J Periodont Res 2009; 44: 543–549

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2008.01149.x

Porphyromonas gingivalis fimbriae induce unique dendritic cell subsets via Toll-like receptor 2

S. Kanaya[1], E. Nemoto[1], T. Ogawa[2], H. Shimauchi[1]

[1]Division of Periodontology and Endodontology, Tohoku University Graduate School of Dentistry, Sendai, Japan and [2]Department of Oral Microbiology, Asahi University School of Dentistry, Gifu, Japan

Kanaya S, Nemoto E, Ogawa T, Shimauchi H. Porphyromonas gingivalis *fimbriae induce unique dendritic cell subsets via Toll-like receptor 2. J Periodont Res 2009; 44: 543–549. © 2008 The Authors. Journal compilation © 2008 Blackwell Munksgaard*

Backgound and Objective: Dendritic cells (DCs) play a critical role in the activation of T cells as well as in shaping immune responses. We have reported previously that *Porphyromonas gingivalis* lipopolysaccharides (*Pg* LPS) induced a $CD14^+CD16^+$ DC subset with a weak immuno-stimulatory activity. In contrast, *Escherichia coli* LPS (*Ec* LPS) induced fully matured DCs with strong immuno-stimulatory activities. Since *Pg* LPS as well as *Pg* fimbriae have been indicated to work as Toll-like receptor (TLR) 2 ligands, we speculate that the TLR usage of bacterial antigens may be critical for DC maturation.

Material and Methods: We investigated the effect of *Pg* fimbriae on the phenotype and function of human peripheral blood DCs in comparison with a TLR2 ligand, peptidoglycan, and a TLR4 ligand, *Ec* LPS.

Results: Flow cytometry revealed that *Pg* fimbriae and peptidoglycan but not *Ec* LPS induced CD14 and CD16 expression on peripheral blood DCs ($CD14^-CD16^-$). A monoclonal antibody against TLR2 abrogated this induction, but an antibody against TLR4 had no effect. Dendritic cells stimulated with *Pg* fimbriae had a weaker capability to induce allogenic T cell proliferation and exhibited a weaker production of interleukin-8 and regulated upon activation, normal T cell expressed and secreted (RANTES) than DCs stimulated with *Ec* LPS.

Conclusion: These results indicate that different TLR usage affects mature DC phenotype and function and is thus crucial to the regulation of immunity to the pathogen.

Hidetoshi Shimauchi, DDS, PhD, Division of Periodontology and Endodontology, Tohoku University Graduate School of Dentistry, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan
Tel: +81 22 717 8333
Fax: +81 22 717 8339
e-mail: simauti@mail.tains.tohoku.ac.jp

Key words: Toll-like receptor 2; human dendritic cell; *Porphyromonas gingivalis*; fimbria

Accepted for publication June 20, 2008

Porphyromonas gingivalis, one of the major pathogenic organisms in periodontitis (1), is known to possess a panel of virulence factors. Among these factors, fimbriae (2) and lipopolysaccharides (LPS) have broad and unique biological activities on various host tissue cells, resulting in the provocation of inflammatory immune responses as well as tissue destruction. It is well recognized that *P. gingivalis* LPS (*Pg* LPS) and fimbriae-specific antibody levels are elevated in periodontitis patients (3). Furthermore, previous reports revealed that *Pg* LPS and fimbriae-specific antibody-secreting cells were detected in the inflamed gingival tissues of patients (4), indicating that acquired immune responses are generated against LPS and fimbriae during the process of *P. gingivalis* infection in periodontal tissues. Although the magnitude of immune responses, e.g. specific antibody levels, increased in proportion to disease severity, the bodies of infected patients seemed to fail to clear the invading

P. gingivalis infection from the periodontal lesion, leading to low-level bacteraemia (5). In this respect, a new concept has emerged that certain periodontal pathogens, including *P. gingivalis*, practice a component of so-called 'stealth technology' to gain access to an immune privileged site using these molecular tools (6).

Dendritic cells (DCs) are distributed widely in both lymphoid (7) and non-lymphoid organs, including periodontal tissues (8). The generation of the primary immune response depends on the efficient activation of naïve T cells by DCs. After capturing an antigen in their immature state, DCs undergo maturation and express a panel of cytokines to stimulate primary T cell responses in the lymphoid organs and antigen-specific secondary responses at inflammatory sites (9,10). The initial recognition of pathogens is mediated by Toll-like receptors (TLRs). Mammalian TLRs are a large family, consisting of 13 members known to date. Among these receptors, TLR4 plays an important role as a receptor of bacterial LPS and its active centre, lipid A. Toll-like receptor 2 recognizes a variety of microbial components, including lipoproteins/lipopeptides, peptidoglycan (PGN), lipoteichoic acid, lipoarabinomannan and zymosan (11,12).

It has been reported that *P. gingivalis* stimulated the maturation of DCs and led to the release of counter-regulatory cytokines and the formation of T cell–DC foci in the gingival mucosa of periodontitis patients (13). We have reported previously that *Pg* LPS preferentially induced a unique DC subset with the $CD14^+CD16^+$ phenotype and weak immuno-stimulatory activities *in vitro* (14). In contrast, *Escherichia coli* LPS (*Ec* LPS) induced fully matured DCs with strong immuno-stimulatory activity. Previous reports suggested that *Pg* LPS as well as fimbriae works as a TLR2 ligand (15–19). These studies led us to speculate that TLR usage of bacterial antigens may be critical for DC maturation. To confirm this hypothesis, we examined: (1) TLR usage of *Pg* fimbriae in human peripheral blood DCs (PBDCs); and (2) the effects of *Pg* fimbriae on DC maturation in comparison with specific ligands for TLR2 and TLR4 (PGN and *Ec* LPS, respectively).

Material and methods

Media and reagents

The culture medium consisted of RPMI-1640 (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 2 mM L-glutamine, 10 mM $NaHCO_3$, 25 μg/mL penicillin (Gibco BRL, Tokyo, Japan) and 5% (v/v) heat-inactivated fetal calf serum (FCS; ICN Biomedical, Costa Mesa, CA, USA). Lympho Separation Medium was purchased from ICN Biomedical. Recombinant human (rh) interleukin-4 (IL-4) and granulocyte macrophage colony stimulating factor (GM-CSF) were obtained from Pepro Tech (London, UK). Bovine serum albumin, mitomycin C, LPS derived from *E. coli* O55:B5 (*Ec* LPS), PGN from *Staphylococcus aureus*, and propidium iodide were from Sigma (St Louis, MO, USA). Fluorescein isothiocyanate (FITC)-conjugated anti-CD14 (clone: MY4), phycoerythrin (PE)-conjugated anti-CD16 (clone: 3G8), anti-CD1a (clone: BL6) and anti-HLA-DR (clone: B8.12.2) were from Immunotech, a Beckman Coulter Company (Marseille, France). Anti-CD80 (B7-1, clone: L307.4), anti-CD86 (B70/B7-2; clone: 2331 (FUN-1)), and anti-CD83 (clone: HB15e) were obtained from BD PharMingen (Hamburg, Germany). We used neutralizing antibodies for TLR2 (clone: T2.5; eBioscience, San Diego, CA, USA) and TLR2 (clone: TL2.1; CASCADE BioScience, Winchester, MA, USA), and a neutralizing antibody for TLR4 was purchased from MBL (Nagoya, Japan). An immunoglobulin G1 isotype control antibody was purchased from R&D Systems (Minneapolis, MN, USA), and an immunoglobulin G2a isotype control was obtained from CHEMICON (Temecula, CA, USA).

Preparation of *Pg* fimbriae

Porphyromonas gingivalis fimbriae were purified as described previously (20). Briefly, *P. gingivalis* strain 381 cells were grown in Gifu anaerobic medium (Nissui Pharmaceutical Co.) at 37°C for 24 h. Bacterial cells were collected by centrifugation at 10,000 ***g*** for 30 min and resuspended in 20 mM Tris-buffered saline (pH 7.4). Fimbriae were separated from the cells by gentle pipetting followed by gentle agitation with a magnetic stirrer bar for 15 min. The supernatant obtained by centrifugation at 10,000 ***g*** for 30 min was subjected to ammonium sulphate precipitation at 40% saturation. The precipitate was then purified by DEAE-Sepharose FAST Flow® (Pharmacia, Uppsala, Sweden). The fractions containing the fimbrial protein were concentrated by ammonium sulphate precipitation followed by dialysis against 10 L of 20 mM Tris-hydrochloride buffer (pH 8.0). Endotoxicity of the fimbriae was not detected by a colorimetric *Limulus* amoebocyte lysate assay (Endospecy®, Seikagaku Co., Tokyo, Japan).

Generation, purification and stimulation of DCs

Immature DCs were prepared from human peripheral blood mononuclear cells (PBMCs) as described previously (14). Briefly, PBMCs were prepared from healthy donors, after receiving written informed consent, using Lympho Separation Medium® (ICN Biomedical, Costa Mesa, CA, USA). Monocytes were separated from PBMCs by adherence to six-well plastic plates in 5% FCS RPMI-1640 for 2 h. Adherent cells were cultured for 7 days in 5% FCS RPMI-1640 with rhIL-4 (500 U/mL) and rhGM-CSF (800 U/mL). $CD14^+$ cells were removed by magnetic sorting using the VarioMACS® technique (Milteny Biotec GmbH, Bergisch Gladbach, Germany) with CD14 (MY4) monoclonal antibody (mAb). Purified immature DCs were stimulated with the indicated stimulants in 5% FCS RPMI-1640 in 24-well (2.0×10^5 cells/well), 48-well (8.0×10^4 cells/well) or 96-well plates (3.0×10^4 cells/well) for the indicated times. The experimental procedures were approved by the Ethical Review Board of Tohoku University Graduate School of Dentistry (Sendai, Japan) and comply with the declaration of Helsinki.

Flow cytometry (FACS)

Dendritic cells (5×10^4 cells) were stained with FITC-conjugated or PE-conjugated mAbs at 4°C for 15 min. The FACS analyses were performed using a FACScan® (Becton Dickinson, Mountain View, CA, USA) and using propidium iodide to exclude dead cells. Data were collected for 10 000 events, which were sorted in list mode and then analysed using Lysis II software (Becton Dickinson).

Allogenic T cell proliferation assay

T cells were separated by magnetic sorting using the VarioMACS Separator® (Milteny Biotech GmbH). Briefly, PBMCs were incubated with a cocktail of hapten-conjugated anti-CD11b (rat IgG2b), CD16 (mouse IgM), CD19 (mouse IgG1), CD36 (mouse IgG2a) and CD56 (mouse IgG1) mAbs for 15 min on ice and washed in phosphate-buffered saline containing 2 mM EDTA and 0.5% (w/v) bovine serum albumin. Then, cells were incubated with anti-hapten microbeads (Milteny Biotech GmbH) for 15 min on ice, and applied to the 25 MS column®. Cells were eluted with washing buffer from the magnetic columns. These cells were found to be > 99% CD3 positive by FACS analysis. Dendritic cells were treated with mitomycin C (50 μg/mL) for 45 min at 37°C, washed extensively, and cocultured for 5 days with T cells (50 000 cells/well) at a ratio of DC:T cells of 1:50 in RPMI-1640 containing 5% FCS in round-bottomed 96-well multiplates. Fifteen hours before the end of culture, [^{3}H] thymidine (1 μCi/well) (Amersham Biosciences Co., Piscataway, NJ, USA) was added to each well. The cells were harvested onto a glass fibre filter, and the radioactivity was counted using a liquid scintillation β-counter (Hewlett Packard, Palo Alto, CA, USA).

Enzyme-linked immunosorbent assay (ELISA)

Supernatants from cell 24 h cultures in 48-well multiplates were harvested. Interleukin-8 (IL-8) and regulated upon activation, normal T cell expressed and secreted (RANTES) were measured using human ELISA kits (Endogen, Woburn, MA, USA). The assays were performed precisely as instructed by the ELISA manufacturer. The concentrations of chemokines in the supernatants were determined using the Softmax data analysis program (Molecular Devices, Menlo Park, CA, USA). Each sample was assayed in triplicate.

Statistical analysis

All experiments in this study were performed at least three times to test the reproducibility of the results, and representative findings are shown. The statistical significance of differences between two means was evaluated by ANOVA ($p < 0.05$).

Results

Induction of CD14 and CD16 expression in *Pg* fimbriae-stimulated DCs via TLR2

We analysed CD14 and CD16 expression levels in DCs after stimulation with *Pg* fimbriae, PGN or *Ec* LPS for 2 days. Purified immature DCs (fresh control) were negative for CD14 and CD16 expression. *Porphyromonas gingivalis* fimbriae and PGN induced $CD14^+$ $CD16^-$ and $CD14^+$ $CD16^+$ expression in DCs (*Pg* fimbriae, 26.49 and 17.82%; PGN, 18.01 and 16.58%, respectively), whereas *Ec* LPS did not induce CD14 or CD16 expression (Fig. 1A). Induction of CD14 and CD16 by *Pg* fimbriae and PGN was inhibited completely by pretreatment with anti-TLR2 mAb. However, pretreatment with anti-TLR4 mAb or isotype control antibody had no inhibitory effects (Fig. 1B). These findings indicated that *Pg* fimbriae and PGN induced $CD14^+$ $CD16^-$ and $CD14^+$ $CD16^+$ DC phenotypes via the TLR2 signalling pathway.

Surface molecule expression in stimulated DCs

We have reported previously that the *Pg* LPS-induced $CD14^+$ $CD16^+$ subset belongs to a DC lineage with a weak expression of co-stimulatory molecules (14). To examine whether *Pg* fimbriae-stimulated DCs belong to the monocyte/macrophage or DC lineage, we analysed the expression of cell surface molecules for the DC lineage. As shown in Fig. 2, significant expression of CD1a on the surface of *Pg* fimbriae- and PGN-stimulated DCs was observed, although some population of cells reduce the expression, suggesting that these cells belong to the DC lineage. The mature DC marker CD83 was induced weakly by *Pg* fimbriae or PGN, unlike *Ec* LPS, which induced CD83 significantly, suggesting that *Pg* fimbriae and PGN induced minimal DC maturation. We examined the expression of molecules (HLA-DR, CD80 and CD86) that affect the antigen presentation ability of the cells. *Porphyromonas gingivalis* fimbriae and PGN induced CD80 expression as potently as *Ec* LPS; however, weaker expression of HLA-DR and CD86 was observed compared with *Ec* LPS (Fig. 2).

Induction of T cell proliferation by *Pg* fimbriae-stimulated DCs

To investigate the ability of *Pg* fimbriae-stimulated DCs to induce T cell responses, *Pg* fimbriae-, PGN- and *Ec* LPS-stimulated DCs were cocultured with allogenic T lymphocytes at a ratio of DC:T cells of 1:50. As shown in Fig. 3, DCs stimulated with *Pg* fimbriae or PGN induced higher T cell proliferation levels in a dose-dependent manner, but significantly weaker ($p < 0.05$) levels than the response induced by *Ec* LPS. These results suggested a limited capability of *Pg* fimbriae- and PGN-stimulated DCs in terms of the induction of T cell responses.

Chemokine production via TLR2

We next investigated the possible induction of chemokine production by *Pg* fimbriae. Figure 4A shows that IL-8 and RANTES were induced significantly at 1 and 10 μg/mL *Pg* fimbriae, respectively. Dendritic cells stimulated with PGN and *Ec* LPS released much

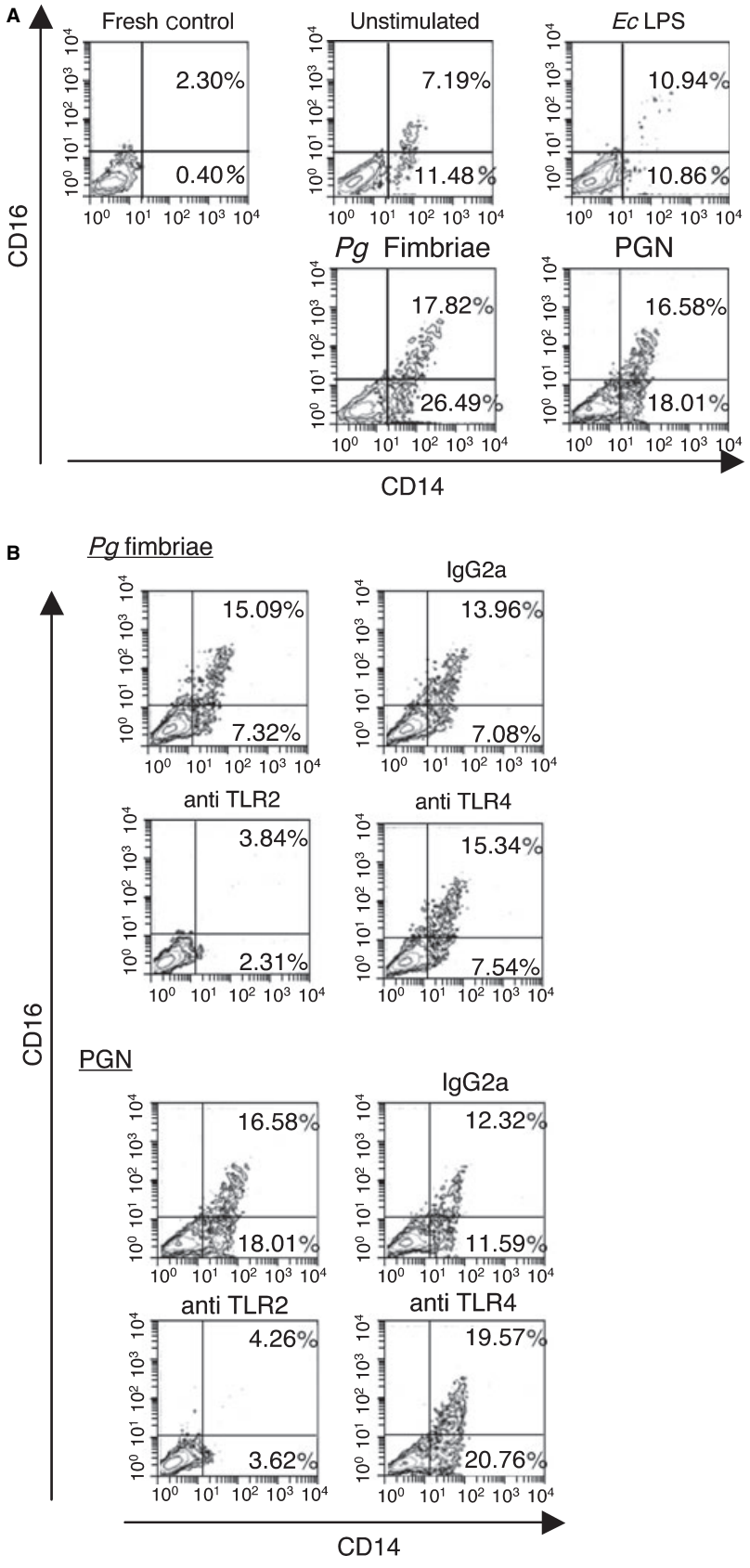

Fig. 1. Expression of CD14 and CD16 molecules on *Pg* fimbriae-, PGN- and *Ec* LPS-stimulated DCs. Immature DCs (fresh control) were prepared by culturing peripheral blood monocytes with GM-CSF and IL-4 for 7 days. (A) After 2 days stimulation with/without 1 μg/mL of the indicated bacterial components, cells were stained using FITC-conjugated anti-CD14 (MY4) and PE-conjugated anti-CD16 (3G8). Dot plot analyses show representatives of four independent experiments. (B) Immature DCs were pretreated with 20 μg/mL anti-TLR2 or anti-TLR4 mAb, as well as 20 μg/mL of control antibodies for 30 min, followed by stimulation with 1 μg/mL *Pg* fimbriae or PGN for 2 days. Expression of CD14 and CD16 on DCs was analysed using FACScan®. Data are representative of three independent experiments.

higher levels of IL-8 and RANTES than *Pg* fimbriae-simulated DCs. Concentrations of 0.01–0.1 μg/mL of PGN and *Ec* LPS were sufficient to induce these chemokines (Fig. 4A). Interleukin-8 production from *Pg* fimbriae- and PGN-stimulated DCs, but not *Ec* LPS-stimulated DCs, was impaired by pretreatment with anti-TLR2 mAb (Fig. 4B), indicating that the production of IL-8 was mediated via TLR2.

Discussion

In human chronic periodontal lesions, abundant infiltration of immune cells, including antigen-presenting cells and T cells, is a prominent feature, indicating the strong induction of antigen-specific immune responses (21). Cutler *et al.* (8) reported that *P. gingivalis* gained entry into immature dendritic cells *in situ* in the diseased gingiva, suggesting that the interaction of immature DCs with *P. gingivalis* and its components is likely to occur in human periodontal lesions.

It has been established that the engagement of TLRs on the surface of DCs by pathogens is critical for skewing responses towards T-helper (Th) 1 and Th2 (22), and that TLR4 leads to the production Th1-associated IL-12, whereas TLR2 promotes Th2-biased responses (23). *Porphyromonas gingivalis* LPS has been reported to stimulate TLR2 rather than TLR4 (15,16),

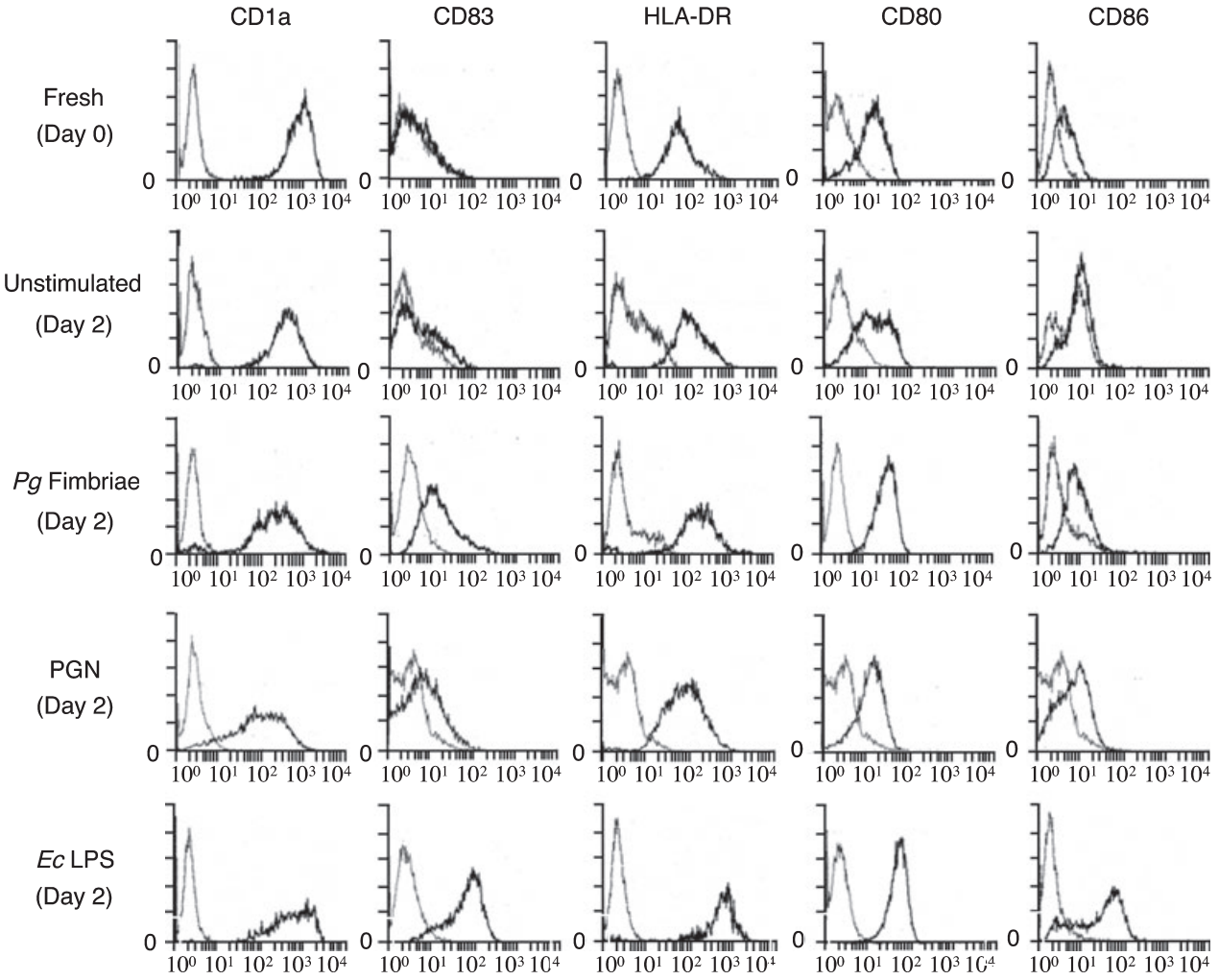

Fig. 2. Pg fimbriae-, PGN- and *Ec* LPS-stimulated DCs. Immature DCs (fresh control) were stimulated with/without 1 μg/mL of the indicated bacterial components for 2 days. The expression levels of CD1a, CD83, HLA-DR, CD80 (B7-1) and CD86 (B7-2) were analysed using FACScan®. Control immunoglobulin G of the same isotype was used as a negative control (thin line). Data are representative of four independent experiments.

in a similar manner to fimbriae (18,19) and other components, such as lipoprotein (24). A recent review paper (25) suggested that the triggering of TLR2 by pathogens induced an immunosuppression status through IL-10 induction, blockade of TLR recognition and TLR-mediated induction of viral replication that serves as an escape mechanism from host defense. These reports led us to hypothesize that *P. gingivalis* mainly triggers TLR2 on DCs, which contributes to Th2-biased responses and immune evasion by this bacterium in periodontal lesions.

In this study, we showed that *Pg* fimbriae and PGN, but not *Ec* LPS, induced upregulation of CD14 and CD16 in DCs. Furthermore, an anti-TLR2 antibody, but not an anti-TLR4 antibody, abrogated the upregulation of CD14 and CD16. Therefore, our findings strongly suggest that: (1) immature DCs recognize fimbriae via TLR2 as well as PGN; and (2) signalling after the ligation of TLR2, but not TLR4, leads to a unique subset of $CD14^+$ $CD16^+$ DCs. Toll-like receptor 2 forms a heterodimer not only with TLR6 but also with TLR1 to recognize a variety of pathogen-associated molecular patterns (26). *Porphyromonas gingivalis* LPS and recombinant fimbrillin required heterotypic receptor complexes of TLR1/TLR2/CD14/CD11b/CD18 for cell activation; in contrast, wild-type fimbriae additionally required TLR6 in the complex (16). However, these results were obtained by transient transfection of these molecules; therefore, further studies are necessary to examine the requirement for other TLRs for the recognition of *Pg* LPS/fimbriae and induction of the $CD14^+CD16^+$ DC subset.

The fimbriae-induced $CD14^+$ $CD16^+$ DC subset showed weak expression of CD83, a marker of mature DCs, suggesting the impairment of DC maturation. The antigen presenting and co-stimulatory activities of fimbriae-activated DCs seemed to be almost equal to those of *Ec* LPS-activated DCs, as indicated by HLA-DR and CD80 expression levels. Furthermore, the present results also revealed the limited capability of *Pg*

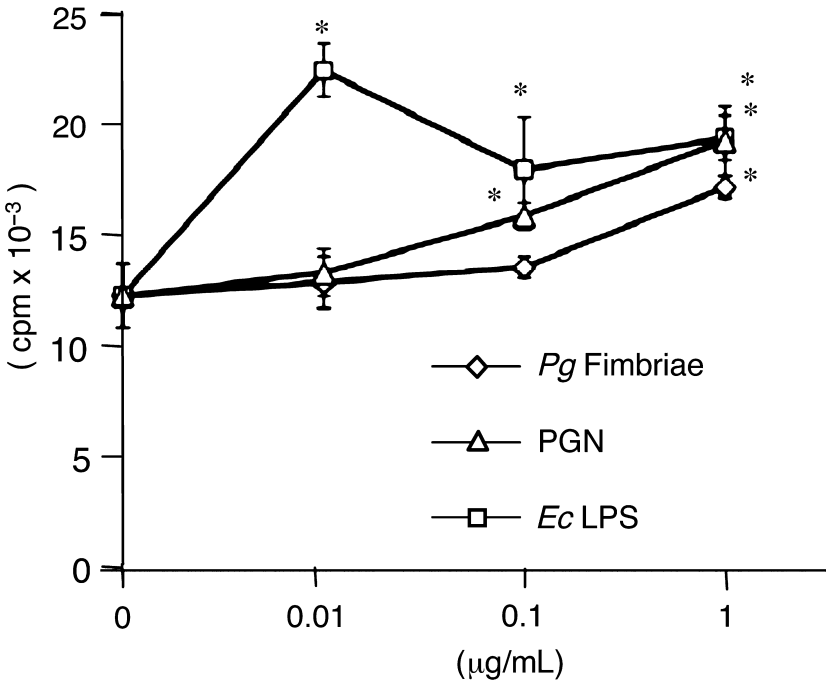

Fig. 3. Allogenic T cell proliferative response induced by *Pg* fimbriae-, PGN- and *Ec* LPS-stimulated DCs. The T cells were separated from PBMCs by magnetic sorting as described in the Material and methods. Dendritic cells stimulated with 0.01–1 μg/mL of *Pg* fimbriae, PGN or *Ec* LPS for 24 h were treated with mitomycin C followed by coculturing for 5 days with T cells (50 000 cells/well) in a ratio of DC:T cells of 1:50. Fifteen hours before culture termination, [^{3}H] thymidine (1 μCi/well) was added, and the level of radioactivity was counted using a liquid scintillation β-counter. Representative data of three separate experiments are shown as means (c.p.m.) ± SE of triplicate assays. Statistical significance is shown (*$p < 0.05$ vs. control).

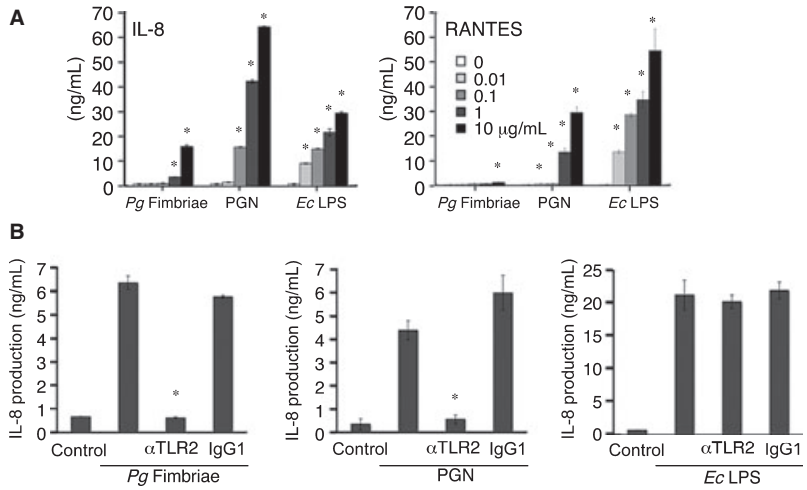

Fig. 4. Chemokine production of *Pg* fimbriae-, PGN- and *Ec* LPS-stimulated DCs. (A) Immature DCs were stimulated with 0.01–10 μg/mL of *Pg* fimbriae, PGN or *Ec* LPS for 24 h and the supernatants were harvested. The levels of IL-8 and RANTES were measured by ELISA. (B) After pre-incubation with 0.5 μg/mL of anti-TLR2 mAb (αTLR2), immature DCs were stimulated with 10 μg/mL *Pg* fimbriae, 100 ng/mL PGN or 100 ng/mL *Ec* LPS for 24 h and supernatants were harvested. The level of IL-8 was measured by ELISA. Representative data of three separate experiments are shown as means ± SE of triplicate assays. Statistical significance is shown (*$p < 0.05$ vs. control).

fimbriae- and PGN-stimulated DCs in the induction of allogenic T cell responses. These patterns of surface molecule expression and allo-T cell stimulatory activity are almost identical to the *Pg* LPS-induced DC subset that we have reported previously (14). Weaker induction of CD86 by *Pg* fimbriae/LPS than by *Ec* LPS in DCs may indicate differential regulation of CD80 and CD86 in the maturation of DCs. Dilioglou *et al*. (27) have shown that CD80 blockage has a weaker effect on naïve T cell activation by monocyte-derived DCs compared with CD86 blockage, which may suggest a weak T cell stimulation by fimbriae-stimulated DCs.

The discrepancy between antigen-presenting and *in vitro* T cell stimulatory activity may be partly due to the weaker expression of co-stimulatory molecules and due to weak chemokine production upon stimulation with *Pg* fimbriae and PGN, since these cytokines are important in regulating the capacity of DCs to initiate immune responses (28). We have shown here that fimbriae could induce IL-8 and RANTES only at higher doses (1 and 10 μg/mL, respectively). In contrast, DCs stimulated with PGN and *Ec* LPS released much higher levels of IL-8 and RANTES at lower doses (0.01–0.1 μg/mL), suggesting that *Pg* fimbriae are a weaker inducer of these chemokines, similar to *Pg* LPS. An anti-TLR2 antibody almost completely abrogated IL-8 and RANTES production from both *Pg* fimbriae- and PGN-stimulated DCs but not from *Ec* LPS-stimulated DC, suggesting that *Pg* fimbriae and PGN induce these chemokines via TLR2. Differential induction of production of these chemokines by *Pg* fimbriae and PGN was observed. Zhou *et al*. (15) investigated cytokine profiles of mouse macrophages exposed to live *P. gingivalis* or *Pg* LPS/fimbriae, and reported that *Pg* LPS/fimbriae induced most cytokines only via TLR2, with the exception of some chemokines, including RANTES. These chemokines were produced by both TLR2 and TLR4 triggering, and only the TLR2 pathway was completely inhibited by live *P. gingivalis*. Although the precise mechanism is still unknown, these

findings, including those of the present study, suggest that host cells sense *P. gingivalis* and its components differently via TLRs. Furthermore, it is possible that unique biological characteristics of *P. gingivalis* components may affect the signalling after TLR recognition by the host immune system.

In this study, we clearly showed that *Pg* fimbriae induced $CD14^+$ $CD16^+$ DCs via TLR2 signalling. The weak immunostimulatory activity of this DC subset may contribute to the escape of *P. gingivalis* from the immune system. However, further studies are necessary to elucidate the pathophysiological role of this subset in the development of periodontal diseases.

Acknowledgements

This work was supported by Grant-in-Aid for Exploratory Research (19659546) and Grant-in-Aid for Young Scientists (18791583 and 20791605) from the Japan Society for the Promotion of Sciences. We thank D. Mrozek (Medical English Service, Kyoto, Japan) for reviewing this manuscript.

References

1. Lamont RJ, Jenkinson HF. Life below the gum line: pathogenic mechanisms of *Porphyromonas gingivalis*. *Microbiol Mol Biol* 1998;**62:**1244–1263.
2. Yoshimura F, Takahashi K, Nodasaka Y *et al*. Purification and characterization of a novel type of fimbriae from the oral anaerobe bacteroides gingivalis. *J Bacteriol* 1984;**160:**949–957.
3. Ogawa T, Kusumoto Y, Hamada S *et al*. Bacteroides gingivalis-specific serum IgG and IgA subclass antibodies in periodontal diseases. *Clin Exp Immunol* 1990;**82:**318–325.
4. Ogawa T, Kono Y, McGhee ML *et al*. *Porphyromonas gingivalis*-specific serum IgG and IgA antibodies originate from immunoglobulin-secreting cells in inflamed gingiva. *Clin Exp Immunol* 1991;**83:**237–244.
5. Gibson FC III, Yumoto H, Takahashi Y *et al*. Innate immune signaling and *Porphyromonas gingivalis*-accelerated atherosclerosis. *J Dent Res* 2006;**85:**106–121.
6. Darveau RP, Belton CM, Reife RA *et al*. Local chemokine paralysis, a novel pathogenic mechanism for *Porphyromonas gingivalis*. *Infect Immun* 1998;**66:**1660–1665.
7. Hart DN. Dendritic cells: unique leukocyte populations which control the primary immune responses. *Blood* 1997;**90:**3245–3287.
8. Cutler CW, Jotwani R, Palucka KA *et al*. Evidence and a novel hypothesis for the role of dendritic cells and *Porphyromonas gingivalis* in adult periodontitis. *J Periodont Res* 1999;**34:**406–412.
9. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature* 1998;**392:**245–252.
10. Cella M, Sallusto F, Lanzavecchia A. Origin, maturation and antigen presenting function of dendritic cells. *Curr Opin Immunol* 1997;**9:**10–16.
11. Takeda K, Akira S. Toll-like receptors in innate immunity. *Int Immunol* 2005;**17:**1–14.
12. Akira S. Mammalian Toll-like receptors. *Curr Opin Immunol* 2003;**15:**5–11.
13. Jotwani R, Palucka AK, Al-Quotub M *et al*. Mature dendritic cells infiltrate the T cell-rich region of oral mucosa in chronic periodontitis: in situ, in vitro, and in vivo studies. *J Immunol* 2001;**167:**4693–4700.
14. Kanaya S, Nemoto E, Ogawa T *et al*. *Porphyromonas gingivalis* lipopolysaccharides induce maturation of dendritic cells with $CD14^+CD16^+$ phenotype. *Eur J Immunol* 2004;**34:**1451–1460.
15. Zhou Q, Desta T, Fenton M *et al*. Cytokine profiling of macrophages exposed to *Porphyromonas gingivalis*, its lipopolysaccharide, or its FimA protein. *Infect Immun* 2005;**73:**935–943.
16. Hajishengalis G, Tapping RI, Harokopakis E *et al*. Differential interactions of fimbriae and lipopolysaccharide from *Porphyromonas gingivalis* with the Toll-like receptor 2-centred pattern recognition apparatus. *Cell Microbiol* 2006;**8:**1557–1570.
17. Eskan MA, Hajishengalis G, Kinane DF. Differential activation of human gingival epithelial cells and monocytes by *Porphyromonas gingivalis* fimbriae. *Infect Immun* 2007;**75:**892–898.
18. Asai Y, Ohyama Y, Gen K *et al*. Bacterial fimbriae and their peptides activate human gingival epithelial cells through Toll-like receptor 2. *Infect Immun* 2001;**69:**7387–7395.
19. Ogawa T, Asai Y, Hashimoto M *et al*. Bacterial fimbriae activate human peripheral blood monocytes utilizing TLR2, CD14 and CD11a/CD18 as cellular receptors. *Eur J Immunol* 2002;**32:**2543–2550.
20. Ogawa T, Shimauchi H, Hamada S. Mucosal and systemic immune responses in BALB/c mice to *Bacteroides gingivalis* fimbriae administered orally. *Infect Immun* 1989;**57:**3466–3471.
21. Ebersole JL, Taubman MA. The protective nature of host responses in periodontal disease. *Periodontol 2000* 1994;**5:**112–141.
22. Mazzoni A, Segal DM. Controlling the Toll road to dendritic cell polarizatison. *J Leukoc Biol* 2004;**75:**721–730.
23. Muthukuru M, Jotwani R, Cutler CW. Oral mucosal endotoxin tolerance induction in chronic periodontitis. *Infect Immun* 2005;**73:**687–794.
24. Sacre SM, Lundberg AM, Andreakos E *et al*. Selective use of TRAM in lipopolysaccharide (LPS) and lipoteichoic acid (LTA) induced NF-κB activation and cytokine production in primary human cells: TRAM is an adaptor for LPS and LTA signaling. *J Immunol* 2007;**178:**2148–2154.
25. Netea MG, Van der Meer JW, Kullberg BJ. Toll-like receptors as an escape mechanism from the host defense. *Trends Microbiol* 2004;**12:**484–488.
26. Kaisho T, Akira S. Regulation of dendritic cell function through toll-like receptors. *Curr Mol Med* 2003;**3:**759–771.
27. Dilioglou S, Cruse JM, Lewis RE. Function of CD80 and CD86 on monocyte- and stem cell-derived dendritic cells. *Exp Mol Pathol* 2003;**75:**217–227.
28. de Saint-Vis B, Fugier-Vivier I, Massacrier C *et al*. The cytokine profile expressed by human dendritic cells is dependent on cell subtype and mode of activation. *J Immunol* 1998;**160:**1666–1676.