J Periodont Res 2006; 41; 73–79

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2005.00837.x

Increased infiltration of CD1d$^+$ and natural killer T cells in periodontal disease tissues

R. Amanuma[1,3], T. Nakajima[2], H. Yoshie[3], K. Yamazaki[1]

[1]Periodontology and Immunology, Department of Oral Health and Welfare, Niigata University Faculty of Dentistry,[2]General Dentistry and Clinical Education Unit, Niigata University Medical and Dental Hospital, [3]Division of Periodontology, Department of Oral Biological Science, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

Amanuma R, Nakajima T, Yoshie H, Yamazaki K. Increased infiltration of CD1d$^+$ and natural killer T cells in periodontal disease tissues. J Periodont Res 2006; 41: 73–79. © Blackwell Munksgaard 2005

Background: Natural killer T (NKT) cells are a unique T lymphocyte subset that has been implicated in the regulation of immune responses associated with a broad range of diseases including autoimmunity, infectious diseases, and cancer. In contrast to conventional T cells, NKT cells are reactive to major histocompatibility complex (MHC) class I-like molecule CD1d. Considering the periodontitis having both aspects of infection and autoimmunity in nature, CD1d and reactive NKT cells are of particular importance.

Objective: The aim of the present study was to examine whether the expression of CD1 isoforms and Vα24$^+$ invariant NKT cells is associated with different disease entities, namely gingivitis and periodontitis.

Material and Methods: Immunohistochemical analysis was performed on cryostat sections of gingival tissues from 19 patients with periodontitis and eight patients with gingivitis using antibodies to CD1a, b, c, d, Vα24$^+$ invariant NKT cells, CD83, CD3 and CD19.

Results: Although all four subsets of CD1 molecules were expressed in periodontal lesions, CD1d was most abundant. CD1d expression was more frequent in periodontitis than gingivitis and increased together with increase of invariant NKT cell infiltration. Double immunohistochemical staining showed co-expression of CD1d and CD19 on identical cells and proximate infiltration of CD1d$^+$ and invariant NKT cells.

Conclusion: These findings suggest that CD1d-expressing B cells could activate NKT cells by CD1d-restricted manner and this NKT cell activation may play roles in pathogenesis of periodontal diseases.

Kazuhisa Yamazaki, Periodontology and Immunology, Department of Oral Health and Welfare, Niigata University Faculty of Dentistry, 5274 Gakkocho 2-ban-cho, Niigata 951–8514, Japan
Tel: +81 25 227 0744
Fax: +81 25 227 0744
e-mail: kaz@dent.niigata-u.ac.jp

Keywords: CD1d; immunohistochemistry; natural killer T cell; periodontal disease

Accepted for publication July 19, 2005

Periodontitis is a chronic inflammation caused by infection with periodontopathic bacteria. Although the importance of peptide antigens in the pathogenesis is well-recognized (1), the involvement of lipid antigens derived from either bacteria or self is largely unknown. Increasing evidence suggests that immune response to lipid antigens play crucial roles in host defense and the maintenance of tolerance (2–6). The recent turning point discovery is that T cells recognize not only peptides but also lipid-containing molecules, become activated and facilitate immune response (2). Although peptide antigens are recognized in major histocompatibility complex (MHC)-restricted manner, lipid-containing antigens such as lipoprotein and lipopolysaccharide (LPS) are recognized in CD1-restricted manner.

Group 1 CD1 (CD1a, b, and c) molecules are expressed mainly by professional antigen-presenting cells such as dendritic cells and B cells (mainly CD1c), whereas CD1d is more broadly expressed, being present on monocytes, macrophages and a proportion of B cells, as well as some

non-hematopoietic cells (7–9). Although antigen-specific CD1-restricted T cells recognize structurally diverse microbial lipid as CD1-presented molecules, many CD1-restricted T cells do not require foreign antigens for activation (10–12). Various ubiquitous lipid structures in mammalian cells, such as phospholipids and sphingolipid, can be presented to individual self-reactive CD1-restricted T cells.

Whereas group 1 CD1 proteins present both self and foreign lipid antigens to conventional T lymphocytes, CD1d predominantly presents self lipids to specialized T cells (13) called natural killer T (NKT) cells (14, 15). Human NKT cells express common markers for natural killer cells and the invariant Vα24JαQ T cell receptor (TCR) (12, 13, 16) and are a unique lymphocyte subtype implicated in the regulation of autoimmune response. We have previously shown that Vα24JαQ invariant NKT cells infiltrated periodontal lesions, suggesting their role to down-regulate the autoimmune response against self-components (17). However, the expression of lipid antigen-presenting CD1 molecules and reactive T-cell populations has not been extensively investigated.

Therefore, in the present study, we investigated the expression and localization of CD1 isoforms and Vα24JαQ invariant NKT cells by immunohistochemistry.

Material and methods

Patients and biopsies

Nineteen patients with moderate to advanced chronic periodontitis, referred to the Periodontal Clinic of Niigata University Medical and Dental Hospital, took part in this study. Gingival biopsies were obtained at the time of periodontal surgery or extraction of severely involved teeth. As a control, eight gingivitis tissues showing no supporting tissue destruction were also obtained from sites requiring extraction for reasons other than periodontitis, such as orthodontic treatment or pericoronitis. The clinical status of the biopsy sites is shown in Table 1. The experimental protocol was approved by the Institutional Review Board of Niigata University and informed consent was obtained from all patients prior to inclusion in this study.

Antibodies

Purified monoclonal antibodies to human invariant NKT cell that recognize invariant Vα24JαQ TCR (6B11), CD1a (HI 149), CD1b (M-T 101), CD1c (11.86), CD1d (CD1d42; all from Pharmingen, San Diego, CA, USA), CD3 (UCHT1), CD19 (HIB19; Glostrup, Denmark), and CD83 (HB15e; eBioscience, San Diego, CA, USA) were used as described below.

Immunohistochemistry

As previously described, serial sections (5 μm in thickness) were cut and stored at −20°C until used (18). Single immunohistochemical staining for the expression of invariant Vα24JαQ TCR, CD1a, CD1b, CD1c, CD1d, and CD83 was performed using an alkaline phosphatase–anti-alkaline phosphatase (APAAP) system (DakoCytomation) on the serial gingival sections as described previously (18).

As negative controls, isotype-matched primary antibodies were used and the specificities were confirmed.

Further, double staining of invariant NKT cell/CD1d, CD1d/CD19, CD1d/CD83, and CD3/CD19 was carried out on all specimens by using combined an avidin–biotin–immunoperoxidase (ABC-PO) system (Vector, Burlingame, CA, USA) and APAAP system (18). Nuclei were counterstained with hematoxylin or methyl green.

Cell analysis

Cell analysis was performed as described previously (19). Briefly, the degree of inflammation was confirmed by hematoxylin–eosin staining. Areas of significant round cell infiltrate in the connective tissues, which contained both T cells and B cells subjacent to the pocket epithelium as determined on CD3/CD19 double-stained slides, were selected. Six areas were selected on each stained section per specimen. Sections from each gingivitis and periodontitis specimen were analyzed for four areas because the cell infiltrates were not large enough to select six areas. Those cells which surface being labeled with coloring substrate were identified as positive cells. Positive cells were counted for these selected foci with an ocular grid (0.04 mm^2) at a magnification of ×400. The area selected for counting was re-located on the serial sections from each specimen using an ocular grid and histological landmarks. In each area, the total number of invariant NKT cells, $CD1a^+$, $CD1b^+$, $CD1c^+$, $CD1d^+$, and $CD83^+$ and mononuclear cells were counted. Counts were repeated at least three times and minimal variation was confirmed. The proportions of each antibody-positive cell were calculated by dividing the positive cell numbers by total mononuclear cell counts.

Statistical analysis

The differences of the expression of each molecule between disease types were analyzed using Mann–Whitney

Table 1. Clinical profile of gingival biopsy sites

	Periodontitis ($n = 19$)	Gingivitis ($n = 8$)
Gender (male/female)	7/12	6/2
Age	55.2 ± 13.6	43.9 ± 19.7
Gingival index	1.1 ± 0.8	0.5 ± 0.5
Probing depth (mm)	6.3 ± 2.2	2.7 ± 0.7
Loss of attachment (mm)	7.5 ± 2.3	3.3 ± 0.7
Tooth mobility	1.9 ± 1.0	0.0 ± 0.0
Bleeding on probing (% site)	52.6	50.0
Bone loss (%)	79.5 ± 24.9	18.6 ± 18.6

Data are expressed as mean ± SD except for gender and bleeding on probing.

U-test. Within each disease group, the proportions of CD1a$^+$, CD1b$^+$, CD1c$^+$, CD1d$^+$, and CD83$^+$ cells were compared by Wilcoxon's signed rank test. Correlation coefficients were analyzed between the proportions of CD1d$^+$ cells and invariant NKT cells. The statistical significance risk rate was set at $p < 0.05$.

Results

More CD1d$^+$ and natural killer T cells infiltrated into periodontitis than gingivitis lesions

A total of 112 and 46 areas for each single staining or each double staining were analyzed in 19 periodontitis and eight gingivitis specimens, respectively. Cellular infiltrates as characterized by T and B lymphocytes distribution in the tissues were similar to those reported previously (18–20). All four isoforms of CD1 molecules were expressed in connective tissues subjacent to periodontal pocket/gingival crevice of periodontal lesions (Figs 1 and 2). It is notable that CD1d was the most abundant in periodontal lesions (Fig. 3). A significantly higher proportion of CD1d was observed compared with group 1 CD1 and CD83 in periodontitis ($p < 0.001$). Although CD1d expression was also significantly higher than group 1 CD1 in gingivitis ($p < 0.05$), it was comparable to CD83 ($p = 0.478$).

Also, the proportion of CD1d$^+$ cells in the inflammatory cell infiltrates was significantly higher in periodontitis than in gingivitis ($p = 0.038$). Furthermore, more invariant NKT cells were observed in periodontitis lesions than gingivitis ($p = 0.018$).

Simultaneous increase of CD1d$^+$ and natural killer T cells

The proportion of CD1d$^+$ cells positively correlated with that of invariant NKT cells in the infiltrates ($r^2 = 0.242$, $p = 0.033$) (Fig. 4).

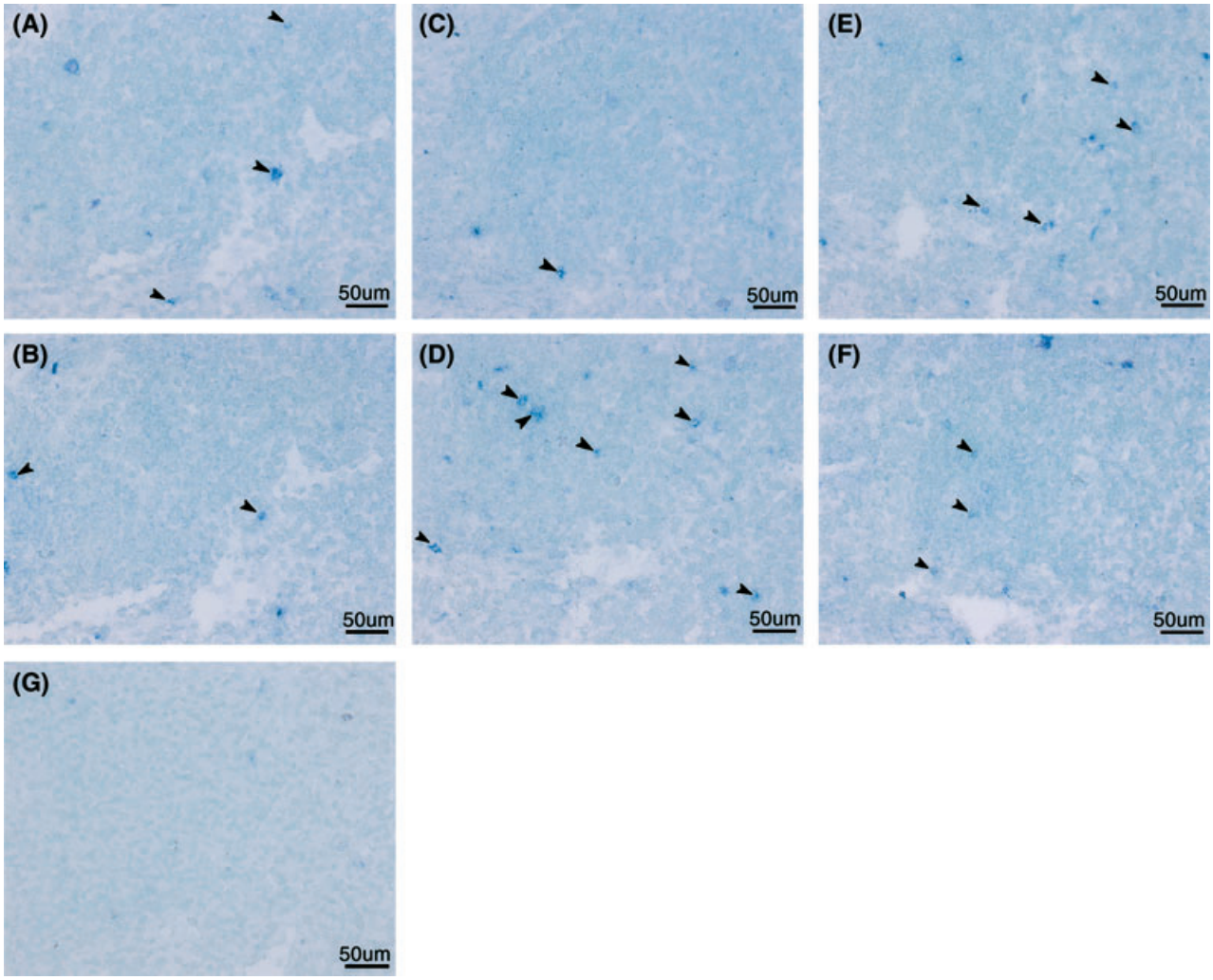

Fig. 1. Immunohistochemistry in the connective tissue subjacent to the pocket epithelium in the periodontitis lesion. A series of serial sections were stained for (A) CD1a, (B) CD1b, (C) CD1c, (D) CD1d, (E) CD83, and (F) invariant natural killer T cell by alkaline phosphatase–anti-alkaline phosphatase (APAAP) method. Negative control using a section from a periodontitis specimen is shown in (G), where mouse IgG1 was used as primary antibody. Arrow heads indicate typical positive cells.

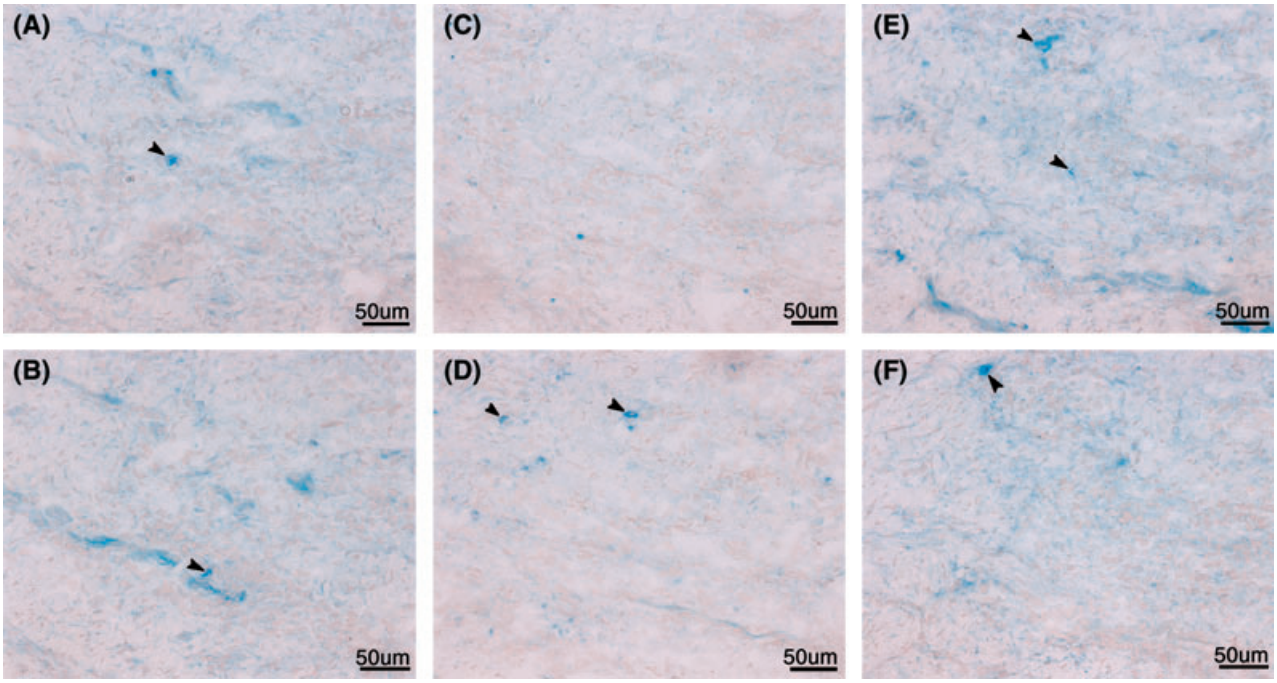

Fig. 2. Immunohistochemistry in the connective tissue subjacent to the pocket epithelium in the gingivitis lesion. A series of serial sections were stained for (A) CD1a, (B) CD1b, (C) CD1c, (D) CD1d, (E) CD83 and (F) invariant natural killer T cell by alkaline phosphatase–anti-alkaline phosphatase (APAAP) method. Arrow heads indicate typical positive cells.

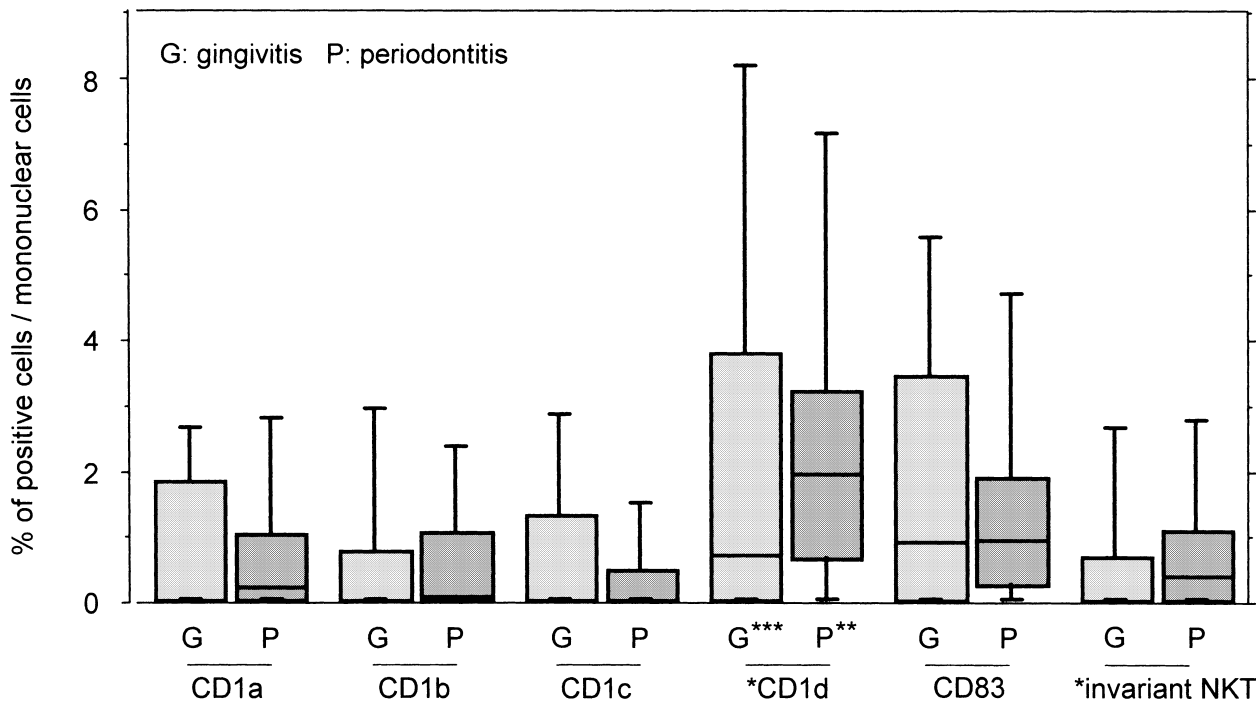

Fig. 3. Comparison of expressions of CD1 isoforms, CD83 and invariant natural killer T cell between periodontitis and gingivitis lesions. The box plots show medians, 25th and 75th percentiles as boxes, 10th and 90th percentiles as whiskers. G, gingivitis ($n = 46$); P, periodontitis ($n = 112$). *The expression of CD1d and invariant NKT was significantly higher in periodontitis compared with gingivitis ($p = 0.038$ and $p = 0.018$, respectively). **CD1d expression was significantly higher than CD1a, b, c and CD83 in periodontitis ($p < 0.001$). ***CD1d expression was significantly higher than CD1a, b, and c in gingivitis ($p < 0.05$).

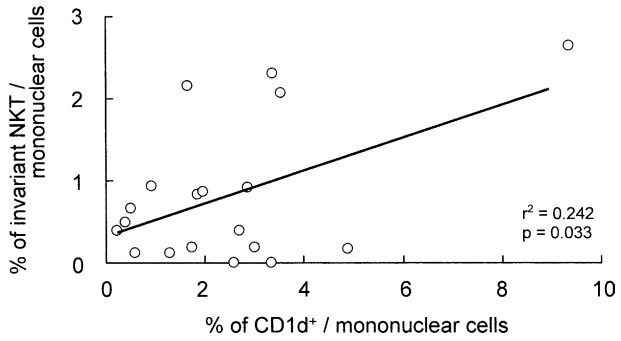

Fig. 4. Correlation of CD1d$^+$ cells with invariant natural killer T cells (NKT cells). Each dot represents the average value of each periodontitis specimen. The values correspond to the mean proportions of all analyzed areas of each specimen. Significantly positive correlations were observed between proportions of CD1d$^+$ cells and invariant NKT cells ($r^2 = 0.242$, $p = 0.033$).

CD19$^+$ B cells expressed CD1d and interacted with natural killer T cells

CD1d expression was co-localized with CD19 expression. Double immunohistochemical staining clearly demonstrated co-expression of CD1d and CD19 on identical cells (Fig. 5A). In contrast, CD83, the marker of mature dendritic cells, was neither co-localized nor co-expressed on the identical cell with CD1d (data not shown). Double immunohistochemical staining showed proximity between some CD1d$^+$ cells and invariant NKT cells (Fig. 5B).

Discussion

In the present study, we demonstrated that the proportions of CD1d$^+$ cells and invariant NKT cells were significantly higher in periodontitis lesion compared with in gingivitis lesion. These findings clearly indicate that concomitant and preferential infiltration of CD1d$^+$ and invariant NKT cells at the site of chronic inflammation induced by microbial infection in humans. In periodontitis lesion, the proportion of CD1d$^+$ cells was also significantly higher than that of CD83$^+$, possible mature dendritic cells, suggesting comparable importance of CD1d$^+$ cells with mature dendritic cells as antigen-presenting cells. Although CD1d can be expressed on dendritic cells, double staining of CD83 and CD1d showed that positive cells for these two markers were distinct. This suggests that mature dendritic cells are not CD1d$^+$ in periodontal lesions. Although the full repertoire of antigens presented by CD1 molecules, particularly by CD1d, has not been characterized, they are known to present lipid-containing molecules such as phospholipids and sphingolipids (6). This implies that T cells in periodontitis lesion might be activated by lipid-containing antigens in CD1-mediated manner, as well as by peptide antigens by MHC on mature dendritic cells.

Although the importance of NKT cells in microbial infection and autoimmunity has been well recognized, physiological antigens for NKT cells has not been clarified. In this context, Brigl *et al.* reported that NKT cells recognized self- but not microbial antigens (21). Recently, Mattner *et al.* have shown that gram-negative LPS-positive *Salmonella typhimurim* activate NKT cells through the recognition

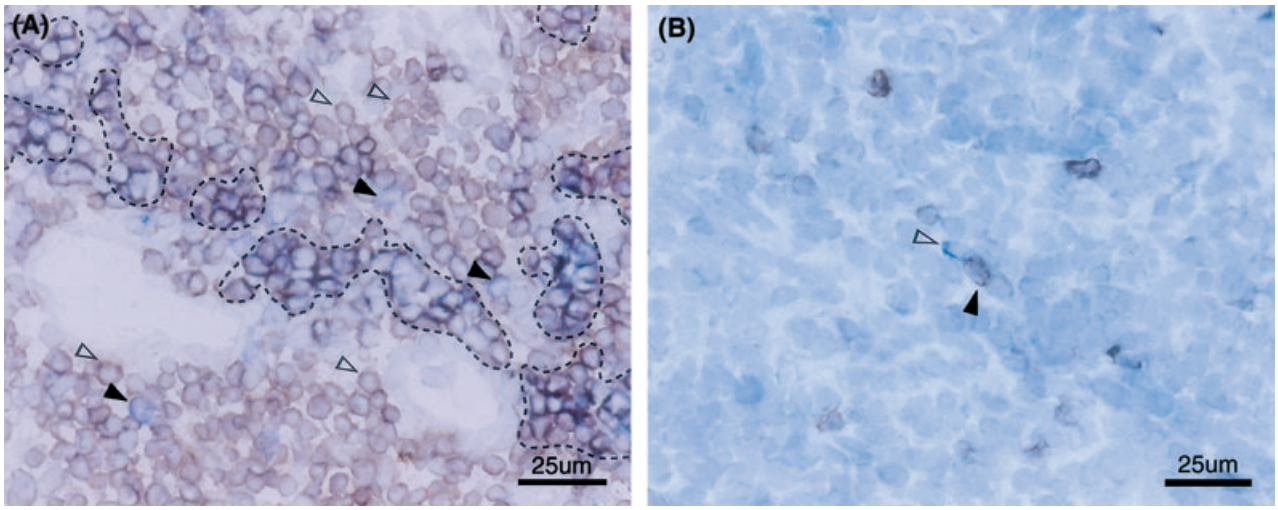

Fig. 5. Double immunohistochemistry of CD1d/CD19 (A) and invariant natural killer T cells (NKT cells)/CD1d (B) in the connective tissue subjacent to the pocket epithelium of periodontitis specimens. (A) CD19-single-positive (brown) and CD1d-single-positive cells (blue) are indicated by open arrow heads and closed arrow heads, respectively. Clusters of and some isolated CD1d-expressing CD19$^+$ cells (double positive) can be seen in the areas surrounded by dotted lines. The number of CD1d-expressing CD19$^+$ cells in the entire area of this photomicrograph is 107. (B) Cognate interaction between invariant NKT cell (blue; open arrow head) and CD1d$^+$ cell (brown; closed arrow head) is demonstrated.

of recently identified endogenous ligand, isoglobotrihexosylceramide (22). Isoglobotrihexosylceramide is an endogenous lysosomal glycolipid and not only expanded human Vα24$^+$ NKT cells but also stimulated Th1 and Th2 cytokine secretion (23). It has also been demonstrated that most mouse and human NKT cells directly recognize glycosphingolipid from *Sphyngomonas*, gram-negative bacteria that do not contain LPS (22, 24). Thus it is becoming clear that both bacterial and endogenous glycolipids presented by CD1d can be recognized by human NKT cells, but in some instances, the recognition of bacteria by NKT cells may be indirect. One study suggested that salmonella infection may lead to an altered environment, where NKT cells are stimulated by inflammatory cytokines in combination with CD1d-mediated presentation of autologous ligands, induced in response to infection (21). This scenario could also be applicable to chronic inflammatory periodontal diseases in which inflammatory cytokines are up-regulated.

Although there are some conflicting results, NKT cell numbers are reduced in peripheral blood of patients with a variety of autoimmune diseases (25). Because of this, NKT cells have been considered to implicate in the suppression of harmful autoimmunity in humans. Effective NKT cell regulation is reported to correlate with the secretion of Th2 cytokines and subsequent suppression of Th1-mediated tissue destruction (25). They also promote tolerance induction by generation of tolerogenic dendritic cells (6, 26). However, target antigens of NKT cells in these diseases have not been characterized. In periodontitis, several lines of evidence suggest that endogenous heat shock protein 60 (27, 28) and collagen type I (29, 30) could act as autoantigens. In addition, increased Th2 responses in periodontitis lesion have been reported (18, 31, 32), although other studies have shown the predominance of Th1 (33, 34). Therefore, it is reasonable to consider that NKT cells in periodontitis lesion are likely to play protective role against autoimmune-mediated tissue destruction, even though the exact roles of and the antigens recognized by NKT cells are yet to be determined.

Another function of NKT cells is on B cells. Galli *et al*. demonstrated that human invariant NKT cells can provide direct help for B-cell proliferation and antibody production through CD1d-restricted mechanisms (35). In fact, many CD19$^+$ B cells expressed CD1d and cognate interaction between CD1d$^+$ cells and NKT cells was seen. In humans, B cells expressing CD1d are found in peripheral blood (36, 37) and in the mantle zone of secondary lymphoid follicles (36). However, such a structure can not be found in periodontitis lesions, though lymphoid foci are developed. Nevertheless, this finding is the first to show CD1d expression of B cells in chronic inflammatory lesion and may be important in understanding the nature of B-cell lesion in periodontitis. In order to confirm this, further analysis using *in vitro* co-culture experiment or triple staining would be needed.

Although the definitive roles of CD1d-restricted NKT cells have not been clarified, the increase of CD1d$^+$ and invariant NKT cells in periodontitis lesion may imply both protective role against infection by periodontopathic bacteria and regulatory role to impede harmful immune response.

Acknowledgements

We thank G. J. Seymour (Oral Biology and Pathology, Department of Dentistry, The University of Queensland) for critical discussion. This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (16390613, 16592066 and 17659655) and the Promotion of Niigata University Research Project.

The authors have declared that no conflict of interest and financial disclosure exist.

References

1. Yamazaki K, Nakajima T. Antigen specificity and T-cell clonality in periodontal disease. *Periodontol 2000* 2004;**35:**75–100.
2. Beckman EM, Porcelli SA, Morita CT, Behar SM, Furlong ST, Brenner MB. Recognition of a lipid antigen by CD1-restricted $\alpha\beta^+$ T cells. *Nature* 1994;**372:**691–694.
3. D'Andrea A, Goux D, De Lalla C *et al*. Neonatal invariant Vα24$^+$ NKT lymphocytes are activated memory cells. *Eur J Immunol* 2000;**30:**1544–1550.
4. Park SH, Benlagha K, Lee D, Balish E, Bendelac A. Unaltered phenotype, tissue distribution and function of Vα14$^+$ NKT cells in germ-free mice. *Eur J Immunol* 2000;**30:**620–625.
5. Matsuda JL, Kronenberg M. Presentation of self and microbial lipids by CD1 molecules. *Curr Opin Immunol* 2001;**13:**19–25.
6. Vincent MS, Gumperz JE, Brenner MB. Understanding the function of CD1-restricted T cells. *Nat Immunol* 2003;**4:**517–523.
7. Blumberg R, Terhorst C, Bleicher P *et al*. Expression of a nonpolymorphic MHC class I-like molecule, CD1D, by human intestinal epithelial cells. *J Immunol* 1991;**147:**2518–2524.
8. Brossay L, Jullien D, Cardell S *et al*. Mouse CD1 is mainly expressed on hemopoietic-derived cells. *J Immunol* 1997;**159:**1216–1224.
9. Spada FM, Borriello F, Sugita M, Watts GF, Koezuka Y, Porcelli SA. Low expression level but potent antigen presenting function of CD1d on monocyte lineage cells. *Eur J Immunol* 2000;**30:**3468–3477.
10. Porcelli S, Brenner MB, Greenstein JL, Balk SP, Terhorst C, Bleicher PA. Recognition of cluster of differentiation 1 antigens by human CD4$^-$CD8$^-$ cytolytic T lymphocytes. *Nature* 1989;**341:**447–450.
11. Bendelac A. Mouse NK1$^+$ T cells. *Curr Opin Immunol* 1995;**7:**367–374.
12. Exley M, Garcia J, Balk SP, Porcelli S. Requirements for CD1d recognition by human invariant Vα24$^+$ CD4$^-$CD8$^-$ T cells. *J Exp Med* 1997;**186:**109–120.
13. Dellabona P, Padovan E, Casorati G, Brockhaus M, Lanzavecchia A. An invariant Vα24-JαQ/Vβ11 T cell receptor is expressed in all individuals by clonally expanded CD4$^-$8$^-$ T cells. *J Exp Med* 1994;**180:**1171–1176.
14. Porcelli SA, Modlin RL. The CD1 system. antigen-presenting molecules for T cell recognition of lipids and glycolipids. *Annu Rev Immunol* 1999;**17:**297–329.
15. Joyce S, Van Kaer L. CD1-restricted antigen presentation: an oily matter. *Curr Opin Immunol* 2003;**15:**95–104.
16. Porcelli S, Yockey CE, Brenner MB, Balk SP. Analysis of T cell antigen receptor (TCR) expression by human peripheral blood CD4$^-$8$^-$ α/β T cells demonstrates preferential use of several Vβ genes and an invariant TCR α chain. *J Exp Med* 1993;**178:**1–16.

17. Yamazaki K, Ohsawa Y, Yoshie H. Elevated proportion of natural killer T cells in periodontitis lesions: a common feature of chronic inflammatory diseases. *Am J Pathol* 2001;**158:**1391–1398.
18. Yamazaki K, Nakajima T, Hara K. Immunohistological analysis of T cell functional subsets in chronic inflammatory periodontal disease. *Clin Exp Immunol* 1995;**99:**384–391.
19. Orima K, Yamazaki K, Aoyagi T, Hara K. Differential expression of costimulatory molecules in chronic inflammatory periodontal disease tissue. *Clin Exp Immunol* 1999;**115:**153–160.
20. Yamazaki K, Nakajima T, Aoyagi T, Hara K. Immunohistological analysis of memory T lymphocytes and activated B lymphocytes in tissues with periodontal disease. *J Periodont Res* 1993;**28:**324–334.
21. Brigl M, Bry L, Kent SC, Gumperz JE, Brenner MB. Mechanism of CD1d-restricted natural killer T cell activation during microbial infection. *Nat Immunol* 2003;**4:**1230–1237.
22. Mattner J, Debord KL, Ismail N *et al*. Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. *Nature* 2005;**434:** 525–529.
23. Zhou D, Mattner J, Cantu C. 3rd *et al*. Lysosomal glycosphingolipid recognition by NKT cells. *Science* 2004;**306:**1786–1789.
24. Kinjo Y, Wu D, Kim G *et al*. Recognition of bacterial glycosphingolipids by natural killer T cells. *Nature* 2005;**434:**520–525.
25. Hammond KJ, Kronenberg M. Natural killer T cells: natural or unnatural regulators of autoimmunity? *Curr Opin Immunol* 2003;**15:**683–689.
26. Gumperz JE, Brenner MB. CD1-specific T cells in microbial immunity. *Curr Opin Immunol* 2001;**13:**471–478.
27. Tabeta K, Yamazaki K, Hotokezaka H, Yoshie H, Hara K. Elevated humoral immune response to heat shock protein 60 (hsp60) family in periodontitis patients. *Clin Exp Immunol* 2000;**120:**285–293.
28. Yamazaki K, Ohsawa Y, Tabeta K *et al*. Accumulation of human heat shock protein 60-reactive T cells in the gingival tissues of periodontitis patients. *Infect Immun* 2002;**70:**2492–2501.
29. Hirsch HZ, Tarkowski A, Miller EJ, Gay S, Koopman WJ, Mestecky J. Autoimmunity to collagen in adult periodontal disease. *J Oral Pathol* 1988;**17:**456–459.
30. Wassenaar A, Reinhardus C, Thepen T, Abraham-Inpijn L, Kievits F. Cloning, characterization, and antigen specificity of T-lymphocyte subsets extracted from gingival tissue of chronic adult periodontitis patients. *Infect Immun* 1995;**63:**2147–2153.
31. Manhart SS, Reinhardt RA, Payne JB *et al*. Gingival cell IL-2 and IL-4 in early-onset periodontitis. *J Periodontol* 1994;**65:**807–813.
32. Lappin DF, MacLeod CP, Kerr A, Mitchell T, Kinane DF. Anti-inflammatory cytokine IL-10 and T cell cytokine profile in periodontitis granulation tissue. *Clin Exp Immunol* 2001;**123:**294–300.
33. Salvi GE, Brown CE, Fujihashi K *et al*. Inflammatory mediators of the terminal dentition in adult and early onset periodontitis. *J Periodont Res* 1998;**33:**212–225.
34. Takeichi O, Haber J, Kawai T, Smith DJ, Moro I, Taubman MA. Cytokine profiles of T-lymphocytes from gingival tissues with pathological pocketing. *J Dent Res* 2000;**79:**1548–1555.
35. Galli G, Nuti S, Tavarini S *et al*. CD1d-restricted help to B cells by human invariant natural killer T lymphocytes. *J Exp Med* 2003;**197:**1051–1057.
36. Brigl M, Brenner MB. CD1: antigen presentation and T cell function. *Annu Rev Immunol* 2004;**22:**817–890.
37. Fais F, Morabito F, Stelitano C *et al*. CD1d is expressed on B-chronic lymphocytic leukemia cells and mediates α-galactosylceramide presentation to natural killer T lymphocytes. *Int J Cancer* 2004;**109:**402–411.