CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

ANALYSIS OF IMMUNOGLOBULIN LEVELS AFTER EXPOSURE OF COW'S MILK PROTEIN IN MICE

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https://doi.org/10.34302/crpjfst/2019.11.5.10

Article history:
Received:
9 March 2019
Accepted:
20 September 2019

Keywords:
Allergen;
Pregnant Balb/c mice;
Neonates Balb/c mice;
Immunoglobulin levels.

ABSTRACT

This research aimed to analize immunoglobulin levels after exposure of cow's milk protein in mice. We conducted a true experiment with a posttest-only control group method. This experiment used female Balb/C mice and neonate mice (pregnant mice 10 weeks old n=15, neonatal mice 2 weeks old). The sample was divided into low dose group (1 mg CMP per gram weight of mice), high dose group (10 mg CMP per gram weight of mice) and control group that consisted 5 pregnant mice respectively. The cow's milk protein allergens were induced in pregnant mice. We took blood from the pregnant mice, and their fetuses were taken for subclass immunoglobulin examination (Ig A, IgM, IgE, Ig G) by using an ELISA (Enzyme-Linked Immunosorbent Assay) kit. In low dose group we found a significant increase ($p<0,05$) in IgG-1, IgG-2a, and IgG-2b levels in pregnant mice and IgG-1, IgG-2a, IgG-2b, and IgG-3 in neonate mice which described in table 1. While for the high dose group described in table 2, similar results occurred in IgG-1, IgG-2a, and IgG-3 levels ($p<0.05$) in pregnant mice and IgG-1, IgG-2a, IgG-2b, and IgG-3 in neonate mice. In high-dose group we also found an increase in IgA, IgM, and IgE ($p<0.05$) in either the neonate or pregnant mice. However, in low dose group there was only a significant increase in IgM ($p=0.004$) and IgE ($p=0,000$) in neonate mice and only IgE ($P=0,000$) in pregnant mice. In conclusion, in low dose group we found a significant increase in IgG-1, IgG-2a, IgG-2b in both pregnant and neonate mice, IgG-3 and IgE in pregnant mice, IgM and IgE in neonate mice. While for the high dose group similar results occurred in IgG-1, IgG-2a, IgG-3, IgA, IgM, and IgE levels in both pregnant and neonate mice and IgG-2b in neonate mice.

1.Introduction

Allergy is a health problem with a high prevalence. In the human population, the prevalence of allergy has reached 20%, with 5%–15% occurring in children. In Southeast Asia, the prevalence of allergy reached 3.3% in children (Platts-Mills et al., 2003). While the prevalence is getting higher, efforts to prevent allergy is yet to be optimized, and the immunology process of *in utero* prevention has not been understood (Jones, Holloway, and Warner, 2000; Boyle, Robins-Browne, and Tang, 2006; Fusaro et al., 2009). Today, suspected allergic events are associated with the fetus's exposure to allergens, but this has yet to be explained. Some studies suggest that the child of a pregnant mother whose family has a history of dust mite allergy may become allergic to dust mites while exposure to cat hair

may induce tolerance to cat hair within the child (Prescott et al., 2003). It is predicted that different doses and types of allergen will induce different immune responses from the fetus. A study shows that fetal dendritic cells do not express the Th2 allergen, but they express the Th1 allergen (Platts-Mills et al., 2003). This leads to a discussion on how to treat allergy and whether mothers should expose allergens to their child to prevent allergies from developing or avoid them. Allergy is a chronic disease that can alter the growth and development of the child. Therefore, an experiment on pregnant Balb/C mice is conducted exposing them to low-dose and high-dose cow's milk protein allergen to analize immunoglobulin levels after exposure of cow's milk protein in mice (Kunert and Lavitska, 2001; Prescott et al., 2003; Lara-Villoslada, Olivares, and Xaus, 2005).

2. Materials and methods

We conducted a true experiment with a posttest-only control group method to analize immunoglobulin levels after exposure of cow's milk protein in mice.

2.1. Animals

The study was conducted in the Virology and Immunology Laboratory, Department of Microbiology, Airlangga University, Surabaya, East Java.

This experiment used female Balb/C mice and neonates (pregnant mice 10 weeks old n=15, neonatal mice 2 weeks old) acquired from the Farma Center of Veterinary, Surabaya. All the animals were inspected by a veterinary consultant to ensure pregnancy and the neonates' health condition. Animals were excluded if they were pregnant with fetuses with congenital disorders, have differences in meal behavior, or show signs of sickness such as decreased weight, breathing patterns, and diarrhea. Cow's milk protein allergens were induced in pregnant mice. After the pregnant mice gave birth, the neonates were kept for two weeks before having their blood taken, and the serum of both the female mice and their fetuses were examined. The samples were homogeneous in gender, age, and weight. The sample was divided into low dose group (1 mg CMP per gram weight of mice), high dose group (10 mg CMP per gram weight of mice) and control group. Then the data were analyzed using inferential statistics to achieve the research objectives.

2.2. Cows Milk Protein Allergen

Materials used were cow's milk protein allergen (Indoor Biotechnologies, Natural Bos d5 (NA-BD5-1). The cow's milk protein allergens were administered intraperitoneal in pregnant mice 5mg/5ml: low dose 1 mg CMP per gram weight of mice (Jones, Holloway, and Warner, 2000; Kunert and Lavitska, 2001; Prescott et al., 2003; Lara-Villoslada, Olivares, and Xaus, 2005). The high dose used the same reagent with 10 times the low dose.

2.3.ELISA

Immunoglobulin was analyzed using an IgG, IgM, and IgA ELISA kit with the Sandwich method. The ELISA kit (Ig isotyping Mouse Instant: Thermo Fisher Scientific company, catalog number 88-50660-22) was used to examine the optical density (OD) of IgG1, IgG2a, IgG2b, IgG3, IgM, IgA and IgE.

The 80 ml serums were collected into a microplate tube and processed by duplo. A standard 20 µl solution of IgE with a titer of 0, 50, 100, 200, 500, and 1000 was added into each tube, commencing to the A 1 and 2 until the G 1 and 2 columns. The microplate was incubated for an hour in room temperature and then washed three times with buffer washing. Then 100 µl of HRP enzyme was added to each tube. The microplate was then incubated for an hour and washed with buffer washing. Next, 100 µl of TMB was added and incubated for 30 minutes.

Absorbant value was analyzed using the 450 nm wavelength of the ELISA reader and then interpreted by linear regression analysis.

2.4. Statistics Analysis

The experiment was analyzed using ANOVA homogeneity test and Kolmogorov-

Smirnoff and normal probability plot for normality test for normal distribution. For testing differences between each group, we used ANOVA for normal distributed data and Kruskal Wallis A, Brown-Forsythe, and Mann-Whitney for abnormal distributed data.

3.Results and discussions

3.1. Results

In low dose group we found a significant increase in IgG-1, IgG-2a, and IgG-2b levels in pregnant mice ($p<0.05$) and IgG-1, IgG-2a, IgG-2b, and IgG-3 ($p<0,05$) in neonate mice which described in table 1. While for the high dose group described in table 2, similar results occurred in IgG-1, IgG-2a, and IgG-3 levels ($p<0.05$) in pregnant mice and IgG-1, IgG-2a, IgG-2b, and IgG-3 in neonate mice ($p<0.05$). Between high-dose and low-dose group, we found significant differences in IgG-1, IgG-2a, IgG-2b, and IgG-3 in neonate mice ($p(r-t)<0.05$) and IgG-1, IgG-2a, and IgG-2b in pregnant mice ($p(r-t)<0.05$). In high-dose group we found an increase in IgA, IgM, and IgE ($p<0.05$) in either the neonate or pregnant mice. However, in low dose group there was only a significant increase in IgM ($p=0.004$) and IgE ($p=0,000$) in neonate mice and only IgE ($P=0,000$) in pregnant mice. In the high-dose group and the low-dose group, there was a significant increase in IgA, IgM, and IgE in either the neonate or pregnant mice ($p(r-t)<0.05$).

Table 1. Immunoglobulin level after being exposed to low dose cow's milk allergen

	Low Dose Group (p)		Control Group	
	Pregnant mice	Neonate mice	Pregnant mice	Neonate mice
IgG-1	1.85± 0.07 (p = 0.000)	1.90± 0.08 (p = 0.004)	1.26± 0.07	0.18± 0.03
IgG-2a	2.66 ± 0.04 (p = 0.000)	2.88 ± 0.23 (p = 0.000)	1.41 ± 0.05	1.48 ± 0.18
IgG-2b	3.38± 0.08 (p = 0.000)	2.87± 0.42 (p = 0.001)	2.58± 0.06	1.95± 0.18
IgG-3	0.47± 0.14 (p = 0.078)	2.09± 0.47 (p = 0.000)	0.36± 0.04	0.09± 0.01
IgA	0.16 ± 0.05 (p = 0.749)	0.12 ± 0.03 (p = 0.192)	0.15 ± 0.09	0.10 ± 0.04
IgM	1.01 ± 0.05 (p = 0.184)	0.73 ± 0.10 (p = 0.004)	1.16 ± 0.22	0.24 ± 0.08
IgE	183.21± 5.91 (p = 0.000)	109.04±10.61 (p = 0.000)	21.89 ± 0.84	22.37 ± 3.01

Table 2. Immunoglobulin level after being exposed to high dose cow's milk allergen

	High Dose Group (p)		Control Group	
	Pregnant mice	Neonate mice	Pregnant mice	Neonate mice
IgG-1	1.49± 0.04 (p = 0.000)	1.31± 0.09 (p = 0.004)	1.26± 0.07	0.18± 0.03
IgG-2a	1.33 ± 0.46 (p = 0.688)	0.76 ± 0.17 (p = 0.000)	1.41 ± 0.05	1.48 ± 0.18
IgG-2b	2.68± 0.05 (p = 0.009)	2.39± 0.12 (p = 0.001)	2.58± 0.06	1.95± 0.18
IgG-3	0.45± 0.05 (p = 0.007)	0.20± 0.05 (p = 0.001)	0.36± 0.04	0.09± 0.01
IgA	0.32 ± 0.05 (p = 0.002)	0.04 ± 0.03 (p = 0.020)	0.15 ± 0.09	0.10 ± 0.04
IgM	1.55 ± 0.05 (p = 0.007)	0.52 ± 0.11 (p = 0.000)	1.16 ± 0.22	0.24 ± 0.08
IgE	192.96± 7.32 (p = 0.000)	124.26± 9.31 (p = 0.000)	21.89 ± 0.84	22.37 ± 3.01

3.2. Discussion

After exposure to cow's milk allergen, there were different responses from IgG2b both in pregnant mice and neonate mice and with high doses and low doses whereas for IgG3, significant response was only in pregnant and neonate mice with high-dose exposure and neonate mice with low-dose exposure. It can be explained in general that exposure to high doses and low doses of both pregnant and neonate mice gives the same response, meaning that the cow's milk antigen can be transferred through the placenta to the neonate and responded to by neonate plasma cells even with a lower response ability in neonate mice. Neonatal B cells express low levels of coreceptors including CD28 and CD40 ligand on Th2 or follicular T helper cells with their corresponding binding partners HLA-peptide, CD80/86, and CD40 on antigen-specific B cells. This limits their capacity to respond to B cells from neonates and infants aged less than two months. Hence, the immune system is immature and markedly impaired in neonates and more so in fetuses (Mcmichael, Simon, and Hollander, 2015).

Response to the exposure to high-dose cow's milk allergen occurred in IgA and IgM whereas for low-dose cow's milk, there was no significant increase from the control. It can be explained that the immune system remains inferior to exposure whereas the IgE response shows a potent allergy parameter both in pregnant mice and neonates with allergy. In food allergy, it is believed that food-specific IgE antibodies bind to high-affinity FcεRI receptors on mast cells, basophils, macrophages, and dendritic cells, as well as to low-affinity FcεRII receptors on macrophages, monocytes, lymphocytes, eosinophils, and platelets. When food allergens penetrate mucosal barriers and contact IgE antibodies bound to mast cells or basophils, histamine and other mediators that induce symptoms of immediate hypersensitivity are released (Li et al., 2005)

We found significant differences in IgE levels between pregnant mice and neonate mice exposed to low-dose and high-dose allergens. Efforts to induce allergy tolerance in mice exposed to high-dose allergens were unsuccessful. This is contrary to a study conducted by Adel-Patient (2005), where the dose of allergens exposed did not affect IgE regulation. There was an increase in IgE levels both after the exposure to low-dose or high-dose allergens (Adel-Patient, Ah-Leung, Creminon, Nouaille, Chatel, Langella, and Wal, 2005; Wavrin, 2015).

4. Conclusions

In low dose group we found a significant increase in IgG-1, IgG-2a, IgG-2b in both pregnant and neonate mice, IgG-3 and IgE in pregnant mice, IgM and IgE in neonate mice. While for the high dose group similar results occurred in IgG-1, IgG-2a, IgG-3, IgA, IgM, and IgE levels in both pregnant and neonate mice and IgG-2b in neonate mice.

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