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Arginase activity in a murine macrophage cell line (RAW264.7) stimulated with lipopolysaccharide from *Actinobacillus actinomycetemcomitans*

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Aims: The aim of the present study was to determine whether or not lipopolysaccharide from *Actinobacillus actinomycetemcomitans* could stimulate arginase activity in a murine macrophage cell line (RAW264.7 cells).
Methods: RAW264.7 cells were treated with *A. actinomycetemcomitans*-lipopolysaccharide or lipopolysaccharide from *Escherichia coli* for 24 h. The effect of polymyxin B, L-norvaline, DL-norvaline, dexamethasone and cytokines (interferon-γ and interleukin-4) on arginase activity in *A. actinomycetemcomitans*-lipopolysaccharide-stimulated cells was also determined. The cells were pretreated with anti-CD14, anti -toll-like receptor 2, or anti-toll-like receptor 4 antibody prior to stimulation with *A. actinomycetemcomitans*-lipopolysaccharide. Arginase activity was determined by a colorimetric assay.
Results: *A. actinomycetemcomitans*-lipopolysaccharide stimulated arginase activity in RAW264.7 cells in a dose-dependent manner, but was less potent than *E. coli*-lipopolysaccharide. Polymyxin B and L-norvaline, but not DL-norvaline, blocked the arginase activity in *A. actinomycetemcomitans*-lipopolysaccharide-stimulated cells. Dexamethasone and interleukin-4 but not interferon-γ augmented arginase activity in *A. actinomycetemcomitans*-lipopolysaccharide-stimulated cells. Treatment of the cells with anti-CD14 and anti-toll-like receptor 4 but not anti-toll-like receptor 2 antibody decreased arginase activity in *A. actinomycetemcomitans*-lipopolysaccharide-stimulated cells.
Conclusion: The results of the present study suggest that lipopolysaccharide from *A. actinomycetemcomitans* via CD14/toll-like receptor 4 complex molecules and the regulatory control of glucocorticoid and cytokines may stimulate arginase activity in RAW264.7 cells.

Key words: *A. actinomycetemcomitans*; arginase; LPS; macrophage; murine; RAW 264.7

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Arginase is an intracellular enzyme that catalyzes the conversion of L-arginine to L-ornithine and urea. Two isoforms of this enzyme, arginase I (the hepatic isoform) and arginase II (the extrahepatic isoform), are known to exist (6). Arginase I, a cytosolic form, is believed to play a role in ureagenesis. Arginase II, a mitochondrial form, is thought to be involved in the biosynthesis of polyamines and in inflammation (6). Arginase may also play a crucial role in wound healing by enhancing

fibroblast proliferation and collagen production as well as in the immune system by regulating, for example, T-cell proliferation (2, 26). As this enzyme utilizes L-arginine, it may compete with nitric oxide synthase, an enzyme that catalyzes the reaction of L-arginine to nitric oxide. Indeed, previous studies indicated that increased nitric oxide production by activated macrophages inhibited the activity of arginase and vice versa (7, 8, 14), suggesting that nitric oxide synthase and arginase activity are reciprocally modulated. Furthermore, the arginase activity of macrophages was up-regulated by bacterial lipopolysaccharide and cytokines such as interleukin-4 (IL-4) and IL-10 but down-regulated by interferon-γ (3, 10, 21), suggesting that arginase activity may be up-regulated by activated Th2 cells, but down-regulated by activated Th1 cells.

Actinobacillus actinomycetemcomitans, a gram-negative oral bacterium, plays a crucial role in the development of periodontal disease, particularly the destructive stage involving rapid gingival and alveolar bone destruction (16, 28). We have shown that, in a murine model, this periodontopathogen stimulates cellular and humoral immunity *in vivo* and *in vitro* (13, 29). This periodontopathogen may also stimulate alveolar osteoclast activation, leading to periodontal bone destruction (22). These previous results seem to indicate that this periodontopathogen is able to induce an immune response and concurrently stimulate both soft tissues and alveolar bone destruction. We and others have recently shown that lipopolysaccharide from periodontopathogens such as *A. actinomycetemcomitans, Prevotella intermedia* and *Porphyromonas gingivalis* induces nitric oxide production by murine macrophages and splenic macrophages (4, 14, 15, 27, 28, 30, 31). Of interest, nitric oxide production by these cells stimulated with *A. actinomycetemcomitans*-lipopolysaccharide was regulated by different cytokines; thus, interferon-γ and IL-12 up-regulated but IL-4 suppressed nitric oxide production (27, 31). We speculated that in *A. actinomycetemcomitans*-lipopolysaccharide-activated murine macrophages, arginase activity may be increased by IL-4 and depressed by interferon-γ and IL-12 (27, 31). However, arginase activity and its regulation in murine macrophages stimulated by *A. actinomycetemcomitans*-lipopolysaccharide is unknown. Therefore, the aim of the present study was to determine arginase activity in a murine macrophage cell line (RAW264.7) stimulated by *A. actinomycetemcomitans*-lipopolysaccharide.

Material and methods

Preparation of *A. actinomycetemcomitans* lipopolysaccharide

A. actinomycetemcomitans Y4 (serotype b) was grown in Todd-Hewitt broth supplemented with 1% (w/v) yeast extract (Difco Laboratories, Detroit, MI) anaerobically as described elsewhere (34). Lipopolysaccharide from this periodontopathic bacterium was extracted using hot phenol-water, treated with nuclease (Sigma, St. Louis, MO), and then lyophilized as previously described (27).

Cell cultures

A murine macrophage cell line (RAW264.7), a kind gift from Dr. T. Sizfizul (School of Biology, University Sains Malaysia), was grown in Dulbecco's modified Eagle's medium supplemented with 5% fecal calf serum and 1% penicillin-streptomycin (Sigma). After confluence, cells were harvested and washed three times, and viable cells then counted. Culture medium 200 μl, containing 2×10^5 cells was cultured in an incubator in 5% CO_2 at 37°C for 24 h and stimulated with various concentrations of *A. actinomycetemcomitans*-lipopolysaccharide. As a control, the cell cultures were stimulated with lipopolysaccharide from *Escherichia coli* (*E. coli*-lipopolysaccharide) (Sigma). Cultures of cells alone without stimulation were used as a negative control. Cell cultures were also stimulated with 10 μg of *A. actinomycetemcomitans*-lipopolysaccharide in the presence of polymyxin B (Sigma) at various concentrations. To determine whether arginase activity of RAW264.7 cells was induced by *A. actinomycetemcomitans*-lipopolysaccharide, cells were incubated with various concentrations of L-norvaline or DL-norvaline and 10 μg of *A. actinomycetemcomitans*-lipopolysaccharide. Dexamethasone (Sigma), a synthetic glucocorticoid, or cytokines (recombinant murine interferon-γ or IL-4) (R & D Systems, Minneapolis, MN) was added to the cell cultures with or without *A. actinomycetemcomitans*-lipopolysaccharide. In other experiments, cells were incubated with antimurine CD14 or antimurine toll-like receptor 2 and 4 antibody (Santa Cruz Biotech, Santa Cruz, CA) at room temperature for 1 h, washed three times, and then stimulated with 10 μg of *A. actinomycetemcomitans*-lipopolysaccharide. All experiments were repeated three times, each consisting of triplicate cultures.

Measurement of arginase activity

Arginase activity in cell lysate was determined by a colorimetric assay as previously described (21). Briefly, cells were lysed with 50 μl of 0.1% Triton X-100 and shaken for 30 min. The cell lysate was then added with 50 μl of 25 mM Tris-HCl (pH 7.5). To 25 μl of this lysate, 5 μl of 10 mM $MnCl_2$ was added and the mixture was heated at 56°C for 10 min. After adding 50 μl of 0.5 M L-arginine (pH 9.7), the mixture was incubated for 1 h at 37°C. The reaction was stopped with 450 μl of acid solution mixture (1 H_2SO_4: 3 H_3PO_4: 7 H_2O). The color was developed by adding 20 μl of 9% α-isonitropropiophenone (dissolved in 100% ethanol), heated at 100°C for 45 min, and then read at 540 nm by μQuant spectrophotometer (Biotek-Instrument Inc., Winooski, VT). Arginase activity was calculated using the arginase activity index with the following formula (3):

$$\text{Arginase activity index} = (\text{test arginase activity}) \times 100/\text{media only arginase}$$

Arginase from cultured cells alone was assigned an activity index value of 100.

Statistical analysis

The data were analyzed with one-way analysis of variance followed by Fisher's least squares differences using the SPSS statistical package (SPSS Co., Chicago, IL).

Results

Arginase activity by murine macrophages

Increased arginase activity by murine macrophages (RAW264.7 cells) stimulated with *E. coli*-lipopolysaccharide was observed (Fig. 1). Similarly, *A. actinomycetemcomitans*-lipopolysaccharide-stimulated RAW264.7 cells produced detectable arginase activity. At any given lipopolysaccharide concentration, the levels of arginase activity in *A. actinomycetemcomitans*-lipopolysaccharide-stimulated RAW264.7 cells were significantly lower than those in *E. coli*-lipopolysaccharide-stimulated RAW264.7 cells ($P < 0.05$).

Effects of polymyxin B

Increased concentrations of polymyxin B added to the *A. actinomycetemcomitans*-lipopolysaccharide-stimulated RAW264.7 cells were correlated with decreased levels

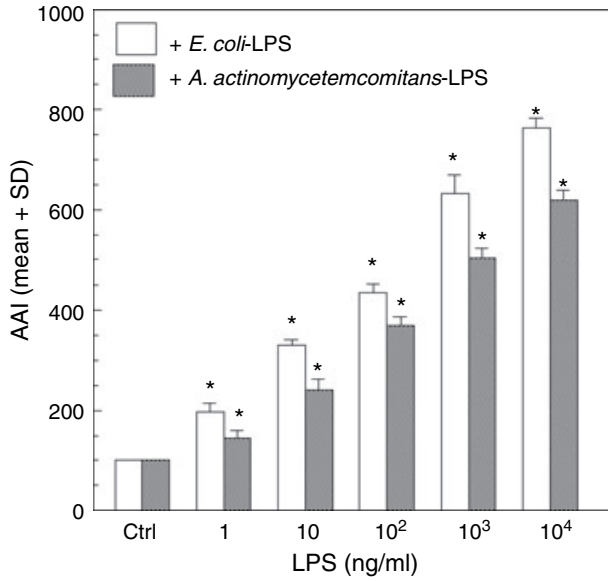

Fig. 1. Arginase activity in RAW264.7 cells stimulated with lipopolysaccharide from *Escherichia coli* and *Actinobacillus actinomycetemcomitans*. The arginase activity index of cells alone was set at 100. ***** Significant difference at $P < 0.05$ between arginase activity in *A. actinomycetemcomitans*- and *E. coli*-lipopolysaccharide-stimulated RAW264.7 cells as well as between various concentrations of lipopolysaccharide from the same bacteria. AAI, arginase activity index; SD, standard deviation.

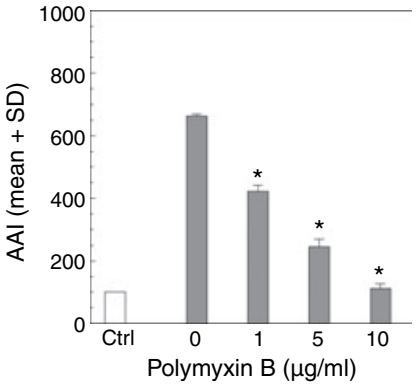

Fig. 2. Effect of polymyxin B on arginase activity in *Actinobacillus actinomycetemcomitans*-lipopolysaccharide -stimulated RAW264.7 cells. *****Significant difference at $P < 0.05$ between arginase activity in RAW264.7 cells with and without the presence of polymyxin B. AAI, arginase activity index; SD, standard deviation.

of arginase activity ($P < 0.05$) (Fig. 2). These results indicated that polymyxin B abolished the stimulatory effect of *A. actinomycetemcomitans*-lipopolysaccharide on arginase activity by RAW264.7 cells.

Effect of norvaline, an arginase inhibitor

L-norvaline, but not its derivative DL-norvaline, suppressed arginase activity by *A. actinomycetemcomitans*-lipopolysaccharide-stimulated RAW264.7 cells ($P < 0.05$) (Fig. 3), indicating that *A. actinomycetemcomitans*-lipopolysaccharide indeed activated arginase activity by RAW264.7 cells.

Effect of dexamethasone, a synthetic glucocorticoid

Dexamethasone has been shown to act on arginase activity by murine macrophages (11, 19). The present study also revealed that dexamethasone increased arginase activity by *A. actinomycetemcomitans*-lipopolysaccharide-stimulated RAW264.7 cells in a dose-dependent fashion ($P < 0.05$) (Fig. 4). However, the addition of dexamethasone failed to augment arginase activity by RAW264.7 cells without stimulation of *A. actinomycetemcomitans*-lipopolysaccharide ($P > 0.05$).

Effect of cytokines

The effects of interferon-γ and IL-4 on arginase activity by *A. actinomycetemcomitans*-lipopolysaccharide-stimulated RAW264.7 cells were assessed, as these cytokines have been shown to regulate arginase activity (3, 10, 21). The results of the present study indicate that increasing concentrations of interferon-γ added to the cultures of *A. actinomycetemcomitans*-lipopolysaccharide-stimulated RAW264.7 cells resulted in decreasing levels of arginase activity (Fig. 5). This cytokine did not augment arginase activity by cells without stimulation of *A. actinomycetemcomitans*-lipopolysaccharide ($P > 0.05$). In sharp contrast, IL-4 promoted arginase activity by RAW264.7 cells with or without the presence of *A. actinomycetemcomitans*-lipopolysaccharide ($P < 0.05$) (Fig. 5).

Role of CD14 molecules

To exert its effect on target cells, lipopolysaccharide binds with lipopolysaccharide-binding protein and this complex of molecules binds to CD14 molecules on the surface of target cells (17). When RAW264.7 cells were coated with anti-CD14 antibody and then stimulated with *A. actinomycetemcomitans*-lipopolysaccharide, arginase activity was significantly reduced ($P < 0.05$) (Fig. 6), suggesting that *A. actinomycetemcomitans*-lipopolysaccharide might bind to CD14 molecules of RAW264.7 cells, leading to activation of arginase activity.

Role of toll-like receptor 2 and 4

Following lipopolysaccharide-CD14 binding, the CD14 molecules concentrate and present lipopolysaccharide to the toll-like receptor 4–MD2 complex, generating signal transduction (17). Indeed, when toll-like receptor 4 but not toll-like receptor 2 molecules were blocked by their respective antibodies, *A. actinomycetemcomitans*-lipopolysaccharide failed to stimulate arginase activity in RAW264.7 cells ($P < 0.05$) (Fig. 7), indicating that the presence of toll-like receptor 4 molecules is a prerequisite for *A. actinomycetemcomitans*-lipopolysaccharide to induce arginase activity in RAW264.67 cells.

Discussion

Our previous study showed that *A. actinomycetemcomitans*-lipopolysaccharide stimulates the production of nitric oxide by RAW264.7 cells and spleen cells (27, 31). The present study showed that *A. actinomycetemcomitans*-lipopolysaccharide is also able to stimulate arginase activity by these cells. It seems plausible that *A. actinomycetemcomitans*-lipopolysaccharide is capable of concurrently stimulating nitric oxide production and arginase activity by murine macrophages. Support can be drawn from the fact that *E. coli*-lipopolysaccharide activates both inducible nitric oxide synthase and arginase in RAW264.7 cells by different transcriptional mechanisms (32). It should be noted that *A. actinomycetemcomitans*-lipopoly-

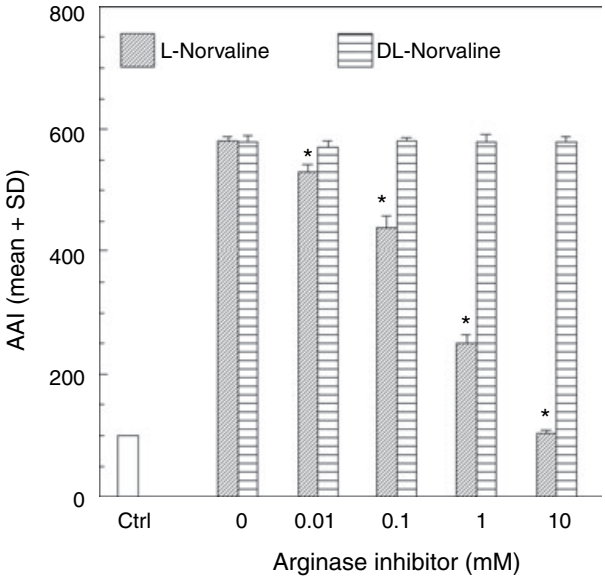

Fig. 3. Effect of L-norvaline or DL-norvaline on arginase activity in *A. actinomycetemcomitans*-lipopolysaccharide-stimulated RAW264.7 cells. The cells were incubated with or without L-norvaline or DL-norvaline for 1 h at room temperature, washed, cultured and stimulated with 10 μg of *A. actinomycetemcomitans*-lipopolysaccharide. *Significant difference at $P < 0.05$ between arginase activity in RAW264.7 cells with and without the presence of L-norvaline or DL-norvaline. AAI, arginase activity index; SD, standard deviation.

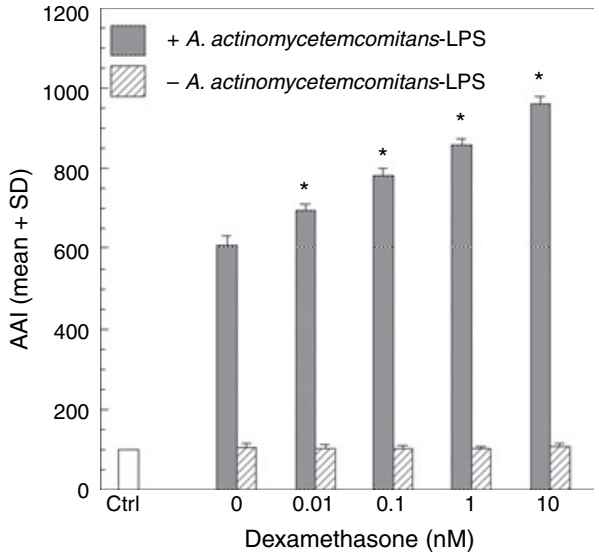

Fig. 4. Effect of dexamethasone on arginase activity in *Actinobacillus actinomycetemcomitans*-lipopolysaccharide-stimulated RAW264.7. *Significant difference at $P < 0.05$ between arginase activity in RAW264.7 cells with and without the presence of dexamethasone. AAI, arginase activity index; SD, standard deviation.

saccharide at any concentration induced significantly less arginase activity than *E. coli*-lipopolysaccharide, indicating that *A. actinomycetemcomitans*-lipopolysaccharide may be less potent than *E. coli*-lipopolysaccharide in inducing arginase activity in murine macrophages.

The present study indicated that polymyxin B abrogated the stimulatory effects of *A. actinomycetemcomitans*-lipopolysaccharide on macrophage-derived arginase activity. Polymyxin B, derived from *Bacillus polymyxa,* binds to the lipid A portion of lipopolysaccharide with 1 : 1 stoichiometry, leading to abolishment of lipopolysaccharide activity (20). Therefore, one may assume that the lipid A portion of *A. actinomycetemcomitans*-lipopolysaccharide may be responsible for murine macrophage activation, leading to arginase activation. However, this notion remains to be investigated further.

Arginase activity in *A. actinomycetemcomitans*-lipopolysaccharide-stimulated RAW264.7 cells was significantly suppressed in the presence of L-norvaline, but not DL-norvaline, in accordance with a previous report that arginase activity in murine macrophage cell line (J744A.1 cells) stimulated with lipopolysaccharide from enteric bacteria was inhibited by L-norvaline (7). The exact mechanism by which L-norvaline inhibited arginase activity seen in the present study is unknown. L-Norvaline might mimic the structure of ornithine and/or inhibit ornithine transcarbamylase, leading to accumulation of ornithine, which in turn might down-regulate arginase (5, 23–25). However, this notion remains to be clarified.

In the present study, arginase activity in *A. actinomycetemcomitans*-lipopolysaccharide-stimulated RAW264.7 cells was augmented by dexamethasone, suggesting that the activity may be up-regulated by glucocorticoid, as seen in our previous study (11, 19). Alternatively, dexamethasone might inhibit the activation of phosphodiesterase 4, augmenting cyclic-AMP levels (1) and hence promoting arginase activity (11, 18, 33).

The results of the present study showing that arginase activity in *A. actinomycetemcomitans*-lipopolysaccharide-stimulated RAW264.7 cells was up-regulated by IL-4 but suppressed by interferon-γ were not unexpected. Interferon-γ is known to up-regulate the expression of inducible nitric oxide synthase without altering arginase expression (11, 19, 32). Indeed, our previous study also indicated that interferon-γ enhances nitric oxide production by *A. actinomycetemcomitans*-lipopolysaccharide-stimulated RAW264.7 and splenic cells (27, 31). There is therefore a strong possibility that endogenous L-arginine is predominantly converted by interferon-γ and *A. actinomycetemcomitans*-lipopolysaccharide-activated inducible nitric oxide synthase to produce nitric oxide. In contrast, IL-4 stimulates arginase activity to catalyze L-arginine to produce urea (9, 21). Our previous data demonstrated that this cytokine suppresses the production of nitric oxide in *A. actinomycetemcomitans*-lipopolysaccharide-stimulated RAW264.7 and splenic cells (27, 31). Furthermore, the expression of arginase I and II is up-regulated by IL-4 and lipopolysaccharide, respectively (19, 21). It seems plausible that the increased levels

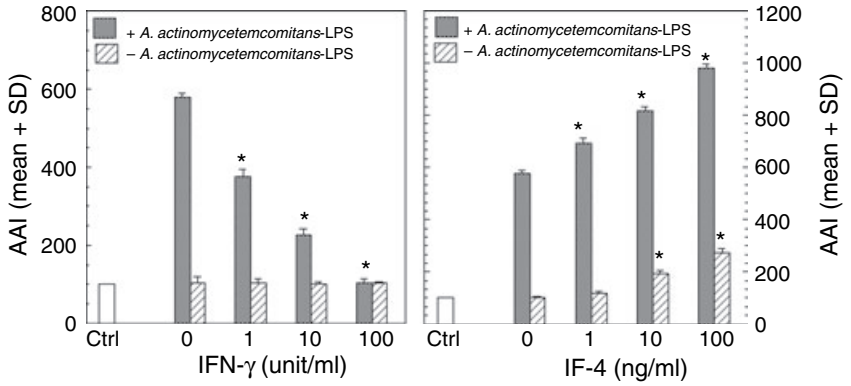

Fig. 5. Effect of interferon-γ and interleukin (IL)-4 on arginase activity in *Actinobacillus actinomycetemcomitans*-lipopolysaccharide-stimulated RAW264.7 cells. *****Significant difference at $P < 0.05$ between arginase activity in RAW264.7 cells with and without the presence of interferon-γ or IL-4 as well as between arginase activity in the cells with IL-4 alone. AAI, arginase activity index; SD, standard deviation.

of arginase activity by both IL-4 and *A. actinomycetemcomitans*-lipopolysaccharide seen in the present study might be the result of increased expression of both arginase I and II. This remains to be further clarified, as the present study did not assess the levels of arginase isoform expression in RAW264.7 cells after stimulation with *A. actinomycetemcomitans*-lipopolysaccharide.

The present study demonstrated that the ability of *A. actinomycetemcomitans*-lipopolysaccharide to stimulate arginase activity in RAW264.7 cells may be dependent on the presence of CD14 and toll-like receptor 4 molecules. It has been shown that the responsiveness of the cells to *A. actinomycetemcomitans*-lipopolysaccharide is mediated by the CD14–toll-like receptor 4 molecule complex (18). It seems plausible that *A. actinomycetemcomitans*-lipopolysaccharide might initially bind to CD14 molecules on RAW264.7 cells, which in turn might present *A. actinomycetemcomitans*-lipopolysaccharide to the toll-like receptor–MD2 complex, thereby inducing signal cascades activating arginase (17). This speculation remains to be investigated further, however.

The extrapolation of the present study to the immunopathogenesis of periodontal disease remains speculative. A study by Güllü and colleagues demonstrated that the levels of gingival arginase activity in periodontally diseased patients were lower than those of gingival inducible nitric oxide synthase expression and this balance of enzyme levels was reversed after periodontal treatments (12). These results are supported by the previous (19) and the present study, which showed that reciprocal levels of nitric oxide and arginase activity occurred due to the regulatory role of IL-4 and interferon-γ on inducible nitric oxide synthase and arginase. Furthermore, expression of inducible nitric oxide synthase and activity of arginase seen in the inflamed gingival tissues (12) indicate that periodontopatic bacteria may stimulate both enzymes in the arginine metabolism during the course of periodontal disease. The possibility that *A. actinomycetemcomitans* may also induce arginase activity in gingival macrophages, determining the course of periodontal disease, can not be ruled out and needs to be elucidated.

In conclusion, the present study showed that arginase activity could be observed in *A. actinomycetemcomitans*-lipopolysaccharide-stimulated RAW264.7 cells and was inhibited by polymyxin B and L-norvaline. Dexamethasone and IL-4 upregulated and interferon-γ suppressed arginase activity in *A. actinomycetemcomitans*-lipopolysaccharide-stimulated RAW-264.7 cells. Pretreatment of RAW264.7 cells with anti-CD14 and anti-toll-like

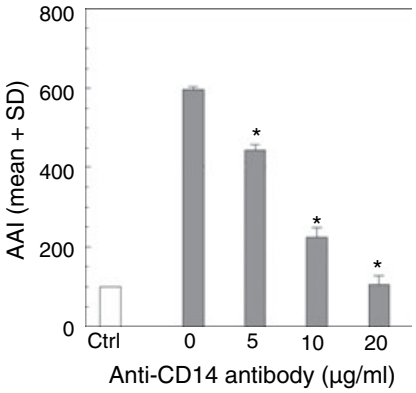

Fig. 6. Effect of CD14 molecules on arginase activity in *Actinobacillus actinomycetemcomitans*-lipopolysaccharide-stimulated RAW264.7 cells. *****Significant difference at $P < 0.05$ between arginase activity in anti-CD14 antibody-treated and -untreated RAW264.7 cells in the presence of *A. actinomycetemcomitan*-lipopolysaccharide. AAI, arginase activity index; SD, standard deviation.

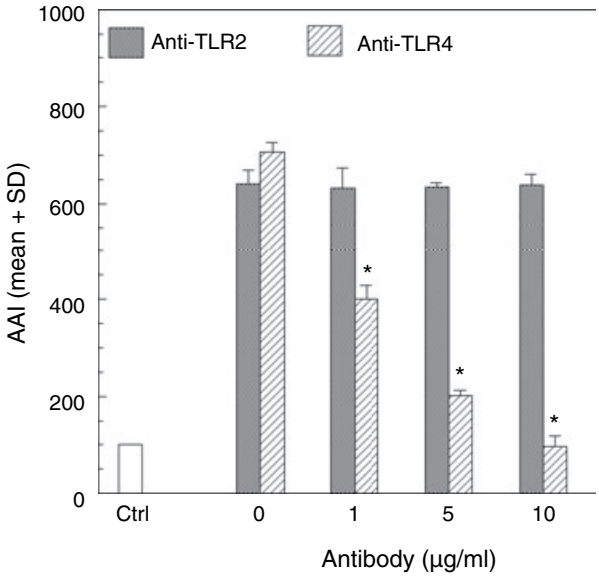

Fig. 7. The role of toll-like receptor 2 and toll-like receptor 4 molecules on arginase activity in *A. actinomycetemcomitans*-lipopolysaccharide-stimulated RAW264.7 cells. *****Significant difference at $P < 0.05$ between arginase activity in anti-toll-like receptor antibody-treated and -untreated RAW264.7 cells in the presence of *A. actinomycetemcomitan*-lipopolysaccharide. AAI, arginase activity index; SD, standard deviation.

receptor 4 but not anti-toll-like receptor 2 antibody suppressed arginase activity in these cells after stimulation with *A. actinomycetemcomitans*-lipopolysaccharide. The results suggest that arginase activity in *A. actinomycetemcomitans*-lipopolysaccharide-stimulated RAW264.7 cells may be dependent on the CD14–toll-like receptor 4 molecule complex and may be regulated by Th1 and Th2 cytokines and glucocorticoid.

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References

1. Ahlstrom M, Pekkinen M, Huttunen M, Lamberg-Allardt C. Dexamethasone down-regulates cAMP-phosphodiesterase in human osteosarcoma cells. Biochem Pharmacol 2005: **69**: 267–275.
2. Bansal V, Ochoa JB. Arginine availability, arginase and the immune response. Curr Opin Clin Nutr Metab Care 2003: **6**: 223–228.
3. Barksdale AR, Bernard AC, Maley ME, Gellin GL, Kearney PA, Boulanger BR, Tsuei BJ, Ochoa JB. Regulation of arginase expression by T-helper II cytokines and isoproterenol. Surgery 2004: **135**: 527–535.
4. Blix IJS, Helgeland K. LPS from *Actinobacillus actinomycetemcomitans* and production of nitric oxide in murine macrophages J774. Eur J Oral Sci 1998: **106**: 576–581.
5. Bode AM, Foster JD, Nordlie RC. Glycogenesis from glucose and ureagenesis in isolated perfused rat livers. J Biol Chem 1994: **269**: 7879–7886.
6. Cederbaum SDYuH, Grody WW, Kern RM, Yoo P, Iyer RK. Arginase I and II. do their function overlap? Mol Genet Metab 2004: **81**: S23–S44.
7. Chang C-I, Liao JC, Kuo L. Arginase modulates nitric oxide production in activated macrophages. Am J Physiol (Heart Circ Physiol) 1998: **43**: H342–H348.
8. Chang C-I, Liao JC, Kuo L. Macrophage arginase promotes tumor cell growth and suppress nitric oxide-mediated tumor cytotoxicity. Cancer Res 2001: **61**: 1100–1106.
9. Corraliza IM, Soler G, Eichmann K, Modollel M. Arginase induction by suppressor of nitric oxide synthesis (IL-4, IL-10 and PGE_2) in murine bone marrow-derived macrophages. Biochem Biophys Res Commun 1995: **206**: 667–673.
10. Gotoh M, Mori M. Arginase II down regulates nitric oxide (NO) production and prevents NO-mediated apoptosis in murine macrophage-derived RAW 264.7 cells. J Cell Biol 1999: **144**: 427–434.
11. Gotoh T, Sonoki T, Nagasaki A, Terada K, Takiguchi M, Mori M. Molecular cloning of cDNA for nonhepatic mitochondrial arginase (arginase II) and comparison of its induction with nitric oxide synthase in a murine macrophage-like cell line. FEBS Lett 1996: **395**: 119–122.
12. Güllü C, Ozmeric N, Tokman B, Elgün S, Balos K. Effectiveness of scaling and root planning versus modified Widman flap on nitric oxide synthase in patients with chronic periodontitis. J Periodontal Res 2005: **40**: 168–175.
13. Herminajeng E, Asmara W, Yuswanto A, Barid I, Sosroseno W. Protective humoral immunity induced by surface associated molecule from *Actinobacillus actinomycetemcomitans* in mice. Microbes Infect 2001: **3**: 997–1003.
14. Hrabak A, Bajor T, Temesi A, Meszaros G. The inhibitory effect of nitrite, a stable product of nitric oxide (NO) formation on arginase. FEBS Lett 1996: **360**: 203–206.
15. Kim S-J, Ha M-S, Choi E-Y, Choi J-II, Choi I-S. *Prevotella intermedia* lypopolysaccharide stimulates release of nitric oxideby inducing expression of inducible nitric oxide synthase. J Periodontal Res 2004: **39**: 424–431.
16. Meyer DH, Fives-Taylor PM. The role of *Actinobacillus actinomycetemcomitans* in the pathogenesis of periodontal disease. Trends Microbiol 1997: **5**: 224–228.
17. Miyake K. Innate recognition of lipopolysaccharide by CD14 and toll-like receptor 4-MD-2; unique roles for MD 2. Int Immunopharmacol 2003: **3**: 119–128.
18. Mochizuki S, Kobayashi M, Suzuki T, Oikawa A, Koseki T, Nishihara T, Hasegawa K. γ-Interferon enhances expression of CD14/MyD88 and subsequent responsiveness to lipopolysaccharide from *Actinobacillus actinomycetemcomitans* in human gingival fibroblasts. J Periodontal Res 2004: **39**: 333–343.
19. Morris SM Jr, Kepka-Lenhart D, Chen L-C. Differential regulation of arginases and inducible nitric oxide synthase in murine macrophage cells. Am J Physiol 1998: **275**: E740–E747.
20. Morrison DC, Jacobs DM. Binding of polymyxin B to lipid A portion of bacterial lipopolysaccharide. Immunochemistry 1976: **13**: 813–818.
21. Munder M, Eichmann K, Morán JM, Centeno F, Soler G, Modolell M. Th1/Th2-regulated expression of arginase isoforms in murine macrophages and dendritic cells. J Immunol 1999: **163**: 3771–3777.
22. Nishida E, Hara Y, Kaneko T, Ikeda Y, Ukai T, Kato I. Bone resorption and local interleukin-1γ and interleukin-1β synthesis induced by *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* lipopolysaccharide. J Periodontal Res 2001: **36**: 1–8.
23. Reczkowski RS, Ash DE. Rat liver arginase: kinetic mechanism, alternate substrates, and inhibitors. Arch Biochem Biophys 1994: **312**: 31–37.
24. Rognstad R. Sources of ammonia for urea synthesis in isolated rat liver cells. Biochim Biophys Acta 1977: **496**: 249–254.
25. Saheki T, Sato Y, Takada S, Katsunuma T. Regulation of urea synthesis in rat liver-inhibition of urea synthesis by L-norvaline. J Biochem 1979: **86**: 745–0750.
26. Shearer JD, Richards JR, Mills CD, Caldwell MD. Differential regulation of macrophage arginine metabolism: a proposed role in wound healing. Am J Physiol 1997: **272**: E181–E190.
27. Sosroseno W, Barid I, Herminajeng E, Susilowati H. Nitric oxide production by a murine macrophage cell line (RAW264.7) induced by lipopolysaccharide from *Actinobacillus actinomycetemcomitans*. Oral Microbiol Immunol 2002: **17**: 72–78.
28. Sosroseno W, Herminajeng E. The immunopathology of chronic inflammatory periodontal disease. FEMS Immunol Med Microbiol 1995: **10**: 171–180.
29. Sosroseno W, Herminajeng E. The role of macrophages in the induction of murine immune response to *Actinobacillus actinomycetemcomitans*. J Med Microbiol 2002: **51**: 581–588.
30. Sosroseno W, Herminajeng E, Bird PS, Seymour GJ. L-arginine-dependent nitric oxide production of murine macrophage-like RAW264.7 cell line stimulated with *Porphyromonas gingivalis* lipopolysaccharide. Oral Microbiol Immunol 2004: **19**: 65–70.
31. Sosroseno W, Herminajeng E, Budiarti S, Susilowati H. Nitric oxide production by murine spleen cells stimulated with lipopolysaccharide from *Actinobacillus actinomycetemcomitans*. Anaerobe 2002: **8**: 333–339.
32. Wang WW, Jenkinson CP, Griscavage JM, Kern RM, Arabolos NS, Nyrns RE, Cederbaum SD, Ignarro LJ. Co-induction of arginase and nitric oxide synthase in murine macrophages activated by lipopolysaccharide. Biochem Biophys Res Commun 1995: **210**: 1009–1015.
33. Wei LH, Morris SM, Cederbaum SD, Mori M, Ignarro LJ. Induction of arginase II in human Caco-2 tumor cells by cyclic AMP. Arch Biochem Biophys 2000: **15**: 225–260.
34. Yamaguchi N, Yamashita Y, Ikeda D, Koga T. *Actinobacillus actinomycetemcomitans* serotype b-specific polysaccharide antigen stimulates production of chemotatic factors and in inflammatory cytokines by human monocytes. Infect Immun 1996: **64**: 2563–2570.