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Role of helper T cells in the humoral immune responses against 53-kDa outer membrane protein from *Porphyromonas gingivalis*

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Outer membrane protein with a 53-kDa molecular weight (Ag53) isolated from *Porphyromonas gingivalis* evokes strong humoral immune responses in many periodontitis patients. To examine the effects of cytokines produced by Ag53-specific Th cells on the IgG production against Ag53, we established Ag53-specific Th-cell lines from patients with early onset periodontitis and from healthy volunteers. We then developed a mixed lymphocyte culture system between Ag53-specific Th cells and auto- or allo-derived T-cell-depleted leukocytes produced from the subjects whose HLA class II haplotypes were completely matched. Interferon-γ production was observed in all Th cell lines from patients and healthy subjects. As for Th2 type cytokines, interleukin (IL)-4, IL-5, IL-6 and IL-10 production varied greatly in Th cells regardless of the periodontal condition of the donor. Only Th cell lines with a high Th2/Th1 ratio induced Ag53-specific IgG production when cocultured with T-cell-depleted leukocytes. Thus, the difference in Th2/Th1 balance may regulate the Ag53-specific IgG production.

Key words: B cell; class II HLA; cytokine profile; IgG antibody; outer membrane protein; periodontitis; *Porphyromonas gingivalis*; Th cell line

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Porphyromonas gingivalis is the most frequently implicated pathogen in periodontal diseases, possessing many kinds of possible antigens recognized by human T cells (4, 19). Among them, the 53-kDa outer membrane protein (Ag53), one of the highly immunogenic proteins originally isolated from the vesicle surface of *P. gingivalis* 381, reacts strongly with sera from many patients with periodontitis (8, 10). Recently, analytical studies of epitopes recognized by T and B cells from early onset periodontitis patients have revealed that there is a common region on Ag53 recognized by both T and B cells in early-onset periodontitis patients, and that the major B-cell epitope is located within the major T-cell epitope on Ag53 (13, 14). This suggests that a limited region on Ag53 is essential for developing Ag53-specific immune responses, leading to the production of specific antibodies. However, the production of antibodies against Ag53 differs greatly even among periodontitis patients, although *P. gingivalis* was detected as the predominant microorganism in their periodontal pockets (3). Therefore, it is suggested that, besides T- and B-cell epitopes, other factors are involved in the establishment of the individually distinct production of IgG antibody against Ag53.

Generally, the difference in helper T (Th) cell subsets specific for antigenic proteins is one of the possible determinants in the production of IgG antibody from B cells, since the antigen-specific antibody production requires the help of Th cells, including the expression of CD40 ligand (CD40L) and the secretion of Th2-type cytokines. Th cells are classified into Th1 and Th2 cells on the basis of their cytokine profiles. Th1 cells secrete Th1-type cytokines such as interleukin (IL)-2 and interferon (IFN)-γ and are associated with cell-mediated immune responses, whereas Th2 cells secrete Th2-type cytokines such as IL-4, IL-5, IL-6, and IL-10

and are associated with the antibody production from B cells. The profile of cytokines produced by Th cells is antigen specific, and varies among individuals. Thus, cytokine profiles of Th cells are likely to be crucial in antibody production.

In the present study, we examined the cytokine profiles of the Ag53-specific Th-cell line established from early-onset periodontitis patients and from periodontally healthy subjects, and the effects of these Th cells with different cytokine profiles on the production of Ag53-specific IgG in response to Ag53.

Material and methods

Antigen preparations

Purified Ag53 protein from *P. gingivalis*, maltose binding protein-Ag53 fusion protein (MBP-Ag53) and Ag53-derived peptides were used as antigens. The purified Ag53 protein was prepared from whole cells of *P. gingivalis* FDC381 according to the method described by Kokeguchi et al. (9). MBP-Ag53 was generated by using a Protein Fusion and Purification System (New England Biolabs Inc., Beverly, MA) according to the method described by Ohyama et al. (13). In short, XL-1 Blue *Escherichia coli*-expressed MBP-Ag53 was sonicated and purified by MBP-specific affinity chromatography, followed by ion-exchange chromatography and gel-filtration chromatography. Ag53-derived peptides were prepared according to the method described by Ohyama et al. (13). Briefly, 45 peptides (sequential 11 amino acids overlapping peptides composed of 21 amino acids) representing the complete Ag53 sequence were synthesized based on the amino acid sequence of Ag53 reported by Hongyo et al. (6), using a solid-phase simultaneous multiple peptide synthesizer PSSM-8 (Shimadzu, Kyoto, Japan) based on F-moc strategy. All peptides were purified using C18 reverse-phase HPLC (Waters, Milford, MA). A mixture of these peptides was used as the Ag53 antigen.

Peripheral blood mononuclear cell donors

Ag53-specific Th-cell lines were established from peripheral blood mononuclear cells (PBMCs) of early-onset periodontitis patients (D1–D6) and healthy individuals (D7–D22). Informed consent was obtained from each donor prior to this study. The PBMC donors included six Japanese early-onset periodontitis patients (five men, mean age: 27.8 ± 7.4 years), and 16 healthy Japanese individuals (14 men, mean age: 30.1 ± 4.3 years). Early-onset periodontitis patients were diagnosed according to the description of Murayama et al. (12).

Lymphocyte preparation

Ag53-specific Th-cell lines were generated by the methods described in our previous study (13). PBMCs (1×10^5/well) were stimulated with 5 μg/ml MBP-Ag53 in RPMI 1640 medium (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 2 mM L-glutamine, 100 units/ml of penicillin, 100 μg/ml of streptomycin, and 10% pooled, heat-inactivated autologous plasma in 96-well flat-bottomed culture plates (Falcon, Becton Dickinson, Lincoln Park, NJ). PBMCs were isolated from heparinized blood by Ficoll-Paque gradient density centrifugation. After 7–9 days, irradiated (3000 cGy) autologous PBMC (1.5×10^5/well) pulsed with mixed-overlapping Ag53-derived peptides (0.5 μM each) as antigen-presenting cells, and human rIL-2 (50 U/ml), human rIL-4 (10 U/ml), were added to the culture wells carrying T-cell blasts, and the cells were maintained for a further 7 days. After specificity assay against the mixed-overlapping Ag53-derived peptides, aliquots of growing cultures specific to Ag53 were combined as the Th-cell line. To maintain the Th-cell activity, antigen-presenting cells, rIL-2, and rIL-4 were added to the culture wells every 6 days.

T-cell-depleted leukocyte fractions were obtained from PBMCs of the donors and were used as the cell fractions enriched with antibody-producing cells. PBMCs were incubated with 150-fold higher amounts of neuraminidase-treated sheep red blood cells (Immune-Biological Laboratories, Gunma, Japan) for 1 h on ice. The cell fraction at the interface after Ficoll-Paque centrifugation was collected to isolate T cells forming rosettes with sheep red blood cells. The T-cell-depleted lymphocytes were obtained by the depletion of $CD2^+$ lymphocytes from the interface-cell fraction, using Dynabeads M450 Pan-T (Dynal, Oslo, Norway), and resuspended in RPMI 1640 medium supplemented with 10% heat-inactivated pooled human serum from each donor, penicillin (100 U/ml), and streptomycin (100 μg/ml). The T-cell-depleted leukocyte fraction exhibited < 1% $CD3^+$ cells as determined by a flow cytometer (EPICS XL, Coulter, Hialeath, FL).

Lymphocyte culture to induce Ag53-specific antibody

T-cell-depleted leukocyte fractions were cultured with autologous or allogeneic Ag53-specific Th cells to induce Ag53-specific IgG antibodies. Freshly isolated T-cell-depleted lymphocytes (4×10^5/well) were cultured with Ag53-specific Th cells (2×10^5/well) in 48-well plates (Coster, Cambridge, MA) with recombinant Ag53 proteins (final concentration: 5 μg/ml) in RPMI 1640 medium supplemented with 2 mM L-glutamine, 100 units/ml of penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated fetal bovine serum (Irvine Scientific, Santa Ana, CA). After 3 days, culture supernatants were collected to measure cytokine levels produced from the cells in the response to Ag53. After 5 days of culture, the medium was changed for fresh medium without antigens and the cells were further cultured. After an additional 9 days, culture supernatants were collected for quantification of the Ag53-specific IgG titer.

Quantification of Ag53-specific IgG

Ag53-specific IgG antibodies in the donors' sera and the culture supernatants were measured by the enzyme-linked immunosorbent assay (ELISA) according to the method described in our previous study (12). ELISA plates (Dynateck Laboratory, Plochingen, Germany) were coated with 10 ng/μl of purified Ag53 protein in $NaHCO_3/Na_2CO_3$ buffer (pH 9.6). After an overnight incubation at 4°C, the plates were washed four times with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (0.05% T-PBS: 0.02% NaN_3, 136.9 mM NaCl, 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 2.7 mM KCl, pH 7.4). The plates were then incubated for 2 h at 37°C after 200-fold diluted sera or undiluted culture supernatants were added to the wells. For the detection of specific IgG, 1 μg/ml biotin-conjugated mouse anti-human IgG (Sigma Chemical Co, St. Louis, MO) was added (2 h, 37°C). After washing with 0.05% T-PBS four times, 1 μg/ml alkaline-phosphatase-labeled streptavidin was added and the plates incubated for 1 h at 37°C. After incubation, the plates were washed four times with 0.05% T-PBS. The plates were incubated for 30 min at 37°C after the substrate was added. The substrate consisted of 2.7 mM p-nitrophenyl phosphate (Sigma) in diethanolamine buffer (9.7% diethanolamine, 0.02% NaN_3, 0.5 mM $MgCl_2$, pH 9.8). Absorbance at 405 nm was measured by using Novapath Mini Reader (Bio-Rad Laboratories, Hercules, CA). Titers of Ag53-specific IgG were expressed as arbitrary units on the basis of the pooled standard serum obtained from periodontal healthy subjects.

Measurement of cytokine production

Antigen-induced cytokine production from the Ag53-specific Th-cell line was assayed by culturing the T cells (5×10^4/well) in 96-well flat-bottomed culture plates (Falcon) in the presence of the mixed-overlapping Ag53-derived peptides (0.1 μM each), and 3000 cGy-irradiated autologous PBMCs (1.5×10^5/well). After 3 days, the culture supernatants were collected for quantification of cytokines by the IFN-γ ELISA kit (Otsuka, Tokyo, Japan), Quantikine human IL-4 and IL-6 Immunoassay Kits (R & D Systems, Minneapolis, MN), and human IL-5 and IL-10 ELISA kits (Endogen, Woburn, MA) according to the manufacturers' instructions. The sensitivity of each assay was as follows: 10 Pg/ml for IFN-γ, 10 Pg/ml for IL-4, 10 Pg/ml for IL-5, 1 Pg/ml for IL-6, 15 Pg/ml for IL-10.

Statistical analysis

The correlation between the amounts of Ag53-induced cytokine and serum Ag53-specific IgG titers of each donor was determined by analyzing correlation factors using Student's *t*-test.

The ratio of each Th2 cytokine to IFN-γ (Th2/Th1) in the culture supernatants of Ag53-specific Th-cell lines was calculated, and the differences in the total amounts of the cytokines and in the ratio of Th2/Th1 between the subjects were determined by the Mann–Whitney *U*-test.

Results

Serum anti-Ag53 IgG titers of the subjects

First, the serum anti-Ag53 IgG titers of all donors were measured. Each titer was indicated as arbitrary ELISA units assuming that the serum IgG titer of donor #7

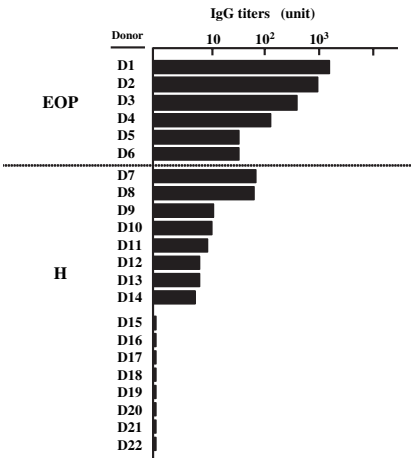

Fig. 1. Serum anti-Ag53 IgG titers of all subjects as measured by ELISA and described in Material and methods. Dotted line separates the donors into two groups, a patient group and a healthy control group (D1–D6, early-onset periodontitis patients; D7–D22, periodontal healthy individuals). Each titer was indicated as arbitrary ELISA units assuming that the serum anti-Ag53 IgG titer of donor #7 (D7) was '100' as a standard. The serum anti-Ag53 IgG titers of early-onset periodontitis patients were significantly higher than those of healthy subjects ($P < 0.05$, Mann–Whitney *U*-test). EOP, early-onset periodontitis patients. H, healthy patients.

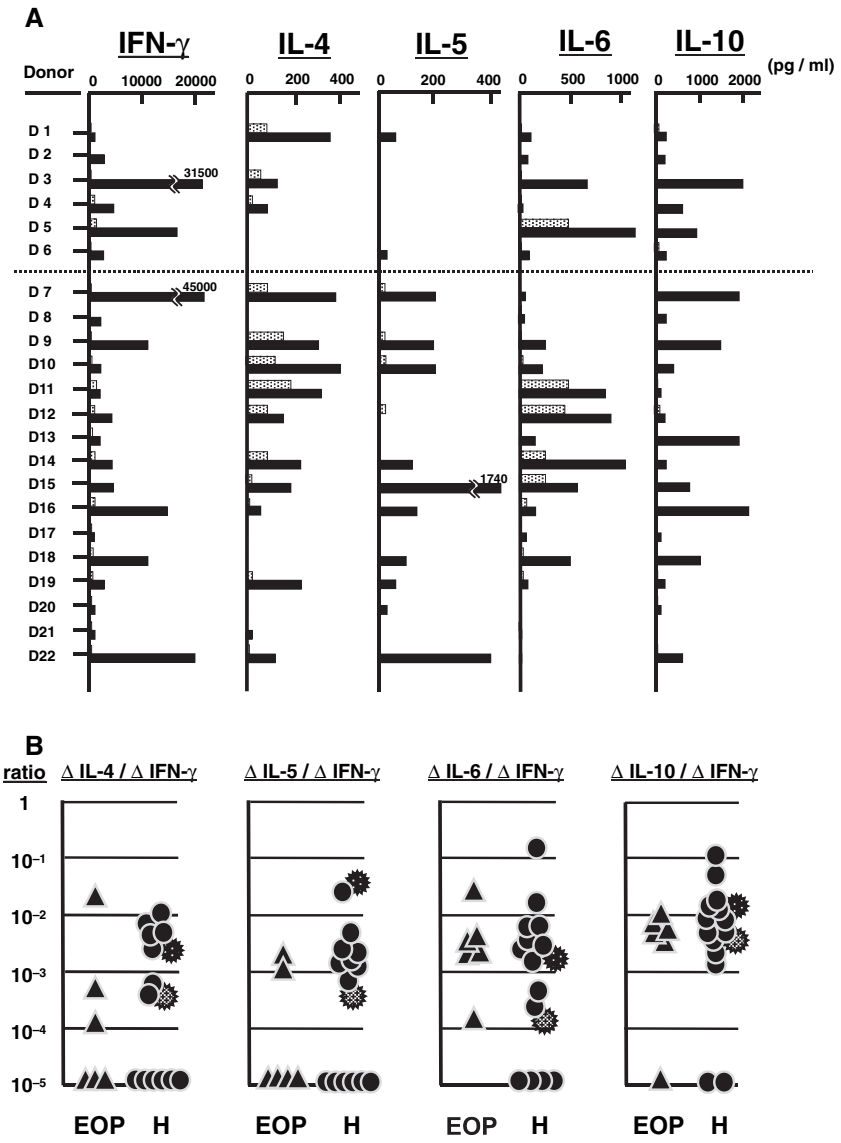

Fig. 2. Cytokine profile of Th-cell line specific to Ag53. A) Amounts of cytokine produced from Ag53-specific Th-cell line. The profile of cytokines produced from 22 Ag53-specific Th-cell lines was determined by measuring each cytokine level including IL-4, -5, -6, and -10 and IFN-γ in the supernatants after the culture of the lines with irradiated autologous PBMC pulsed with (■ black bar) or without (▨ speckled bar) Ag53-derived peptides. The amounts of cytokine were indicated as the mean value of duplicate cultures. Patient and healthy control groups are separated by the dotted line (D1–D6, early-onset periodontitis patients; D7–D22, periodontally healthy individuals). B) Th2/Th1 cytokine ratios of Ag53-specific Th-cell line. The ratio of each Th2-type cytokine including IL-4, -5, -6, and -10 to the Th1-type cytokine, IFN-γ, all of which were produced from the Th-cell lines, was calculated and demonstrated. The ratios of all Th2 cytokines to IFN-γ (Th2/Th1) in Th-cell lines established from D15 (●) were significantly higher than those in Th-cell lines from D7 (●) ($P < 0.05$, Mann-Whitney *U*-test). All experiments were done in duplicate, and the mean calculated. Each ratio was calculated following subtraction of the mean value of the produced cytokines in the absence of the antigen from the mean value of the cytokine in the presence of antigen. The experiment was repeated four times and similar results were obtained. The representative data of four separate experiments are shown. EOP, early-onset periodontitis patients. H, healthy patients.

(D7) was '100' as a standard (Fig. 1). Serum anti-Ag53 IgG titers of early-onset periodontitis patients were significantly higher than those of healthy subjects ($P < 0.05$, Mann–Whitney *U*-test).

Profiles of cytokines produced from Ag53-specific Th-cell line

The profile of cytokines produced from 22 Ag53-specific Th-cell lines was determined by measuring each cytokine level. These include IL-4, -5, -6, and -10 and IFN-γ in the culture supernatant after the coculture of the cell lines with irradiated autologous PBMC pulsed with Ag53-derived peptides (Fig. 2A). The Th1-type cytokine IFN-γ was produced from all Th-cell lines, whereas the production of Th2-type cytokines greatly varied among the Th-cell lines. The ratio of Th2-type cytokines (IL-4, -5, -6, and -10) to the Th1-type cytokine IFN-γ in each T-cell line was calculated, and is shown in Fig. 2B. The total amounts of each cytokine and the Th2/Th1 ratios did not differ significantly in early-onset periodontitis patients and periodontally healthy subjects. Correlation analysis also indicated that there was no positive or negative relationship between any of the Ag53-induced cytokine levels from T-cell lines and the serum Ag53-specific IgG titers.

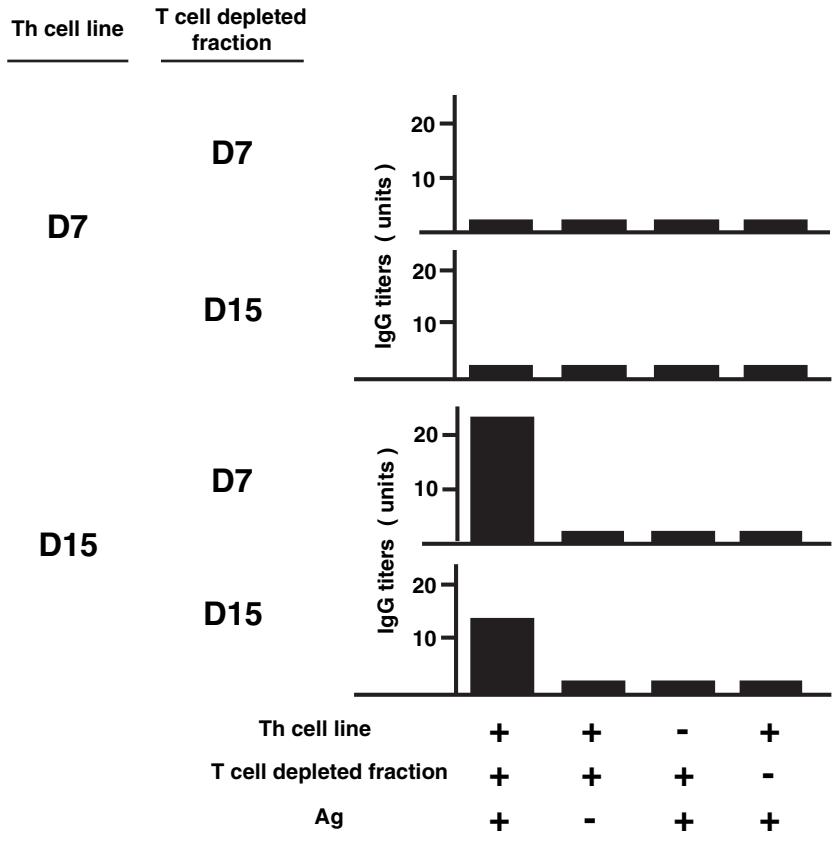

Fig. 3. Ag53-specific IgG production induced by the culture of Ag53-specific Th cell line with HLA-matched T-cell-depleted lymphocytes. Ag53-specific Th cells (2×10^5/well) from periodontally healthy donors (D7 and D15) were cultured with autologous or allogeneic freshly isolated T-cell-depleted lymphocytes (4×10^5/well) in 48-well plates with recombinant Ag53 proteins (final concentration: 5 μg/ml). Culture supernatants were collected at day 14 for quantification of Ag53-specific IgG titer. Each titer was indicated as arbitrary ELISA units assuming that the serum anti-Ag53 IgG titer of D7 was '100' as a standard. Ag, antigen.

Ag53-specific Th-cell line with high Th2/Th1 ratio induces production of Ag53-specific IgG

Fortunately, it was found that periodontally healthy donors #7 (D7) and #15 (D15) shared completely matched HLA class II haplotypes (DRB1*0101–DQA1*0101–DQB1*0501/DRB1*0406–DQA1*0301–DQB1*0302), and that the Th-cell line established from these two subjects had quite different cytokine productivity patterns. The ratio of all Th2/Th1 (IL-4/IFN-γ, IL-5/IFN-γ, IL-6/IFN-γ and IL-10/IFN-γ) from D15 was significantly higher than that from D7 ($P < 0.05$, Mann–Whitney *U*-test) (Fig. 2B). Thus, it was suspected that D15 produced higher amounts of Ag53-specific IgG. However, as shown in Fig. 1, the serum IgG titer specific to Ag53 was much higher in D7 than in D15.

To see whether this difference in cytokine profile is actually reflected in Ag53-specific antibody production, we employed the mixed lymphocyte culture system between Ag53-specific Th cells and auto- or allo-derived T-cell-depleted leukocytes from D7 and D15(Fig. 3). When Th cells from D7 showing a lower Th2/Th1 ratio were cultured with T-cell-depleted lymphocytes from either D7 or D15, no Ag53-specific IgG was detected in the culture supernatants. However, Ag53-specific IgG was produced from the T-cell-depleted leukocyte fraction in both D7 and D15 when Th cells from D15 with higher Th2/Th1 ratio were employed, suggesting that the induction of Ag53-specific IgG depends on the Th2/Th1 ratio.

We also examined whether PBMCs alone could induce specific antibody against Ag53 when cultured with Ag53. Following *in vitro* culture of PBMCs with the antigen protein, Ag53-specific antibody was not detected in the culture supernatant, even in the culture of PBMCs from the donors who showed a high serum antibody titer against Ag53 (data not shown).

Cytokine production during Ag53-specific IgG induction

Besides a high Th2/Th1 ratio, to determine the essential cytokines influencing the Ag53-specific IgG production, we measured cytokine levels in the supernatants after 3-day culture of Th cells with T-cell-depleted lymphocytes, and calculated the ratio of Th2/Th1 cytokines. Of note, no IL-4 was detected in any culture supernatants examined. Higher Th2/Th1 ratios (IL-5/IFN-γ, IL-6/IFN-γ and IL-10/IFN-γ) were observed in cultures where Ag53-specific IgG was produced than in the cultures where it was not (Fig. 4), indicating that IL-5, IL-6 and IL-10 are important cytokines inducing antibody production.

Discussion

In this study, we established Th-cell lines specific to Ag53 and examined their cytokine profiles in response to Ag53. The comprehensive approach that we have conducted so far on Ag53 (6, 8–10, 13, 14), including the current study, is valuable for obtaining a better understanding of the immune responses specific to the pathogenic antigens. Our present study

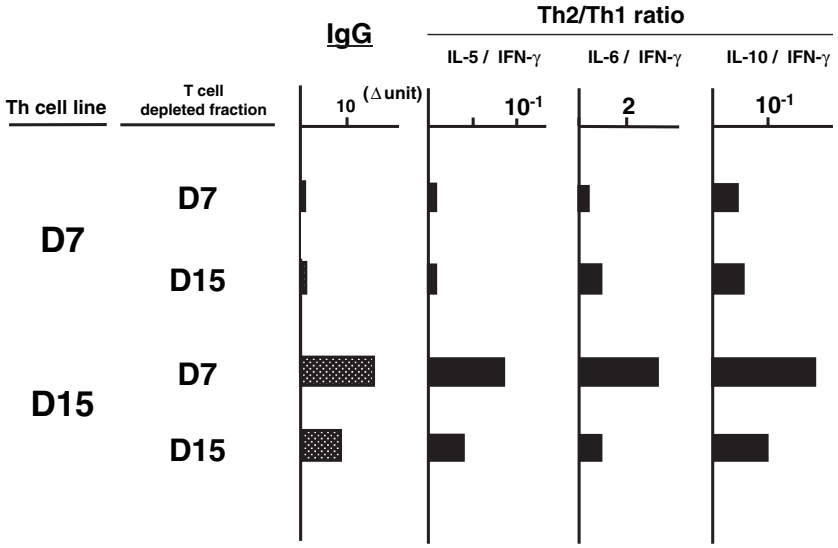

Fig. 4. Th2/Th1 ratio of cytokines in the mixed lymphocyte culture between Th cells and auto- or allo-derived T-cell-depleted leukocytes. Ag53-specific Th cells (2×10^5/well) were cultured with autologous or allogeneic freshly isolated T-cell-depleted lymphocytes (4×10^5/well) in 48-well plates with recombinant Ag53 proteins (final concentration: 5 μg/ml). Culture supernatants were collected on day 3 for quantification of cytokines and the ratio of Th2/Th1 in the culture supernatants of Ag53-specific Th-cell lines were calculated (■ black bar). The amount of Ag53-specific IgG titer was (▩ speckled bar) indicated as the mean value of duplicate cultures, after subtraction of the mean value obtained from cultures without antigens. Ag, antigen.

demonstrated that IFN-γ of Th1-type cytokine was produced from all Th-cell lines obtained from periodontitis patients and healthy subjects. The Th1-type immune response plays an important role in the protection against several intracellular bacteria (5, 16). In fact, in recent studies, it was reported that *P. gingivalis* can actually invade oral epithelial cells *in vitro* or in the organ culture system (17, 18). Thus, Ag53-specific T-cell subsets exhibiting IFN-γ production in response to Ag53 may contribute to the development of specific cellular immunity against *P. gingivalis* infection. This speculation appears reasonable, as Ag53 has been observed on the vesicle surface of *P. gingivalis* (10).

We also demonstrated that Th2-type cytokine production varied in the response to Ag53. Although IL-4 and IL-5 were detected in the culture supernatants of only a limited number of subjects, IL-6 and IL-10 were frequently detected in the supernatants of most subjects. Since IL-6 and IL-10 are cytokines produced from both T cells and monocytes, the observed results in this study may comprise the total amount of the cytokines produced by both types of the cells. In the experimental systems employed in this study, it seemed difficult to distinguish exactly how much cytokine is produced by specific cell types. Further studies will be needed, such as treating the PBMCs with the protein synthesis inhibitor, e.g. emetine, when using these cells as antigen-presenting cells.

A major determinant of the development of either the Th1- or Th2-type cytokine profile is the combination of cytokines produced locally, as these cytokines not only promote each type of immunity but also suppress each other's activity. A predominance of IL-10 favors the development of Th2 cells and suppresses the expansion of Th1 cells producing IFN-γ. In our present study, a high amount of IFN-γ was often detected in the culture supernatants that also contained high amounts of IL-10. Therefore, the results are contradictory. However, we measured the amount of cytokine in the supernatants collected after short-term culture of the T cells, and therefore did not observe the polarized profile. With long-term culture, it is possible to observe the polarized cytokine profile in the culture supernatants, as either cytokine may dominate and promote either type of immunity.

It has been reported that in subjects exposed to antigens, including ovalbumin, tetanus toxoid and filarial parasite (7, 22, 24), and subjects vaccinated against influenza virus and poliovirus (20, 23, 25) their PBMCs are easily able to produce the antigen-specific antibodies following culture with each antigen. In terms of Ag53, following *in vitro* culture of PBMCs with the antigen protein, antigen-specific antibody was not detected in the culture supernatant, even in the culture of PBMCs from the donors who exhibited a high serum antibody titer against Ag53 (data not shown). However, it became possible to detect Ag53-specific IgG by employing the culture system of T-cell-depleted lymphocytes with Ag53-specific Th-cell line with a high Th2/Th1 ratio. This finding suggests that T-cell activation is required for the induction of antigen-specific antibody production. The memory T cells specific to Ag53 did not appear to be activated in the culture of PBMCs with antigen protein; the number of clones of T cells specific to Ag53 might be either too small (if present at all) to activate themselves in such an *in vitro* situation. To overcome this problem, we established an Ag53-specific Th-cell line and used it to study *in vitro* Ag53-specific IgG induction. This also has the advantage that it minimizes individual differences in the number of T-cell clones specific to Ag53 from their previous immune exposure to Ag53.

It was likely that whether Ag53 specific IgG was produced depends upon T-cell phenotypes. When the T-cell line showing a high Th2/Th1 ratio was cultured with the T-cell-depleted leukocyte fraction, IgG antibody production was detected in the culture supernatant. However, the T-cell line with a low Th2/Th1 ratio did not have the ability to induce IgG production. Thus, the Th2/Th1 ratio of Th cells may be a factor regulating the Ag53-specific IgG production in response to Ag53.

With respect to Ag53-specific IgG production, we obtained contrary results between *in vitro* assays and serum data. In *in vitro* assays, Ag53-specific IgG production was only induced when Th cells showing a higher ratio of Th2/Th1 were cultured with the T-cell-depleted leukocyte fraction. Because the assays were performed in a closed space, cytokines produced from Th cells could easily have influenced the T-cell-depleted leukocyte fraction, causing it to induce specific IgG. In addition, high amounts of Ag53 were added to the cultures. Thus, one can consider these *in vitro* assays to be a model of immunological events occurring in local periodontal lesions. However, in the case of serum IgG, the elevation of serum antibody level may require a certain amount of infection with particular antigens. We could not detect serum IgG specific to Ag53 in D15. However, if D15 is infected with Ag53, we believe that the level of serum IgG specific to Ag53 would easily elevate.

Recently, it has been reported that B-cell proliferative responses are observed in the presence of *P. gingivalis* and IL-10 (21), a phenomenon known as polyclonal B-cell activation, leading to elevated numbers of the B cells in periodontal lesions. Polyclonal B-cell activation induced by periodontopathic bacteria has been suggested to contribute to an increase in IgG antibodies in periodontal lesions (2). In the present study, however, Ag53-specific IgG production was not observed in the culture without the help of Th cells. Thus the antibody production against Ag53 occurred in an immunologically antigen-specific manner, and was not simply a result of polyclonal B-cell activation.

To distinguish the possible cytokines affecting the Ag53-specific IgG production, we evaluated the amounts of several cytokines in the culture supernatants. Surprisingly, IL-4 was not detected under our experimental conditions and may therefore not be essential for the induction of Ag53-specific antibody production. Production in the absence of IL-4 could be due to several factors. For example, endogenously produced IL-6 and IL-10 are involved in the differentiation of CD40-activated B cells and production of immunoglobulins (1), and IL-13 can also compensate the function of IL-4 (11, 15).

In conclusion, we examined cytokine profiles of Ag53-specific Th-cell lines and their effects on Ag53-specific IgG production in response to Ag53. The data suggest that differences in the profile of Th2-type cytokine may explain individual differences in antibody titer against Ag53, and that differences in the ratio of Th2/Th1 cytokines produced from Th cells regulate the production of Ag53-specific IgG.

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