Shifting Immune Response and Cytokine Profiles After *Porphyromonas gingivalis* Lipopolysacaride Exposure

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Abstract

There is a tendency that child who doesn't have any family history of atopic disease will easily suffer asthma or hay fever when they lived with people who have. It is believed that hygiene hypothesis should be revisited again, while some research suggest that periodontal pathogens *Porphyromonas gingivalis* (Pg) play significant role in the presence ofsome atopic diseases.

We compared level of cytokine profile for type I hypersensitivity markers in wistar rats after exposure of lipopolysaccharide (LPS) of *Porphyromonas gingivalis* (Pg).

An experimental study with pretest-posttest controlled group design were done in 1 January-10 December 2016. We used 3 groups of wistar rats, given exposure in the various dose of LPS Pgand 1 group of wistar rats given placebo. 11 days later, we measured level of cytokine profiles IFN-γ, IL-2, IL-4, IL-5, IgE and IgG4 by ELISA.

There is a shifting from a non-atopy rat became an atopy rat. After 11 days, there is no significant different in Th1 activity (represented by IFN-γ and IL-2), whereas there is a significant different in Th2 activity(represented by IL-4 and IL-5). Exposure of LPS Pg also stimulates increasing of IgE and IgG4.

There is a significant shifting immune response and cytokine profiles after LPS Pg exposures. Nevertheless, manifestation of atopic diseases and allergic asthma are not clearly appears due to the increasing level of IgG4 as counter-response to increasing of IgE.

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Introduction

Several years ago, we recognized hygiene hypothesis stated that fewer opportunities for infections and microbial exposures have resulted in more widespread asthma and other atopic disease. Dispute to that hypothesis, we found a tendency that an increasing infectious oral and dental agents over the past half century have coincided with increases in the prevalence of asthma and other allergic diseases[1].

Asthma is an inflammatory disease of respiratory tract which are most often found as often as a chronic obstructive pulmonary disease, the worldwide incidents of asthma increased. The prevalence of asthma in developed countries in adults is approximately 10% and more children, while in developing countries the prevalence is lower, but increased rapidly year by year[2].

According to World Health Organization (WHO), in 2005 there were approximately 300 million people with asthma and there are 250,000 people die from asthma every year, an estimation that in 2025 people with asthma will grow to 100 million people[3].

In Indonesia, the research on school children aged 13-14 years using a questionnaire ISAAC (International Study on Asthma and Allergy in Children) in 1995 reported that the prevalence of asthma was 2.1%, while in 2003 it increased to 5.2%. A survey of asthma in school children in several cities in Indonesia (Medan, Palembang, Jakarta, Bandung, Semarang, Yogyakarta, Surabaya, and Denpasar) showed

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that the prevalence of asthma in school children (6 to 12 years) ranged from 6.4 to 3.7% while in junior high school children in Jakarta by 5.8%. Based on this description, it appears that atopic diseases has become a public health problem that needs serious attention[3].

Asthma transmission concept proposed by Yoo (2007) is interesting to be showed. This concept explain why non-atopic children may get atopic diseases, even though there were no family history. Similarly, the prevalence of asthma as high in children result of the adoption by the foster mother who was also suffering from asthma, such as between a biological child with a mother who suffered from asthma. Also the findings in the people of New Guinea, asthma occurs first in adults after he returned to the village after working in Europe.

In line with some of the findings above, in the field of dentistry the rare some germs responsible for the occurrence of asthma. Tanner reported that periodontal pathogens found in the dental plague are also contagious[6].These anaerobic species of periodontal pathogens are found at the beginning in toothless baby's mouth. It is believed that mothers' saliva or caregivers' saliva act as a source of these anaerobic bacteria[7].

The concept of pathogen transmission from one to another, linked by research done by Lee et al, in which the research shows that periodontal pathogen also contagious[8]. Itis revealed that if a child has periodontal germs, then at least one of the parents has the bacteria with the same genotype. Therefore, there is a possibility that allergic asthma is not only inherited, but also passed on to non- allergic individuals through periodontal pathogens.

In connection with the role of bacteria periodontal pathogens against allergic asthma, the research results by Wiyarni(2007) showed that gram negative rod-shaped dental bacteria is more dominant in subjects with atopic diseases who still had symptoms of asthma[8]. According to the paradigm of Th1 / Th2 balance, person with allergy has a Th2 immune response[9].

Th2 immune responses induced by TLR2 signaling pathway and stimulates the release of cytokines IL-4, IL-13. This paradigm in line with concept LPS as TLR ligand, *Porphyromonasgingivalis* is one ofTLR2 ligand[10]. These bacteria may play a role in atopic diseases are based on a report that low doses (<1U / mL) exposure of LPS *P.gingivalis* will inhibit toll-like receptor 2 (TLR2) pathway. LPS *P.gingivalis* could inhibit TLR4 activation pathway which acts as T helper 1 (Th1) resulting in production bottle necks interferon-gamma (IFN gamma). TLR2 activation sustainable will encourage the immune response Th2(IL-4and IL-5)[11].

P. gingivalis have several kinds of LPS with different ion mass and acyl groups which determine the heterogeneity of the lipid A component of the most bioactive LPS[11,12], specifically found that LPS *P.gingivalis* with major ion mass Lipid A in 1435 and 1450 m / z (pgLPS1435/1450) can serve as an immune modulatory in TLR4. Similarly, research in mice Balb / c neonates showed that Ovalbumin injection of L P S *P.gingivalis* together with low doses can aggravate allergic reactions characterized by meaningful elevated levels of serum immunoglobulin E than control group[13].

Periodontal pathogens may be involved in the transmission of asthma in several ways:(1) direct transmission from parents and caregivers through induces respiratory infections and type 1 hypersensitivity, (2) indirectly through the placenta and stimulate prematurebirth[13].

Due to that theory, including allergic asthma and other atopic diseases, exposure to infectious agents couldn't be maintained. The fact that almost all children with allergic asthma, but only a small percentage of children have allergic asthma, at least raise the possibility that means there are some additional factors that are involved.

One of the studies that support the phenomenon of bacterial infection through periodontal pathogens in atopic diseases and allergic asthma is found by Utomo (2010) in a study of non-allergic mice induced with chronic gingivitis PgLPS. After LPS Pg injected in non-allergic mice on day 4 IgE examination with positive monoclonal ovalbumin antibody and in extra-pulmonary bronchial tissue there is an increased expression of ECP and LTC4[14].

Based on the literature mentioned above, it is possible the occurrence of asthma in non-atopic subjects periodontal pathogens caused by bacteria. However, this mechanism is remain unclear.

Materials and methods

Materials

1. Lipopolysaccharide (LPS) of *Porphyromonasgingivalis* (Astarte Biologics, WA,USA,in intra-sulcular injectionoflow-dose, medium and high)
2. The monoclonal antibody against IFN-γ, IL-2, IL-4, IL-5, , IG E and IgG_4were measured in plasma by direct-sandwich ELISA (R&D System Europe Ltd, Abingdon, UK) according to manufacturer's instructions. All measurements were done in duplicates and values averaged for analysis.

Ethics statement

Ethical approval to carry out this study was granted by Airlangga Oral and Dental Hospital in collaboration with College of Dentistry Research Ethics Committee (DREC Ref: 72/13) and Universitas Airlangga College of Medicine Research Council (Ref: MREC/A/1768).

Study population

16 male wistar rats (age 8-10 week, weight 120-150 g) were randomized and divided into 4 groups of intervention:

- Group A: Placebo intra-sulcular injected.
- Group B1: 0.3µg/ml LPS *Porpyhromonasgingivalis* intra-sulcular injected.
- Group B2: 1µg/ml LPS *Porpyhromonasgingivalis* intra-sulcular injected.
- Group B3: 3µg/ml LPS *Porpyhromonasgingivalis* intra-sulcular injected.

Experimental procedures

Samples were conducted with euthanasia protocols. Blood serum taken from *plexus retro orbitalis*. The entire sample was taken blood serum 11 days after LPS Pg injected and seen the activity of Th1 (levels of IFN-γ, IL-2); activity of Th2 (levels of IL-4, IL-5); level of IgE and IgG_4 with direct sandwich enzyme-linked immuno sorbent assay (ELISA).

Study design

Pretest-posttest controlled group design was done in this study. We extracted 16wistar rats and randomized them into 4 groups. Within each group, there were no significant age and body weight differences.

Group A were given placebo intra-sulcular. Group B1 were given 0.3 □ g/ml LPS *Porpyhromonasgingivalis* intra-sulcular, Group B2 were given 1 □ g/ml LPS *Porpyhromonasgingivalis* intra-sulcular,Group B3 were given 3 □ g/ml LPS *Porpyhromonasgingivalis* intra-sulcular.

After 4 days and 11 days, serum samples were taken in both groups. Activity of Th1 (levels of IFN-γ, IL-2); activity of Th2 (levels of IL-4, IL-5); level of IgE and IgG_4 were measured by direct-sandwich ELISA.

Statistical analysis

Data analysis was performed using SPSS version 17.0 (IBM corp, Chicago, USA). Statistical analyses were done using student's paired t-test and results were presented as means ± standard errors (SE). The paired t-test was used to test differences between variables of cytokine profile and level of Th1 and Th2 activity among each groups of wistar rats. Pearson's chi-square test was used to test for correlations between laboratory markers. Univariate (crude values) and multiple linear regression (adjusted coefficient) analyses were used to test for association or to identify variables which predict different levels of laboratory markers. A *p*-value of 0.05 was considered significant.

Results

Results of the study include: (1) a comparison table between groups: A - Control (only given placebo); B1 (treated by low dose Pg LPS injection, 0,3µg / ml); B2 (treated by medium dose Pg LPS injection, 1 µg / ml); and B3 (treated by high dose ofLPS injection, 3µg / ml). (2)

Plot profile of Elisa examination on each cytokine.

1. Th1 activity after eleven days LPS Pg injected. Th1 is reflected by the level of IFNγ and IL-2.

There's no different significantly in Th1 activity after 11 days LPS Pg injection, this can seen at the level IFN- γ and IL-2 in all dose.

2. Th2 activity after eleven days LPS Pg injected. Th1 is reflected by the level of IL-4 and IL-5.

There's a different significantly in Th2 activity after 11 days LPS Pg injection, this can seen at the level IL-4 in all dose.

1. Non-atopic profile reflected by Ig G4 level, although Atopy profile reflected by IgE level.

Group	n	Level (pg/ml)	
		IFN-γ	IL-2
Placebo	4	-13,75 ± 13,40	2,55 ± 1,62
LPS Pg 0,3 µg/ml	4	4,01 ± 13,65	26,58 ± 20,60
LPS Pg 1 µg/ml	4	1,71 ± 51,12	20,58 ± 3,02
LPS Pg 3 µg/ml	4	-3,91 ± 8,53	-7,05 ± 8,81
p		0,053	0,131

Table 1. Level IFN-γ and IL-2 after 11 days. *significant at α =0,05.

Group	n	Level (pg/ml)	
		IL-4	IL-5
Placebo	4	-2,97 ± 1,23[a]	22,22 ± 6,56

Table 2. Level IL-4 and IL-5 after 11 days.
*significant at α =0,05
[ab]the same *superscript* in the same column is showing there's no different between group.

Group	n	Level (pg/ml)	
		IgG4	IgE
Placebo	4	3,17 ± 3,13[a]	-0,64 ± 3,77[a]
LPS Pg 0,3 µg/ml	4	28,09 ± 2,57[b]	174,99 ± 21,09[c]
LPS Pg 1 µg/ml	4	51,69 ± 24,53[c]	102,63 ± 7,35[b]
LPS Pg 3 µg/ml	4	53,60 ± 10,80[c]	95,31 ± 22,30[b]
p		0,002*	0,001*

Table 3. Level IgG4 and IgE after 11 days.
*significant at α =0,05
[ab]the same *superscript* in the same column is showing there's no different between group.

There's a different significantly in IgG4 and IgE activity after 11 days LPS Pg injection.

Discussion

Th1 activity reflected by the level of IFNγ and IL-2, where in the all dose the level of IFNγ and IL-2, both of them no different after Pg LPS injection. Th2 activity reflected by the level of IL-4 and IL-5, where in the all dose the level of IL-4 were significant decrease after 11 days LPS Pg injection.

Atopic profiles were reflecte dby increasing of level IgE. Although IgE level is increasing, yet IgG_4 level also increase as a counter response[15]. It means that even rat become susceptible to atopic due to the increasing level of IgE, body mechanism is able to provide protection, with increased IgG_4 as a counter response to prevent manifestation of allergic diseases and type 1 hypersensitivity. Thus, exposure of LPS Pg will develop chance ofatopic and hypersensitivity markers, but manifestation of allergic reaction is a complex pattern[16].

Conclusions

In this study, exposure group with LPSPgis able to shift immune response, with greater level of IgE and IgG4. There is no difference in Th1 activity, but significant changed were observed in Th2 activity, represented by level of IL-4 and IL-5. Further research evaluates manifestation of allergic reaction is need to be done, about complex interaction between IgE and IgG_4 levels after LPS Pg exposure.

Limitations and Strengths

This study had limitations with regard to the animal trials and limited number of samples to associate with the assessed cytokines as well as reported experience of atopic diseases in animals. These factors may have an impact on the interpretation of our results. Thus, the findings should be interpreted within the context of this study and its limitations. The strengths of the study were its high statistical power and the homogeneity of each group to enable comparison with the unexposed subjects.

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Declaration of Interest

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