RESEARCH PAPER

Atopic Biomarker Changes after Exposure to *Porphyromonas gingivalis* Lipopolysaccharide: A Small Experimental Study in Wistar Rat

“Oral pathogen influence atopic disease”

Sindy Cornelia Nelwan DDS, Ph.D^{1*}, Ricardo Adrian Nugraha MD², Anang Endaryanto MD, Ph.D³, Prof. Asti Meizarini DDS, Ph.D⁴, Udijanto Tedjosasongko DDS, Ph.D¹, Prof. Seno Pradopo DDS, Ph.D¹, Haryono Utomo DDS, Ph.D⁵

¹ Department of Pediatric Dentistry, Faculty of Dentistry, Universitas Airlangga;

² Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Airlangga;

³ Division of Pediatric Allergy and Immunology, Department of Child Health, Faculty of Medicine, Universitas Airlangga;

⁴ Department of Dental Materials Science and Technology, Faculty of Dentistry, Universitas Airlangga;

⁵ Department of Orthodontics, Faculty of Dentistry, Universitas Airlangga

* Correspondence to: Sindy Cornelia Nelwan, DDS, Ph.D

Department of Pediatric Dentistry, Faculty of Dentistry, Universitas Airlangga

Address: Jalan Mayjend Prof. Dr. Moestopo 47, Surabaya (60132) - Indonesia

Phone : +62811346979

Email : sindy-c-n@fkg.unair.ac.id

Atopic Biomarker Changes after Exposure to *Porphyromonas gingivalis* Lipopolysaccharide: A Small Experimental Study in Wistar Rat

“Oral pathogen influence atopic disease”

Background. IgE and IgG₄ are implicated in atopic development and clinically utilized as major biomarkers. Atopic responses following certain pathogens such as *Porphyromonas gingivalis* is currently an area of interest for further research. **Purpose.** The aim of this study is to measure the level of IgE, IgG₄, and IgG₄/IgE ratio periodically after exposure of periodontal pathogen *Porphyromonas gingivalis* (Pg) lipopolysaccharide (LPS). **Methods.** We used 16 wistar rats (*Rattus norvegicus*) randomly subdivided into 4 groups, group 1 were injected by placebo, group 2 by LPS Pg 0.3 µg/mL, group 3 by LPS Pg 1 µg/mL, and group 4 by LPS Pg 3 µg/mL. Sera from both groups were taken from retro-orbital plexus before and after exposure. **Results.** Level of IgE and IgG₄ increased significantly following exposure of LPS Pg at day-4 and day-11. Greater increase of IgE rather than IgG₄ contributes to rapid decline of IgG₄/IgE ratio, detected in the peripheral blood at day-4 and day-11. **Conclusion.** Modulation of atopic responses following exposure to LPS Pg is reflected by decrease in IgG₄/IgE ratio that accompanies an increase of IgE.

Keywords: *allergic diseases; atopic inflammatory pathway; immunoglobulin; periodontal pathogen; Porphyromonas gingivalis LPS*

Clinical significance: *Porphyromonas gingivalis*, a keystone pathogen during periodontal disease, may have a tendency to disrupt atopic biomarkers.

INTRODUCTION

Oral cavity is the the habitat of numerous bacterias including *Porphyromonas gingivalis* (Pg). Pg is a gram negative, facultative anaerobic pathogen which is responsible in causing gingivitis or periodontitis (1). In low-income countries, gingivitis and periodontitis can affect up to 90% of the adult population (2). Rather than alveolar bone and ligament destruction, *Porphyromonas gingivalis* is believed to have some linked with the development of atopic responses in a susceptible host (3). Following Pg infection, hosts' adaptive immune response (both cell-mediated and humoral-mediated) could induce systemic inflammatory reaction, not only just local destruction of tooth-supporting tissues (4-5). Although periodontal pathogens such as Pg play major role in the initiation of local and systemic inflammatory reaction (6), the host aberrant immune responses are more interesting subjects to be studied. Since humoral immune responses are stimulated following Pg infection, there might have been linked to the occurrence of atopy.

Despite long-standing research about hygiene hypothesis in several decade, there is an unequivocally-accepted fact that the prevalence of atopy increases greater among children who had periodontal pathogen colonization or infection (7). While endorsing these hygiene hypothesis approaches, there is an alternative hypothesis in which exposure to some periodontal pathogens will exclusively trigger an "Immunoglobulin-E skew" rather than declining it (8). Within the context of the hygiene hypothesis, the most essential microbial exposures need to be studied is biomolecular relationship between host antibody and regulatory T-cell with lipopolysaccharide (LPS), an endotoxin released by Pg to affect host immune reaction.

Hygiene hypothesis principles might not be able to answer all phenomenon of increasing incidence of atopy among children with poor oral hygiene (9). Some studies report a positive association between the colonization/infection of Pg with the development of allergic diseases (10-15), whereas some studies report no association (16-21). Due to lack of conclusive

evidence about the association between Pg and allergic diseases (22), we try to measure the level of atopic biomarkers following Pg infection.

To the best of our knowledge, measuring IgG₄ and IgE antibody may have a closer association to the atopic profiles, since IgG₄ and IgE are released after activation of mature B cells following the modulation of IL-4 and IL-5 released by Th-2 cells during type I hypersensitivity (23). By looking at the alteration of IgG₄ and IgE antibodies level after exposure to these selected components of Pg in an animal model, we hope to understand deeper about the biological mechanism of B-cell production antibodies patterns and humoral immune responses earlier before the clinical manifestation of atopy.

MATERIAL AND METHODS

Ethics approval

Animal experimental study were conducted under the approval of the Institutional Animal Research Ethics Committee of Universitas Airlangga (UNAIR), Surabaya, Indonesia (animal approval no:50/KKEPK.FKG/IV/2015) under the name of Sindy Cornelia Nelwan as the Principal Investigator. Study was carried out in strict accordance to internationally-accepted standards of the Guide for the Care and Use of Laboratory Animals of the National Institute of Health.

Animals

The present study used 16 male Wistar rats (*Rattus norvegicus*) between eight and ten weeks of age (average body weight 120-150 grams). The rats were housed in microisolator cages and maintained in a constant room temperature ranging from 22°C to 25°C, with a 12-h light/12-h dark cycle, under artificially controlled ventilation, with a relative humidity ranging from 50% to 60%. The rats were fed a standard balanced rodent diet (NUTRILAB CR-1®) and water were provided ad libitum.

Experimental design and groups

The present study design was a pre-test post-test controlled group design using quantitative method. We extracted 16 male Wistar rats, randomized and then classified into 4 groups. Each group consists of 4 matched Wistar rats (age, weight, IgE and IgG₄ baseline characteristic). Group 1 were given placebo. Group 2 were given lipopolysaccharide (LPS) of *Porphyromonas gingivalis* (Pg) at dose 0.3 µg/mL. Group 3 were given LPS Pg at dose 1 µg/mL. Group 4 were given LPS Pg at dose 3 µg/mL. The enrolled subjects will receive LPS by an intra-sulcular injection. Intra-sulcular injection has an advantages due to the its direct delivery of LPS to oral cavity in which the tip of needle is injected slowly at the crestal bone. Longitudinal quantitative measurement had been done by repeated checked of IgE level, IgG₄ level, and IgG₄/IgE ratio

in both groups between day-0 (before treatment), day-4, and day-11. An average of 0.2 ml peripheral blood sera was obtained by Pasteur pipette from retro-orbital plexus, using a lateral approach.

Level of IgG₄ and IgE

Sample of the sera were collected and stored at -70°C (-94°F) at Institute of Tropical Diseases Universitas Airlangga (UNAIR). All sera were assessed by direct-sandwich enzyme-linked immunosorbent assay (ELISA) under the manufacturer's (R&D System Europe Ltd, Abingdon, UK) protocol. Briefly, the sera were examined at the microtiter plates with mix supplied 25 ml of 3,3',5,5'-tetramethylbenzidine to 1 ml of phosphate-citrate buffer plus perborate in a mildly acidic buffer (adjust pH 5.7). Level of IgG₄ were detected using monoclonal antibody anti-IgG₄, transferring it to microtiter plates, adding the supplied conjugate, adding blocking solution, diluting plasma sample (1:100,000), and washing between the steps. Level of IgE was detected using monoclonal antibody anti-IgE, similar following steps until diluting plasma sample (1:200). A minimum value of 0.01 pg/mL for IgE and 0.01 ng/mL for IgG₄ were assigned for below the limit of detection.

Statistical analysis

All measurements were performed at least three times. Results were presented as means ± standard errors (SEM). The assumption of the normality for the complete data was assessed by Shapiro-Wilk test. Test of homogeneity of variances was assessed by Levene Statistics. Statistical significance were examined by one-way ANOVA and repeated measure ANOVA using SPSS version 17.0 for Microsoft (IBM corp, Chicago, USA).

RESULTS

General characteristic investigations

Table 1 showed baseline characteristics of 16 Wistar rats (*Rattus norvegicus*). No significant differences had been found for mean age ($p=0.774$), body weight ($p=0.700$), baseline IgE ($p=0.071$), baseline IgG₄ ($p=0.770$), and baseline IgG₄/IgE ratio ($p=0.053$) among the four groups. (Table 1)

Comparison of serum IgE level between four groups

Prior to experiments (day-0), there was no difference of serum IgE level between the four groups ($p>0.05$). Meanwhile, four days following experiments, there was a significance difference of serum IgE level between both groups ($p=0.006$). At day-4, the highest average IgE level could be found in the group treated with LPS Pg 1 $\mu\text{g/ml}$ (17.00 ± 1.69 pg/ml) and the lowest average IgE level could be found in the control group (5.31 ± 0.76 pg/ml) respectively. Furthermore, ten days following experiments, there was also a significance difference of serum IgE level between both groups ($p=0.047$). At day-10, the highest average IgE level could be found in the group treated with LPS Pg 0.3 $\mu\text{g/ml}$ (180.34 ± 10.42 pg/ml) and the lowest average IgE level could be found in control group (5.06 ± 1.86 pg/ml) respectively. (Table 2)

Comparison of serum IgG₄ level between four groups

Prior to experiments (day-0), there was no difference of serum IgG₄ level between the four groups ($p>0.05$). Meanwhile, four days following experiments, there was a significance difference of serum IgG₄ level between both groups ($p=0.008$). At day-4, the highest average IgG₄ level could be found in the group treated with LPS Pg 3 $\mu\text{g/ml}$ (23.86 ± 1.59 ng/ml) and the lowest average IgG₄ level could be found in the control group (8.34 ± 0.58 ng/ml) respectively. Furthermore, ten days following experiments, there was a greater difference of serum IgG₄ level between both groups ($p=0.005$). At day-10, the highest average IgG₄ level could be found in the

group treated with LPS Pg 3 $\mu\text{g/ml}$ (63.74 ± 4.74 ng/ml) and the lowest average IgG₄ level could be found in control group (13.91 ± 0.99 ng/ml) respectively. (*Table 3*)

Ratio of IgG₄/IgE antibodies between four groups

The average IgG₄/IgE ratio for the control group at day-0, day-4, and day-11 was 1.87×10^3 , 1.72×10^3 , and 3.62×10^3 . In the low-dose LPS group, the average IgG₄/IgE ratio was 2.20×10^3 , 1.27×10^3 , and 0.22×10^3 . In the medium-dose LPS group, the average IgG₄/IgE ratio was 1.19×10^3 , 0.69×10^3 , and 0.56×10^3 . In the high-dose LPS group, the average IgG₄/IgE ratio was 1.59×10^3 , 1.64×10^3 , and 0.65×10^3 . All groups exhibited significant differences of IgG₄/IgE ratios, except at day-0. The highest IgG₄/IgE ratio at day-4 and day-10 could be found in control group. The lowest IgG₄/IgE ratio at day-4 could be found in LPS Pg 1 $\mu\text{g/ml}$ group, whilst the lowest ratio at day-11 could be found in LPS Pg 0.3 $\mu\text{g/ml}$ respectively. (*Table 4*)

Subgroup analysis in Wistar rats treated with of 0.3 $\mu\text{g/ml}$ LPS Pg

Level of IgE were increased dramatically from day-0 to day-11 after experiments (5.36 ± 0.19 pg/ml to 180.34 ± 10.42 pg/ml; $p=0.011$). Level of IgG₄ were also increase significantly from day-0 to day-11 after experiments (11.76 ± 0.85 ng/ml to 39.85 ± 2.14 ng/ml; $p=0.006$). On the other hand, IgG₄/IgE ratio were decreased following experiments ($2.20 \pm 0.13 \times 10^3$ to $0.22 \pm 0.01 \times 10^3$; $p=0.014$). (*Table 5*)

Subgroup analysis in Wistar rats treated with 1 $\mu\text{g/ml}$ LPS Pg

Level of IgE were raised dramatically from day-0 to day-11 after experiments (10.27 ± 0.70 pg/ml to 112.90 ± 3.87 pg/ml; $p=0.003$). Level of IgG₄ were also increase significantly from day-0 to day-11 after experiments (11.91 ± 1.66 ng/ml to 63.6 ± 10.76 ng/ml; $p=0.027$). On the other hand, IgG₄/IgE ratio were declined following experiments ($1.19 \pm 0.20 \times 10^3$ to $0.56 \pm 0.09 \times 10^3$; $p=0.362$). (*Table 6*)

Subgroup analysis in Wistar rats treated with of 3 µg/ml LPS Pg

Level of IgE were raised dramatically from day-0 to day-11 after experiments (6.69 ± 0.61 pg/ml to 102.01 ± 11.04 pg/ml; $p=0.009$). Level of IgG₄ were also increase significantly from day-0 to day-11 after experiments (10.14 ± 1.44 ng/ml to 63.74 ± 4.74 ng/ml; $p=0.029$). On the other hand, IgG₄/IgE ratio were declined following experiments ($1.59 \pm 0.34 \times 10^3$ to $0.65 \pm 0.08 \times 10^3$; $p=0.113$). (Table 7)

DISCUSSION

Several mechanisms have been suggested to alter to atopic inflammatory responses following LPS Pg infection. One of the mechanisms proven in this study is an elevation of IgE antibody and declining of IgG₄/IgE ratio (24). Other mechanisms which are indirectly proven are shifting of Th-1 to become Th-2 cells along with their cytokines pathway. Down-regulation of the Th-1 cell associated to depress of cell-mediated immune response and stimulate humoral immune response, thus pathogens are able to evade the immune clearance (25).

Porphyromonas gingivalis possess very sophisticated defense mechanisms against host immune responses. These pathogens produce capsules containing long chain LPS which is designed effectively to counter MAC. These long chain LPS can also downgrade cell-mediated immunity by shifting Th-1 into Th-2 which less dangerous to pathogens (26). LPS may have an essential role in switching cell-mediated to humoral-mediated immune responses (27). LPS Pg antigen is processed and presented on its surface again with MHC-II molecule. Recent studies suggest an activation of alternative complement pathway, disruption of classical complement pathway, modulation of antigen presenting cells, and downregulation of anti-inflammatory cytokines are responsible for the Th2-skewed immune response following exposure to LPS Pg. Predominance shifting from Th-1 into Th-2 occurs in several extra-lymphoid tissues; the ideal site for *Porphyromonas gingivalis* is the oral cavity (28).

Interleukin-4 (IL-4), which is produced by naive T cells, acts as autocrine manner known to be responsible for the differentiation and activation of Th-2 phenotype (29). Guo et al (2014) shows upon the occurrence and development of allergic diseases, there is a complex pathobiology which results an imbalance of Th-1/Th-2 (30). In an atopic disease such as bronchial asthma or urticaria, naive T cell can differentiate into Th-2 under the spesific circumstance by IL-4–induced STAT6 and GATA-3 transcription factors (30). Th-2 predominant immune response will automatically stimulate plasma cell to release IgE and IgG₄ (31). Upon re-exposure of antigen or

allergen, binding of the allergen to IgE orchestrates the adaptive immune system to initiate rapid sensitization. Frequent sensitization is a major risk factor for the development of allergic diseases such as urticaria, bronchial asthma, hay fever or atopic dermatitis/ eczema (32).

Our previous study used whole-cell body of *Porphyromonas gingivalis* to study different molecular responses in Wistar rats. Our first project studied the association between periodontal pathogen and host innate immunity. Exposure to *Porphyromonas gingivalis* had been shown to stimulate level of TLR2 and depress level of TLR4 (33). Our findings might indicate that several bacterial properties can turn-off host innate immunity and host inflammatory response. Our second project studied the association between periodontal pathogen and host adaptive immunity. We summarized that high dose CFU of Pg stimulates fold increase of Th-2 cytokines (IL-4, IL-5 and IL-13) and decrease of Th-1 cytokines (IFN- γ and IL-17) (34). These were the cornerstone to continue our project in studying LPS as the most important component of these bacteria.

At this moment, both total IgE or spesific IgE antibodies have little diagnostic value in the occurence of allergic manifestation. Even total or spesific IgE is increasing, yet the manifestation of allergy doesn't usually develop, since IgG₄ level is also increase as a counter-regulator (35). It means that even human or rat become susceptible to atopic due to the increasing level of IgE, body mechanism is able to provide protection, with increased IgG₄ as a counter response to prevent manifestation of allergic diseases and immediate hypersensitivity. Thus, exposure of LPS Pg will develop chance of atopic and hypersensitivity markers, but manifestation of allergic reaction is a complex pattern (36). IgG₄/IgE ratio has closer accuracy to detect any alteration of atopic inflammatory pathway. Increase level of IgE which isn't accompanied by IgG₄ can be seen in patient with urticaria or atopic dermatitis (37). IgE-switched B cells are much more likely to differentiate into plasma cells, whereas IgG₄-switched B cells are less likely to differentiate (38). This reason would explain why IgE antibody is the most dominant antibodies in the development of atopic inflammatory pathway, whereas IgG₄ antibodies become prominent later during chronic

non-atopic stimulation (39). According to this reason, IgG₄/IgE ratio may predict atopic responses more accurately than total or specific IgE level.

Limitations and Strength

Several limitations should be highlighted. First, this study had limitations with regard to very small number of samples which can increase the likelihood of error and imprecision. Second, results from animal model often do not translate into replications in human model (40). IgE antibody responses in Wistar rats are typically transient, whereas the atopic IgE response in human persists for many years (41). Other crucial difference is IgG₄/IgE ratio, which is usually much higher in the Wistar rats than human (42-43). 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 between groups and periods.

Conclusion

Several experiments in rats indicate that exposure to LPS Pg may have a tendency to increase level of IgE and IgG₄. On the contrary, declining of IgG₄/IgE ratio following exposure to LPS Pg suggest the potential role of LPS Pg for isotype switching from IgG₄ to IgE. The results of the present study favor indirect isotype switching route for most IgE as secondary responses from LPS Pg infection that leads to systemic atopic inflammatory pathway.

ACKNOWLEDGMENTS

This project is fully supported by Darmawan Setijanto as Dean of Universitas Airlangga College of Dentistry and Coen Pramono as Director of Airlangga Oral and Dental Hospital. We would like to acknowledge Harjanto from Physiology Department and Indrawati Retno from Biochemistry Department for providing material and reagent generation. We also thank all lecturers and research assistants from Universitas Airlangga, who are willing to help in the technical aspect.

CONFLICT OF INTEREST

All authors declare no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Nelwan SC, Endaryanto A.

Project administration and funding acquisition: Nelwan SC, Endaryanto A, Utomo H.

Data curation and formal analysis: Nugraha RA.

Investigation: Nelwan SC, Nugraha RA.

Methodology: Nelwan SC, Nugraha RA.

Resources and Software: Nelwan SC, Meizarini A, Utomo H.

Supervision and validation: Tedjosasongko U, Pradopo S.

Writing - original draft: Nugraha RA.

Writing - review & editing: Tedjosasongko U, Utomo H.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available online at Universitas

Airlangga Repositroy (<http://repository.unair.ac.id/id/eprint/63285>) for research purposes.

CONSENT FOR PUBLICATIONS

Not applicable (public data).

REFERENCES

1. Herrero ER, Fernandes S, Verspecht T, Ugarte-Berzal E, Boon N, Proost P, Bernaerts K, Quirynen M, Teughels W. Dysbiotic Biofilms Deregulate the Periodontal Inflammatory Response. J Dent Res 2018;97(5):547-55.
2. Mazurek-Mochol M, Majorczyk E, Banach J, Dembowska E, Kuśnierczyk P, Safranow K, Pawlik A. The influence of KIR gene presence/absence polymorphisms on the development of periodontal disease in smokers and non-smokers. Cent Eur J Immunol 2017;42(4):347–53.
3. Sperr M, Kundi M, Tursic V, Bristela M, Moritz A, Andrukhov O, Rausch-Fan X, Sperr WR. Prevalence of Comorbidities in Periodontitis Patients Compared to the General Austrian Population. J Periodontol 2018;89(1):19-27.
4. Tarannum F, Faizuddin M. Effect of gene polymorphisms on periodontal diseases. Indian. J Hum Genet 2012;18(1):9–19.
5. Pathan MM, Bhat KG, Joshi VM. Comparative evaluation of the efficacy of a herbal mouthwash and chlorhexidine mouthwash on select periodontal pathogens: An in vitro and ex vivo study. J Indian Soc Periodontol 2017; 21(4):270-275.
6. Rafiei M, Kiani F, Sayehmiri F, Sayehmiri K, Sheikhi A, Zamanian Azodi M. Study of *Porphyromonas gingivalis* in periodontal diseases: A systematic review and meta-analysis. Med J Islam Repub Iran 2017;31:62.
7. Dowarah R, Verma AK, Agarwal N, Singh P, Singh BR. Selection and characterization of probiotic lactic acid bacteria and its impact on growth, nutrient digestibility, health and antioxidant status in weaned piglets. PLoS One 2018;13(3):e0192978.
8. Fishbein AB, Fuleihan RL. The hygiene hypothesis revisited: does exposure to infectious agents protect us from allergy? Curr Opin Pediatr 2012;24(1)98-102.
9. Tse K, Horner AA. Allergen tolerance versus the allergic march: the hygiene hypothesis revisited. Curr Allergy Asthma Rep 2008;8(6):475-83. Review.
10. Wang P, Duan D, Zhou X, Li X, Yang J, Deng M, Xu Y. Relationship between expression of

human gingival beta-defensins and levels of periodontopathogens in subgingival plaque. J Periodontal Res. 2014;50(1):113–122.

11. Tomitaa S, Komiya-Ito A, Imamura K, Kita D, Ota K, Takayama S, Makino-Oi A, Kinumatsu T, Ota M, Saito A. Prevalence of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Tannerella forsythia* in Japanese patients with generalized chronic and aggressive periodontitis. Microbial Pathogenesis 2013;61-62:11–5.

12. Ting Zh, Hong X, Zhaohui Y. Relationships of five periodontal pathogens causing subgingival plaque in patients with chronic periodontitis under different periodontal conditions. West China J Stomatol 2013;5:518–21.

13. Amano A, Kuboniwa A, Nakagawa I, Akiyama S, Morisaki I, Hamada S. Prevalence of specific genotypes of *Porphyromonas gingivalis* fimA and periodontal health status. J Dent Res 2000;79(9):1664–8.

14. Lu R, Feng L, Gao X, Meng H, Feng X. Relationship between volatile fatty acids and *Porphyromonas gingivalis* and *Treponema denticola* in gingival crevicular fluids of patients with aggressive periodontitis. Beijing Da Xue Xue Bao 2013;45(1):12–6.

15. Scapoli L, Girardi A, Palmieri A, Testori T, Zuffetti F, Monguzzi R, Lauritano D, Carinci F. Microflora and periodontal disease. Dent Res J (Isfahan) 2012;9(2):S202–6.

16. Carinci F, Scapoli L, Girardi A, Cura F, Lauritano D, Nardi GM, Palmieri A. Oral microflora and periodontal disease: new technology for diagnosis in dentistry. Ann Stomatol 2013;5(2):170–3.

17. Avila-Campos MJ, Velasquez-Melendez G. Prevalence of putative periodontopathogens from periodontal patients and healthy subjects in São Paulo SP Brazil. Rev Inst Med trop S. Paulo 2002;44(1):1–5.

18. Wilson M, Lopatin D, Osborne G, Kieser JB. Prevalence of *Treponema denticola* and *Porphyromonas gingivalis* in plaque from periodontally- healthy and periodontally-diseased sites. J Med Microbiol 1993;38:406–10.

19. Riep B, Edesi-Neuss L, Claessen F, Skarabis H, Ehmke B, Flemmig TF, Bernimoulin JP, Göbel UB, Moter A. Are putative periodontal pathogens reliable diagnostic markers? J Clin

- Microbiol 2009;47(6):1705–11.
20. Kato A, Imai K, Ochiai K, Ogata Y. Higher Prevalence of Epstein–Barr Virus DNA in Deeper Periodontal Pockets of Chronic Periodontitis in Japanese Patients. PLoS ONE. 2013;8(8):e71990.
21. Kato A, Imai K, Ochiai K, Ogata Y. Salivary detection of periodontopathic bacteria in Fanconi's anemia patients. Anaerobe. 2013;24:32–5.
22. Rafiei M, Kiani F, Sayehmiri F, Sayehmiri K, Sheikhi A, Zamanian Azodi M. Study of *Porphyromonas gingivalis* in periodontal diseases: A systematic review and meta-analysis. Med J Islam Repub Iran 2017;31:62.
23. Okamoto S, Taniuchi S, Sudo K, Hatano Y, Nakano K, Shimo T, Kaneko K. Predictive value of IgE/IgG₄ antibody ratio in children with egg allergy. Allergy, Asthma & Clinical Immunology 2012;8:9.
24. Portnoy J. IgE in Clinical Allergy and Allergy Diagnosis [Internet]. Available at http://www.worldallergy.org/professional/allergic_diseases_center/ige/ [accessed on 1 July 2018].
25. Herman RA, Ladics GS. Allergenic sensitization versus elicitation risk criteria for novel food proteins - short communication. Regul Toxicol Pharmacol 2018;94:283-5.
26. Matthews CR, Joshi V, de Jager M, Aspiras M, Kumar PS. Host-bacterial interactions during induction and resolution of experimental gingivitis in current smokers. J Periodontol 2013;84(1):32-40.
27. Finlay BB and McFadden G. Anti-Immunology: Evasion of the Host Immune System by Bacterial and Viral Pathogens. Cell 2006;124(4):767-782.
28. Schmid-Hempel P. Immune defence, parasite evasion strategies and their relevance for 'macroscopic phenomena' such as virulence. Philos Trans R Soc Lond B Biol Sci 2009; 364(1513):85–98.
29. Theodorou GL, Marousi S, Ellul J, Mougiou A, Theodori E, Mouzaki A, Karakantza M. T helper 1 (Th1)/Th2 cytokine expression shift of peripheral blood CD4+ and CD8+ T cells in patients at the post-acute phase of stroke. Clin Exp Immunol. 2008;152(3):456–63.

30. Guo HW, Yun CX, Hou GH, Du J, Huang X, Lu Y, Keller ET, Zhang J, Deng JG. Mangiferin attenuates TH1/TH2 cytokine imbalance in an ovalbumin-induced asthmatic mouse model. PLoS One 2014;9:e100394.
31. Ettinger R, Karnell JL, Henault J, Panda SK, Riggs JM, Kolbeck R, Sanjuan MA. Pathogenic mechanisms of IgE-mediated inflammation in self-destructive autoimmune responses. Autoimmunity 2017;50(1):25-36. Review.
32. Varricchi G, Raap U, Rivellese F, Marone G, Gibbs BF. Human mast cells and basophils- How are they similar how are they different? Immunol Rev 2018;282(1):8-34. Review.
33. Nelwan SC, Nugraha RA, Endaryanto A, Retno I. Modulating toll-like receptor-mediated inflammatory responses following exposure of whole cell and lipopolysaccharide component from *Porphyromonas gingivalis* in wistar rat models. Eur J Dent 2017;11(4):422-6.
34. Nelwan SC, Nugraha RA, Endaryanto A, Meizarini A, Tedjosasongko U, Pradopo S, Utomo H. Converging findings from linkage between periodontal pathogen with atopic and allergic immune response. Cytokine 2019;113:89–98. doi: 10.1016/j.cyto.2018.06.015.
35. Saulite I, Hoetzenecker W, Guenova E, Schmid-Grendelmeier P, Glatz M. Skin Test Reactivity to Hymenoptera Venom after Venom Immunotherapy Correlates Inversely with the IgG/IgE Ratio. Int Arch Allergy Immunol 2017;174(3-4):190-9.
36. Garman L, Smith K, Muns EE, Velte CA, Spooner CE, Munroe ME, Farris AD, Nelson MR, Engler RJ, James JA. Unique Inflammatory Mediators and Specific IgE Levels Distinguish Local from Systemic Reactions after Anthrax Vaccine Adsorbed Vaccination. Clin Vaccine Immunol 2016;23(8):664-71.
37. Deo SS, Mistry KJ, Kakade AM, and Niphadkar PV. Role played by Th2 type cytokines in IgE mediated allergy and asthma. Lung India 2010;27(2):66–71. doi: 10.4103/0970-2113.63609.
38. Kubo M. T follicular helper and TH2 cells in allergic responses. Allergol Int 2017;66(3):377-81.
39. Zhang Y, Fear DJ, Willis-Owen SA, Cookson WO, Moffatt MF. Global gene regulation during activation of immunoglobulin class switching in human B cells. Sci Rep

412 2016;6:37988.

413 40. Platts-Mills TA, Vaughan JW, Blumenthal K, Pollart Squillace S, Sporik RB. Serum IgG and
414 IgG₄ antibodies to Fel d 1 among children exposed to 20 microg Fel d 1 at home: relevance
415 of a nonallergic modified Th2 response. *Int Arch Allergy Immunol* 2001;124:126–9.

416 41. Ramadani F, Bowen H, Upton N, Hobson PS, Chan YC, Chen JB, Chang TW, McDonnell
417 JM, Sutton BJ, Fear DJ, Gould HJ. Ontogeny of human IgE-expressing B cells and plasma
418 cells. *Allergy* 2017;72(1):66-76.

419 42. Aalberse RC, Platts-Mills TA, Rispens T. The Developmental History of IgE and IgG₄
420 Antibodies in Relation to Atopy, Eosinophilic Esophagitis, and the Modified TH2 Response.
421 *Curr Allergy Asthma Rep* 2016;16(6):45.

422 43. Hatipoğlu U, Subramanian A, Campbell T, Rice R, Mummadi S, Hu B, Lang DM.
423 Intrasubject Variability in Total IgE Levels in Patients with Moderate to Severe Persistent
424 Allergic Asthma Over 1 Year. *J Allergy Clin Immunol Pract* 2016;4(4):691-6.

425

ABBREVIATIONS

ANOVA	analysis of variant
BCR	B cell receptor
CD-40	Cluster Differentiation-40
CFU	colony-forming unit
CSR	class-switch recombination
df	degree of freedom
ELISA	enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
HDM	house dust mite
IACUC	Institutional Animal Care and Use Committee
IFN- γ	gamma-Interferon
IgE	immunoglobulin-E
IgG ₄	immunoglobulin-G ₄
IL	interleukin
LLPC	long-lived plasma cell
LPS	lipopolysaccharide
mAb	monoclonal antibody
MAC	membrane attack complex
MHC	major histocompatibility complex
NIH	National Institutes of Health
NGS	next-generation sequencing
NK	natural killer cells
OIT	oral immunotherapy
PAMP	pathogen-associated molecular patterns
PCR	Polymerase Chain Reaction
Pg	<i>Porphyromonas gingivalis</i>
SD	standard deviation

454	SEM	standard error of the mean
455	SPSS	Statistical Package for the Social Sciences
456	Th-1	type-1 helper T-cells
457	Th-2	type-2 helper T-cells
458	TNF- α	Tumor Necrosis Factor- α

TABLES

Table 1. General characteristics of study population

Variable	Placebo	LPS Pg 0,3 ug/ml	LPS Pg 1 ug/ml	LPS Pg 3 ug/ml	<i>p value</i>
Age (weeks)	8.87 ± 0.86	9.37 ± 0.95	8.75 ± 0.96	9.12 ± 0.63	0.774
Weight (g)	135.25 ± 9.54	135.25 ± 9.55	137.25 ± 7.93	138.00 ± 10.98	0.700
Male (%)	100	100	100	100	-
IgE (pg/mL)	5.69 ± 0.28	5.36 ± 0.19	10.27 ± 0.70	6.69 ± 0.61	0.071
IgG ₄ (ng/mL)	10.74 ± 1.41	11.76 ± 0.85	11.91 ± 1.66	10.14 ± 1.44	0.770
IgG ₄ /IgE (x10 ³)	1.87 ± 0.18	2.20 ± 0.13	1.19 ± 0.20	1.59 ± 0.34	0.053

Notes:

* Data are presented as mean ± standard error of the mean (SEM)

** One-way ANOVA for categorical variables; significant at $p < 0.05$

*** IgE, immune globulin E; IgG₄, immune globulin G₄; IgG₄/IgE, ratio between averages IgG₄ levels divided by averages IgE levels.

467 **Table 2. Comparisons of total serum IgE (pg/mL) between four groups (mean $\pm$ SEM)**

Group	n	IgE day 0	IgE day 4	IgE day 11
Placebo	4	5.69 $\pm$ 0.28	5.31 $\pm$ 0.76	5.06 $\pm$ 1.86
LPS Pg 0,3 ug/ml	4	5.36 $\pm$ 0.19	9.26 $\pm$ 0.32	180.34 $\pm$ 10.42
LPS Pg 1 ug/ml	4	10.27 $\pm$ 0.70	17.00 $\pm$ 1.69	112.90 $\pm$ 3.87
LPS Pg 3 ug/ml	4	6.69 $\pm$ 0.61	14.79 $\pm$ 0.86	102.01 $\pm$ 11.04
F statistic	/	20.733	26.171	83.758
p value	/	0.071	0.006	0.047

468 **Notes:**

469 * measured by one-way ANOVA (df1=3, df2=12, f table 3.490; significant at $p < 0.05$)

470

471 **Table 3. Comparisons of total serum IgG₄ (ng/mL) between four groups (mean ± SEM)**

Group	n	IgG ₄ day 0	IgG ₄ day 4	IgG ₄ day 11
Placebo	4	10.74 ± 1.41	8.34 ± 0.58	13.91 ± 0.99
LPS Pg 0,3 ug/ml	4	11.76 ± 0.85	11.80 ± 0.83	39.85 ± 2.14
LPS Pg 1 ug/ml	4	11.91 ± 1.66	11.98 ± 1.65	63.6 ± 10.76
LPS Pg 3 ug/ml	4	10.14 ± 1.44	23.86 ± 1.59	63.74 ± 4.74
F statistic	/	0.379	29.265	15.665
p value	/	0.770	0.008	0.005

472 **Notes:**

473 * measured by one-way ANOVA (df1=3, df2=12, f table 3.490; significant at $p < 0.05$)

474

475 **Table 4. Comparisons of IgG₄/IgE ratio (x10³) between four groups (mean ± SEM)**

Group	n	IgG ₄ /IgE day 0	IgG ₄ /IgE day 4	IgG ₄ /IgE day 11
Placebo	4	1.87 ± 0.18	1.72 ± 0.36	3.62 ± 0.85
LPS Pg 0,3 ug/ml	4	2.20 ± 0.13	1.27 ± 0.08	0.22 ± 0.01
LPS Pg 1 ug/ml	4	1.19 ± 0.20	0.69 ± 0.03	0.56 ± 0.09
LPS Pg 3 ug/ml	4	1.59 ± 0.34	1.64 ± 0.20	0.65 ± 0.08
F statistic	/	3.418	5.032	13.600
p value	/	0.053	0.017	<0.001

476 **Notes:**

477 * measured by one-way ANOVA (df1=3, df2=12, f table 3.490; significant at $p < 0.05$)

478

479 **Table 5. Comparisons of total serum IgE, IgG₄, and IgG₄/IgE ratio before and after**
 480 **treatment in the group with exposure of 0.3 µg/mL LPS Pg (mean ± SEM)**

Time-point	n	IgE (pg/mL)	IgG ₄ (ng/mL)	IgG ₄ /IgE (x10 ³)
Day-0 Before treatment	4	5.36 ± 0.19	11.76 ± 0.85	2.20 ± 0.13
Day-4 After treatment	4	9.26 ± 0.32	11.80 ± 0.83	1.27 ± 0.08
Day-11 After treatment	4	180.34 ± 10.42	39.85 ± 2.14	0.22 ± 0.01
F statistic	/	93.924	178.233	71.969
p value	/	0.011	0.006	0.014

481 **Notes:**

482 * measured by repeated measure ANOVA

483 ** df times=2, df error=14, f table 3.739; significant at $p < 0.05$

484

485 **Table 6. Comparisons of total serum IgE, IgG₄, and IgG₄/IgE ratio before and after**
 486 **treatment in the group with exposure of 1 µg/mL LPS Pg (mean ± SEM)**

Time-point	n	IgE (pg/mL)	IgG ₄ (ng/mL)	IgG ₄ /IgE (x10 ³)
Day-0 Before treatment	4	10.27 ± 0.70	11.91 ± 1.66	1.19 ± 0.20
Day-4 After treatment	4	17.00 ± 1.69	11.98 ± 1.65	0.69 ± 0.03
Day-11 After treatment	4	112.90 ± 3.87	63.6 ± 10.76	0.56 ± 0.09
F statistic	/	294.526	35.437	1.760
p value	/	0.003	0.027	0.362

487 **Notes:**

488 * measured by repeated measure ANOVA

489 ** df times=2, df error=14, f table 3.739; significant at $p < 0.05$

490

491 **Table 7. Comparisons of total serum IgE, IgG₄, and IgG₄/IgE ratio before and after**
 492 **treatment in the group with exposure of 3 µg/mL LPS Pg (mean ± SEM)**

Time-point	n	IgE (pg/mL)	IgG ₄ (ng/mL)	IgG ₄ /IgE (x10 ³)
Day-0 Before treatment	4	6.69 ± 0.61	10.14 ± 1.44	1.59 ± 0.34
Day-4 After treatment	4	14.79 ± 0.86	23.86 ± 1.59	1.64 ± 0.20
Day-11 After treatment	4	102.01 ± 11.04	63.74 ± 4.74	0.65 ± 0.08
F statistic	/	111.386	32.929	7.841
p value	/	0.009	0.029	0.113

493 **Notes:** * measured by repeated measure ANOVA ** df times=2, df error=14, f table 3.739;
 494 significant at $p < 0.05$.

495

496 **FIGURE LEGENDS**

497 **Figure 1.** Longitudinal observation of serum IgE levels following exposure to LPS Pg.

498 **Figure 2.** Longitudinal observation of serum IgG₄ levels following exposure to LPS Pg.

499 **Figure 3.** Comparisons of IgG₄/IgE ratio between four groups (mean $\pm$ SEM).