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Characterization of $CD4^+$ $FOXP3^+$ T-cell clones established from chronic inflammatory lesions

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Introduction: Our previous study demonstrated that the gene expression of *FOXP3*, a characteristic marker for $CD4^+$ $CD25^+$ regulatory T cells in mice, is upregulated more in periodontitis than in gingivitis at the messenger RNA (mRNA) level. Furthermore, most of the T-cell clones established from periodontitis lesions expressed *FOXP3* mRNA. However, role of the $FOXP3^+$ gingival T cells has not been elucidated.
Methods: The phenotype of FOXP3-expressing cells in periodontitis lesions was determined immunohistochemically. $CD4^+$ $FOXP3^+$ gingival T-cell clones were established from three patients with advanced periodontitis by using immunomagnetic beads. Gene expression and phenotype analyses were performed by reverse-transcription polymerase chain reactions and flow cytometry, respectively. The effect of $CD4^+$ $FOXP3^+$ T-cell clones on the proliferative response of $CD4^+$ $CD25^-$ T cells was examined by $[^3H]$thymidine incorporation.
Results: FOXP3 expression was found in some $CD4^+$ T cells and $CD25^+$ cells but not in $CD8^+$ T cells by immunohistochemistry. In spite of a substantial expression of the *CD25* gene, the expression level of membrane CD25 on the $CD4^+$ $FOXP3^+$ gingival T-cell clones was low. While peripheral blood $CD4^+$ $CD25^+$ $FOXP3^+$ cells suppressed the proliferation of $CD4^+$ $CD25^-$ T cells, the $CD4^+$ $CD25^{low}$ $FOXP3^+$ gingival T-cell clones enhanced the proliferation significantly.
Conclusion: Our study makes it evident that most, if not all, of the $FOXP3^+$ T cells in periodontitis lesions can be considered to be effector T cells. The effector activity of the gingival T-cell clones could be attributable to the low level of membrane CD25 expression. Further studies are clearly needed to clarify the role of these T cells and their unique characteristics in the pathogenesis of periodontal disease.

Key words: FOXP3; Periodontitis, regulatory T cell; T-cell clone

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T cells are key players in the immune response to infections where they regulate the cellular and humoral responses either positively or negatively. Although the periodontitis lesion is characterized by the infiltration of B cells and plasma cells, a number of T cells are seen in the lesion (25). It has been considered that the activation of T cells specific to periodontopathic bacteria and the subsequent imbalance of the regulatory mechanisms of the T cells may be involved in the pathogenesis of periodontitis (7).

Analysis of the cytokine profile of the T cells in terms of T helper type 1 (Th1) versus Th2 responses demonstrated that the predominance of B cells and plasma cells in periodontitis lesions cannot be explained by Th2 upregulation but may be because of an imbalance between Th1 and Th2 (3). We have also shown that interferon-γ and transforming growth factor-β messenger RNA (mRNA) expressions tended to be higher in periodontitis tissues compared with gingivitis tissues but no differences were found for interleukin-4 (IL-4) and IL-10, although there was an inter-individual variation. These findings suggest that perhaps subtle differences in the balance

of cytokines may result in different disease expressions (8).

It is becoming evident that T cells with a suppressive function, the so-called regulatory T cells, represent a crucial element in the regulatory networks that control immune responses and maintain peripheral tolerance (21). Among the several subsets of regulatory T cells, the naturally occurring $CD4^+$ $CD25^+$ regulatory T cells have drawn much attention because their removal leads to spontaneous development of various autoimmune diseases in otherwise normal animals. Mice carrying the X-linked *scurfy* mutation lack conventional $CD4^+$ $CD25^+$ regulatory T cells (5, 12, 19) and the mutation is found to be in the *forkhead box protein P3* (*Foxp3*) gene. In mice, *Foxp3* has been shown to be expressed exclusively in $CD4^+$ $CD25^+$ regulatory T cells and is not induced upon the activation of $CD25^-$ T cells. When *Foxp3* is introduced via retrovirus or enforced transgene expression, naïve $CD4^+$ $CD25^-$ T cells are converted to regulatory T cells (9). However, the role of FOXP3 in human $CD4^+$ $CD25^+$ regulatory T cells remains controversial (15, 24).

Our previous study demonstrated both an increased proportion of the $CD4^+CD25^+$ T-cell population and elevated *FOXP3* gene expression in periodontitis lesions (16). Furthermore, >90% of the T-cell clones established from periodontitis lesions expressed *FOXP3* (10). Thus, the exact role of $CD4^+$ $FOXP3^+$ T cells in the regulation of the immune response in chronic inflammatory disease urgently requires clarification.

Materials and methods

In situ identification of $FOXP3^+$ T cells

All study protocols hereafter were approved by the institutional review board of Niigata University and informed consent was obtained from all patients and control subjects before their inclusion in this study.

Gingival biopsies of periodontitis were obtained at the time of periodontal surgery or extraction of severely involved teeth. Serial cryostat sections were prepared and subjected to single staining of CD4 (Beckman Coulter, Fullerton, CA) CD8 (eBioscience, San Diego, CA) and CD25 (Beckman Coulter). Double staining of CD4 and FOXP3, CD8 and FOXP3 (goat polyclonal antibody; Abcam, Cambridgeshire, UK), and CD25 and FOXP3 was also carried out according to the method we have used and reported previously (26).

Establishment of gingival T-cell clone from periodontitis patient

Seven T-cell clones from three patients with moderate periodontitis were used. Two T-cell clones (patient A) had been used for gene expression analysis in our previous study (10) whereas the other T-cell clones (three clones from patient B and two clones from patient C) were newly established. Inflamed gingival tissues were obtained at periodontal surgery and cut into small pieces in RPMI 1640 containing penicillin, streptomycin and fungizone, and subjected to Dispase digestion. Tissues were passed through nylon mesh and subsequent single-cell suspensions were subjected to Ficoll–Paque Plus (Amersham Biosciences AB, Uppsala, Sweden) density gradient centrifugation. The resulting mononuclear cells were stimulated with an anti-CD3 monoclonal antibody (30 ng/ml, UCHT1; Immunotech, Marseille, France) in the presence of irradiated (40 Gy; PS-3000SB, Pony Industry, Osaka, Japan) allogeneic peripheral blood mononuclear cells (PBMC; 2×10^6/well), Epstein–Barr virus-transformed allogeneic PBMC (2×10^5/well) and 50 U/ml recombinant human IL-2 (PEPRO TECH, London, UK) in 2 ml culture media in a 24-well culture plate (NUNC, Roskilde, Denmark). After expansion of the cells, $CD4^+$ T cells were purified using magnetic beads (Miltenyi Biotec, Auburn, CA); they were then cloned by limiting dilution, and stimulated as described above. T-cell clones were maintained with IL-2 for at least 3 weeks after stimulation and then used for subsequent experiments.

Purification of $CD4^+$ $CD25^+$ T cells

Peripheral blood was obtained from the same patients whose T-cell clones were established and also from healthy donors. PBMC were prepared by centrifugation over a Ficoll–Paque density gradient and $CD4^+$ T cells were purified using the human CD4 T-Lymphocyte Enrichment Set-DM and BD™ IMagnet cell separation kit (both from BD Biosciences Pharmingen, San Diego, CA). After isolation of $CD4^+$ T cells, $CD25^+$ T cells were purified with anti-human CD25 Particles-DM (BD Biosciences Pharmingen). Remaining cells were used as $CD4^+$ $CD25^-$ T cells and the PBMC depleted of $CD3^+$ cells were irradiated and used as accessory cells.

Gene expression analysis of the T-cell populations

Total RNA was isolated from $CD4^+$ $CD25^+$ and $CD4^+$ $CD25^-$ T cells and from gingival T-cell clones using an RNeasy Micro Kit (Qiagen, Hilden, Germany). The RNA was then reverse-transcribed to complementary DNA using a random primer (TAKARA BIO INC., Shiga, Japan) and Moloney murine leukemia virus reverse transcriptase (Invitrogen, Carlsbad, CA). One microliter of cDNA from each cell population was added to a polymerase chain reaction (PCR) mixture containing GoTaq Green Master Mix (Promega, Madison, WI), and 420 nM oligonucleotide primers (Table 1) in a total volume 20 μl. The reaction was performed using a DNA thermal cycler (GeneAmp® PCR System 9700; Applied Biosystems, Foster City, CA). Ten microliters of each PCR product was electrophoresed on a 2% agarose gel and visualized by ethidium bromide staining. The primer sets used in this study are shown in Table 1.

Analysis of FOXP3 expression of $CD4^+$ $CD25^+$, $CD4^+$ $CD25^-$ T cells and gingival T-cell clones

The $CD4^+$ $CD25^+$ and $CD4^+$ $CD25^-$ T cells and the gingival T-cell clones were

Table 1. Primer sequences for RT-PCR

	Sequence
FOXP3	Forward: 5′-GCAAATGGTGTCTGCAAGTGG-3′
	Reverse: 5′-AGTGCCATTTTCCCAGCCA-3′
CD25	Forward: 5′-GAATGCAAGAGAGGTTTCCGC-3′
	Reverse: 5′-AATAAACCATCTGCCCCACCAC-3′
CTLA-4	Forward: 5′-AGTATGCATCTCCAGGCAAAGC-3′
	Reverse: 5′-CCAGAGGAGGAAGTCAGAATCTG-3′
IL-10	Forward: 5′-TTGCCAAGCCTTGTCTGAGATG-3′
	Reverse: 5′-AGCCCCAGATCCGATTTTGG-3′
TGF-β1	Forward: 5′-GACATCAACGGGTTCACTACCGG-3′
	Reverse: 5′-GAGGCAGAAGTTGGCATGGTAGC-3′
β-actin	Forward: 5′-GCGAGAAGATGACCCAGATCATGTT-3′
	Reverse: 5′-GCTTCTCCTTAATGTCACGCACGAT-3′

CTLA-4, cytotoxic T-lymphocyte associated antigen 4; IL-10, interleukin-10; TGF-β1, transforming growth factor-β1.

directly stained for fluorescein isothiocyanate (FITC)-conjugated anti-FOXP3 (clone PCH 101: eBioscience), phycoerythrin (PE)-conjugated anti-CD4 (eBioscience), FITC-conjugated anti-glucocorticoid-induced TNF receptor family-related protein (GITR; eBioscience), PE-conjugated anti-cytotoxic T-lymphocyte associated antigen-4 (CTLA-4; eBioscience) and respective isotype controls. To stain CD25, we used anti-human CD25 Particles-DM® (BD Biosciences Pharmingen) followed by PE-conjugated anti-mouse immunoglobulin G (eBioscience). The expression levels of each surface molecule were analyzed using a FACScan flow cytometer (Becton Dickinson, San Jose, CA) and CELLQUEST PRO software (BD Biosciences).

Cell culture and proliferation assay

Five gingival T-cell clones from two patients (patients A and B) were used in the following experiments. CD4$^+$ CD25$^-$ T cells (5×10^3/well) were cultured with or without autologous CD4$^+$ CD25$^+$ T cells or gingival T-cell clones (each at 5×10^3/well) in the presence of autologous accessory cells (5×10^4/well) in 200 μl RPMI-1640 containing 10% fetal calf serum (Biowest, Nuaillé, France) in a 96-well culture plate (NUNC). To analyze the proliferation of CD4$^+$ CD25$^-$ T cells, the cells other than CD4$^+$ CD25$^-$ T cells were irradiated (40 Gy) before co-culture. For polyclonal stimulation, 0.1 μg/ml soluble anti-CD3 and 0.1 μg/ml soluble anti-CD28 monoclonal antibodies were added to the cultures. The cultures were pulsed with 0.5 μCi/well of [^{3}H]thymidine during the final 12 h of a 6-day assay, this was predetermined in preliminary experiments, and [^{3}H]thymidine incorporation was measured by a liquid scintillation counter.

Results

FOXP3 expression is restricted in CD4$^+$ or CD25$^+$ T cells but not in CD8$^+$ T cells in chronic inflammatory periodontitis lesion

FOXP3-immunoreactive cells were observed in periodontitis lesions. As clearly demonstrated by double immunohistochemistry, FOXP3 expression was restricted to CD4$^+$ T cells (Fig. 1A) and was not found in CD8$^+$ T cells (Fig. 1B). We found that neither CD4$^-$ nor CD8$^-$ cells were FOXP3$^+$. In addition, most if not all FOXP3$^+$ cells were also CD25$^+$ (Fig. 1C), indicating that these cells are CD4$^+$ C25$^+$ FOXP3$^+$.

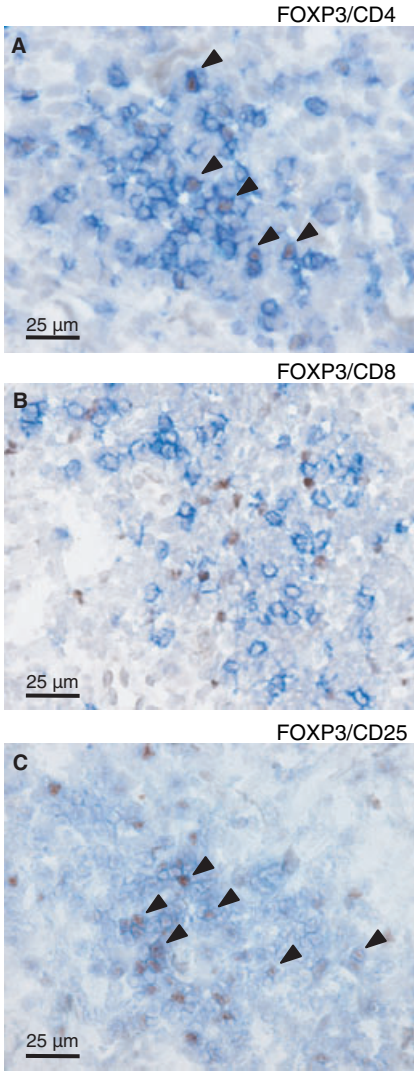

Fig. 1. Double immunohistochemistry of FOXP3/CD4 (A), FOXP3/CD8 (B) and FOXP3/CD25 (C) in the connective tissue subjacent to the pocket epithelium of a periodontitis specimen. FOXP3 was labeled with peroxidase (brown), whereas CD4, CD8 and CD25 were labeled with alkaline phosphatase (blue). Arrows indicate double-positive cells.

Gene expression and phenotypic analyses of gingival T-cell clone, CD4$^+$ CD25$^+$ T cells and CD4$^+$ CD25$^-$ T cells

Gene expression analysis revealed that preferential expression of *FOXP3* was found in both gingival T-cell clones, except clones 4 and 24 from patient B, and CD4$^+$ CD25$^+$ T cells; the expression was much less in CD4$^+$ CD25$^-$ T cells. All the T-cell clones expressed CD25 at a similar level to peripheral blood CD4$^+$ CD25$^+$ T cells irrespective of their *FOXP3* expression level. Cells used as CD4$^+$ CD25$^-$ T cells expressed low levels of CD25, probably as a result of contamination with a small number of CD4$^+$ CD25$^+$ T cells in spite of fractionation using a commercial kit. For CTLA-4 mRNA, no difference was observed between CD4$^+$ CD25$^+$ T cells and CD4$^+$ CD25$^-$ T cells, and substantial expression was observed in all the T-cell clones. For IL-10 mRNA, higher expression was observed in CD4$^+$ CD25$^+$ T cells and CD4$^+$ CD25$^-$ T cells compared with the T-cell clones. No difference was observed for transforming growth factor-β1 (Fig. 2). Flow cytometric analyses demonstrated that the pattern of gene expression of *FOXP3* and *CD25* was confirmed at the protein level in CD4$^+$ CD25$^-$ T cells and CD4$^+$ CD25$^+$ T cells. Although FOXP3 expression in the T-cell clones was usually lower than that in the peripheral blood CD4$^+$ CD25$^+$ T cells, those of two T-cell clones (clone 25, patient B and clone 8, patient C) and peripheral blood CD4$^+$ CD25$^+$ T cells were comparable. However, CD25 expression of the T-cell clone was much lower than that of CD4$^+$ CD25$^+$ T cells. None of the CD4$^+$ CD25$^-$ T cells, CD4$^+$ CD25$^+$ T cells or gingival T-cell clone expressed CTLA-4 on the cell surface. On the other hand, some gingival T-cell clones expressed GITR (Fig. 3).

Upon activation of CD4$^+$ CD25$^-$ T cells by anti-CD3 and anti-CD28 monoclonal antibodies for 3 days, CD25 expression was markedly upregulated. Although the expression of FOXP3 was slightly enhanced, it was much lower than that of CD4$^+$ CD25$^+$ T cells and lower still than that of the gingival T-cell clone. Similarly, the expression of both CD25 and FOXP3 of the gingival T-cell clone were upregulated by the same stimulation; however, the intensity of CD25 was lower than that of CD4$^+$ CD25$^-$ T cells even after stimulation (Fig. 4).

The role of CD4$^+$ CD25$^+$ T cells and gingival T-cell clones in the proliferative response of CD4$^+$ CD25$^-$ T cells to T-cell receptor stimulation

Since both CD4$^+$ CD25$^+$ T cells and gingival T-cell clones are estimated to proliferate in response to T-cell receptor (TCR) stimulation when the cells are co-cultured with CD4$^+$ CD25$^-$ T cells, we examined the effect of irradiation of CD4$^+$ CD25$^+$ T cells or a gingival T-cell clone on the proliferative response of CD4$^+$ CD25$^-$ T cells in a preliminary study. The results demonstrated that irradiated gingival T-cell clones did not proliferate but rather enhanced the proliferation of CD4$^+$ CD25$^-$ T cells in response

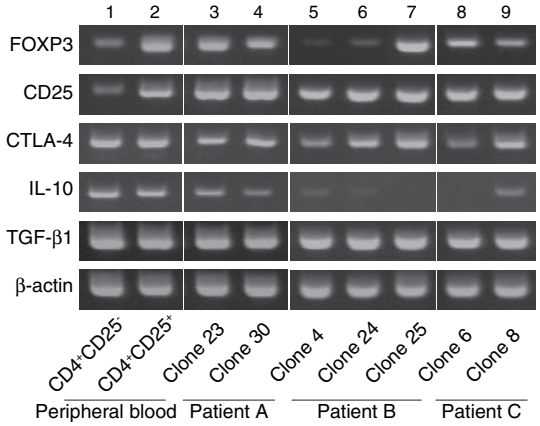

Fig. 2. Gene expressions of $CD4^+$ $CD25^-$ T cells, $CD4^+$ $CD25^+$ T cells, and gingival T-cell clones. Total RNA was extracted from each cell population and the gene expression was analyzed by reverse transcription PCR as described in the Materials and methods. Lane 1, peripheral blood $CD4^+$ $CD25^-$ T cells; lane 2, peripheral blood $CD4^+$ $CD25^+$ T cells; lanes 3–9, gingival T-cell clones.

irrespective of their FOXP3 expression, resulted in enhanced uptake of [^{3}H]thymidine without TCR stimulation. This response was further upregulated by TCR stimulation, particularly in the co-culture with the gingival T-cell clones.

Discussion

We have recently found that many of the $CD4^+$ T-cell clones derived from either gingival tissue or peripheral blood express the *FOXP3* gene. In the present study $FOXP3^+$ cells were also identified in periodontitis lesions. The cells were restricted to the $CD4^+$ population and were not present in the $CD8^+$ population. Furthermore, most $FOXP3^+$ cells were found in the $CD25^+$ fraction. Whereas the frequency of *FOXP3* gene-expressing $CD4^+$ gingival T-cell clones was >90% (10), not all $CD4^+$ T cells were $FOXP3^+$ in the lesion. Discrepancy between *in vivo* and *ex vivo* FOXP3 expression of the $CD4^+$ T cells may be the result of *in vitro* stimulation of the T-cell clones by anti-CD3 and anti-CD28 antibodies. In this regard, Wang et al. found that FOXP3 expression was induced when $CD4^-CD25^-FOXP3^-$ T cells were activa-

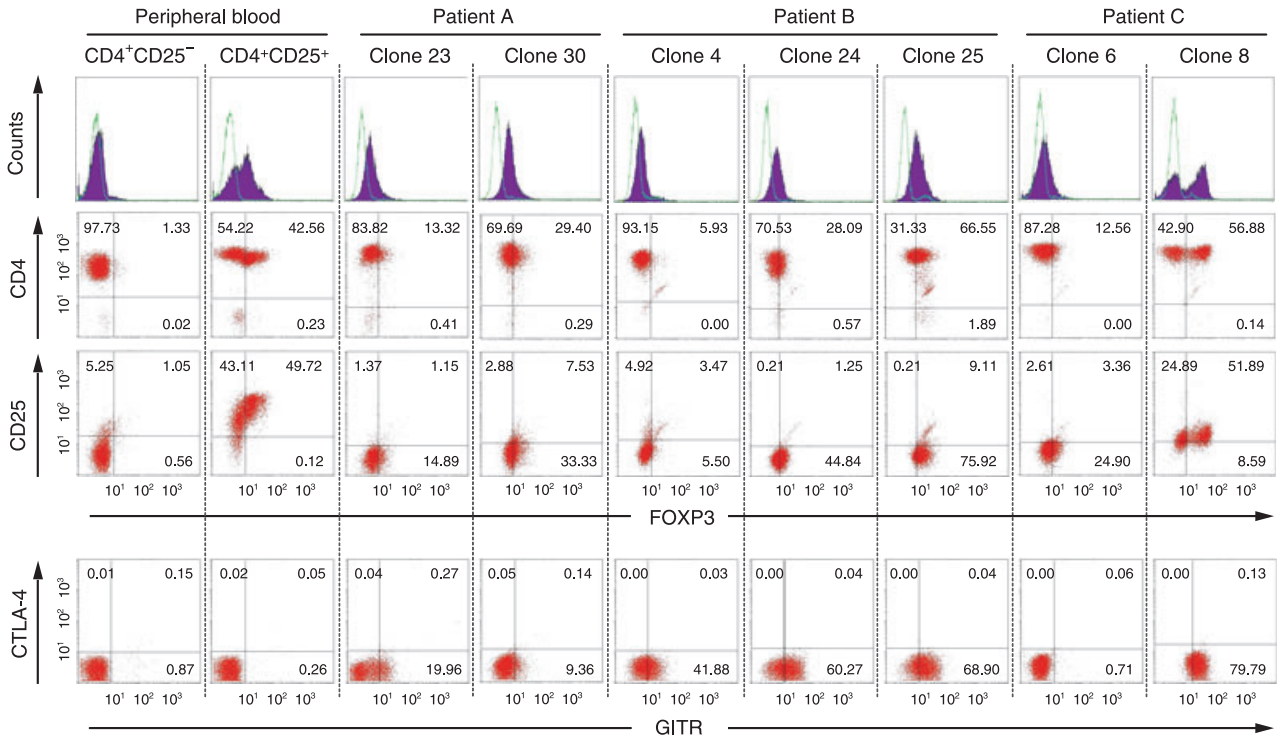

Fig. 3. Expression of FOXP3, CD25, GITR, and CTLA-4 in $CD4^+$ $CD25^-$ T cells, $CD4^+$ $CD25^+$ T cells, and gingival T-cell clones. FOXP3 expression is also represented as histograms. The line represents the isotype control.

to TCR stimulation (data not shown). Therefore, we used irradiated $CD4^+$ $CD25^+$ T cells or gingival T-cell clones in the subsequent co-culture experiments with $CD4^+$ $CD25^-$ T cells. As shown in Fig. 5, $CD4^+$ $CD25^-$ T cells exhibited weak proliferation without TCR stimulation in the presence of autologous accessory cells but a marked proliferative response with TCR stimulation. When $CD4^+$ $CD25^-$ T cells were co-cultured with irradiated autologous $CD4^+$ $CD25^+$ T cells in the presence of anti-CD3 and anti-CD28 antibodies, the proliferative response was clearly suppressed in patient A. However, in the case of patient B the suppressive effect of $CD4^+$ $CD25^+$ T cells was not obvious because the proliferative response of $CD4^+$ $CD25^-$ T cells was weaker compared with patient A. In contrast, co-culture of $CD4^+$ $CD25^-$ T cells with irradiated gingival T-cell clones,

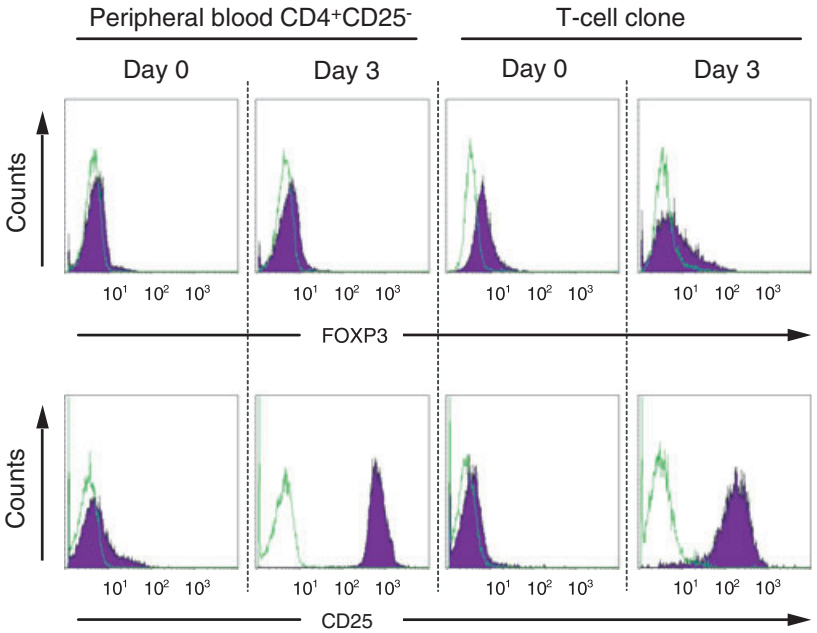

Fig. 4. Expression of CD25 and FOXP3 on $CD4^+$ $CD25^-$ T cells and gingival T-cell clones stimulated with anti-CD3 and anti-CD28 monoclonal antibodies. Immunomagnetic bead-separated $CD4^+$ $CD25^-$ T cells and gingival T-cell clones were stimulated with anti-CD3 and anti-CD28 (both at 0.1 μg/ml) for 3 days. The cells were harvested and analyzed by flow cytometry. Data are represented as histograms. The line represents the isotype control.

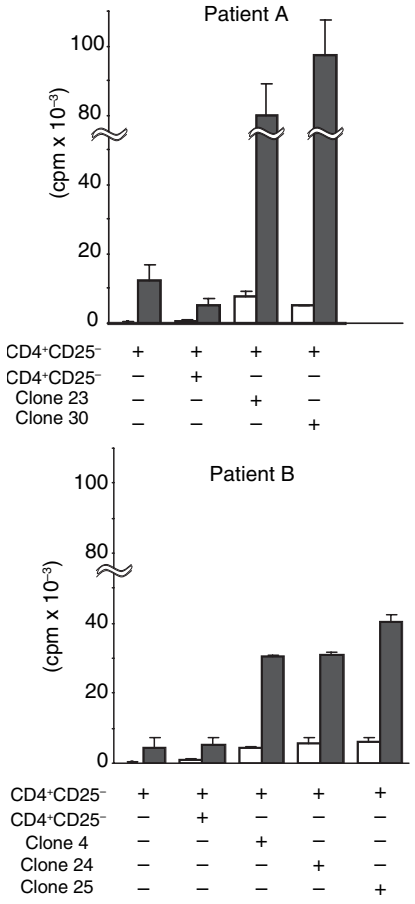

Fig. 5. Proliferative response of $CD4^+$ $CD25^-$ T cells to T-cell receptor stimulation in co-culture. $CD4^+$ $CD25^-$ T cells were cultured alone or co-cultured with irradiated $CD4^+$ $CD25^+$ T cells or gingival T-cell clones, and proliferative responses were determined in the presence or absence of anti-CD3 and anti-CD28 antibodies (both at 0.1 μg/ml) after 6 days. □, unstimulated control; ■, stimulated with anti-CD3 and anti-CD28 antibodies.

ted through TCR cross-linking, expression peaking at day 7 with ~ 90% of the cells being $FOXP3^+$ (23). Similarly, Gavin et al. reported that a small subset of human $CD4^+$ and $CD8^+$ T cells transiently upregulated FOXP3 upon *in vitro* stimulation (6). Although the induction of FOXP3 by TCR ligation was transient (23), repeated stimulation during the cloning process may have induced prolonged FOXP3 expression in some T-cell clones. The level of FOXP3 expression of most T-cell clones was lower than that of $CD4^+$ $CD25^+$ regulatory T cells. Since many of the target genes of *Foxp3* are reported to be key modulators of T-cell activation and function (14), absence of regulatory activity of gingival T-cell clones may be explained by their low level of FOXP3. However, the role of a gingival T-cell clone expressing relatively high levels of FOXP3 remains to be determined (clone 8 from patient C).

There is controversy over the value of FOXP3 as a $CD4^+$ $CD25^+$ regulatory T-cell marker that has arisen from conflicting data of *in vitro* activation experiments using $CD4^+$ $CD25^-$ T cells. Initial work in humans supported the mouse data that Foxp3 is not only a faithful marker for $CD4^+$ $CD25^+$ Tr but is both necessary and sufficient for their development and function (2, 13). However, Walker et al. demonstrated that activation of human $CD4^+$ $CD25^-$ T cells led to the expression of FOXP3 and the expression of FOXP3 in activated $CD4^+$ $CD25^+$ cells correlated with regulatory function (22). This report was followed by others showing that FOXP3 was induced in human $CD4^+$ T cells by either polyclonal or antigen-specific stimulations (1, 15). These studies showed that regulation of FOXP3 expression was different in humans and rodents, but still supported the conclusion that FOXP3 expression in $CD4^+$ T cells was equated with regulatory activity (28). However, activated T cells which became $FOXP3^+$ transiently fail to show regulatory activity (6), (23). Thus, the target genes of FOXP3 in humans may be different from those in mice (14, 27).

Flow cytometric analysis revealed that the expression level of FOXP3 of some gingival T-cell clones was equivalent to or even higher than that of peripheral blood $CD4^+$ $CD25^+$ T cells. In spite of apparent FOXP3 expression, those T-cell clones upregulated the proliferative response of autologous $CD4^+$ $CD25^-$ T cells in the presence of anti-CD3 and anti-CD28 antibodies. These findings indicate that $CD4^+$ $FOXP3^+$ gingival T-cell clones are functionally different from the natural regulatory T cells that express not only CD4 and FOXP3 but also high levels of CD25.

Another possible explanation for the lack of regulatory activity of $FOXP3^+$ gingival T-cell clones is the difference of CD25 expression between $CD4^+$ $CD25^+$ regulatory T cells and gingival T-cell clones. Whereas $CD4^+$ $CD25^+$ regulatory T cells express high levels of CD25, the CD25 expression of the gingival T-cell clones was low. Our previous study demonstrated that most of the T-cell clones established from inflamed gingival tissues expressed the *CD25* gene. The discrepancy between gene expression and cell surface expression of protein is not known. However, it is speculated that CD25 is transcribed and translated but not expressed on the cell surface. In fact, gingival T-cell clones do have intracellular CD25 (data not shown). A number of studies have demonstrated that CD25 is the hallmark

antigen of regulatory T cells in mice and humans (2, 4, 11, 13, 18). The intensity of CD25 expression is considered to be a reliable marker for regulatory T cells in the peripheral blood (2). However, this may not be sufficient to identify regulatory T cells in inflamed tissues because it is not T-cell-restricted and cannot be used to distinguish $CD4^+$ $CD25^+$ regulatory T cells from activated effector T cells. In fact, it is reported that T cells in periodontitis lesions are activated with increased proportions of $CD25^+$ and HLA-DR^+ cells (20).

Immunohistological double staining showed that the most $FOXP3^+$ cells were $CD25^+$. This suggests that the majority of periodontitis lesion-infiltrating $FOXP3^+$ cells are $CD4^+$ $CD25^+$. However, immunohistological procedures can be used to identify the cells that are positive for CD25 but cannot be used to compare the expression levels between each positive cell. Therefore, the exact level of CD25 expression of the cells in the tissues is not known. Considering the involvement of the autoimmune mechanism in the pathogenesis of periodontitis (7), there may be $CD4^+$ $CD25^+$ $FOXP3^+$ regulatory T cells in periodontitis lesion.

$CD4^+$ $CD25^+$ regulatory T cells are also characterized by the constitutive expression of other markers such as CTLA-4 (17). Although CTLA-4 mRNA was expressed in gingival T-cell clones, neither cell-surface expression nor intracellular expression of CTLA-4 was confirmed by flow cytometry. In the present study, $CD4^+$ $CD25^+$ T cells suppressing proliferation of $CD4^+$ $CD25^-$ T cells were also CTLA-4^-. Thus, unlike mice, neither FOXP3 nor CD25 is a definitively characteristic marker for regulatory T cells in humans.

Nonetheless, this is the first study to show that the human $CD4^+$ $CD25^{low}$ $FOXP3^+$ T cells derived from chronic inflammatory lesions function as effector T cells in terms of the proliferation of $CD4^+$ $CD25^-$ T cells in response to TCR stimulation.

In conclusion, most, if not all, of the $FOXP3^+$ T cells in periodontitis lesions are different from natural regulatory T cells. Further studies are needed to clarify the role of this unusual $CD25^{low}$ $FOXP3^+$ T-cell population in periodontal disease.

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