA, the 5-day-old offspring were divided into the following groups: Group FTP_1, Group STP_1, Group