Oral Microbiology Immunology 2006: 21: 411–414
Printed in Singapore. All rights reserved

ORAL MICROBIOLOGY AND IMMUNOLOGY

Short communication

The role of $CD4^+$ and $CD8^+$ T cells on antibody production by murine Peyer's patch cells following mucosal presentation of *Actinomyces viscosus*

W. Sosroseno[1,2], P. S. Bird[3], E. Gemmell[3], G. J. Seymour[3,4]

[1]Department of Oral Biology, School of Dental Sciences, and Department of Immunology, School of Medical Sciences, Universiti Sains Malaysia, Kota Baru, Malaysia, [2]Department of Oral Biology, Faculty of Dentistry, Gadjah Mada University, Yogyakarta, Indonesia, [3]Department of Oral Biology and Pathology, School of Dentistry, The University of Queensland, St Lucia, Brisbane Qld, Australia, [4]Faculty of Dentistry, The University of Otago, Dunedin, New Zealand

Sosroseno W, Bird PS, Gemmell E, Seymour GJ. The role of $CD4^+$ and $CD8^+$ T cells on antibody production by murine Peyer's patch cells following mucosal presentation of Actinomyces viscosus.
Oral Microbiol Immunol 2006: 21: 411–414. © Blackwell Munksgaard, 2006.

The aim of this study was to determine the role of CD4 and CD8 cells on specific antibody production by murine Peyer's patch (PP) cells after oral immunization with *Actinomyces viscosus* in mice. Female DBA/2 mice were orally immunized with three low doses of heat-killed *A. viscosus*. Sham-immunized mice served as a control group. Mice were depleted of CD4 or CD8 cells by intraperitoneal injection of anti-CD4 or anti-CD8 antibodies daily for 3 days before oral immunization. One week after the last oral immunization, PPs were removed and cell suspensions were cultured with *A. viscosus*. Specific antibody production in the culture supernatants was assessed by enzyme-linked immunosorbent assay. The results showed that oral immunization with *A. viscosus* induced a predominant specific immunoglobulin A (IgA) response by PP cells and, to a lesser extent, IgM antibodies. Depletion of CD4 but not CD8 cells suppressed the production of specific antibodies. These results suggest that oral immunization with low doses of *A. viscosus* may induce the production of specific antibodies by murine PP cells in a CD4-cell-dependent fashion.

Key words: *Actinomyces viscosus*; antibody; CD4 cells; CD8 cells; mice; Peyer's patch

W. Sosroseno, Department of Oral Biology, School of Dental Sciences, Universiti Sains Malaysia, 16150 Kota Bharu, Malaysia
Tel.: 60 9 766 3752;
fax: 60 9 764 2026;
e-mail: wihaskoro@kb.usm.my
Accepted for publication February 1, 2006

Intestinal Peyer's patches (PPs) are the main inductive sites of lymphocyte activation against antigens in the gut; these lymphoid nodules contain all the immunocompetent cells necessary for the development of an immune response (9, 12). Murine PP dendritic cells secrete high levels of interleukin-10 (IL-10) and preferentially activate T helper type 2 (Th2) cells, which in turn help B cells to develop into immunoglobulin A^+ (IgA) secreting cells (3, 10). However, both Th1 and CD8 cells in murine PPs have also been shown to be activated following oral immunization (5, 17). This suggests that following oral immunization of mice, the development of a PP immune response, particularly with regards to the generation of IgA^+ B cells, may require the activation of CD4 cells.

Actinomyces viscosus, a gram-positive facultative anaerobic bacterium, is one of the first organisms to colonize the tooth surface and has been associated with root caries and gingivitis in humans (2, 18). A previous study demonstrated that oral immunization of mice with 100 μg of an *A. viscosus* bacterial suspension administered on three sequential occasions leads to the induction of antigen-specific systemic immune tolerance, also known as oral tolerance, mediated by antigen-specific $CD4^+$ and $CD8^+$ T cells (14–16). Since oral immunization is capable of inducing a mucosal immune response (9), the aim of this study was to determine whether oral immunization with *A. viscosus* induces antibody production by murine PP cells and to investigate the role of $CD4^+$ and $CD8^+$ T cells on this PP cell immune response.

Materials and methods

A. viscosus T14, a kind donation from Dr A.C.R. Tanner, The Forsyth Institute, Boston, MA, was grown anaerobically in trypticase soy broth medium (BBL, Microbiology System, Cockeyville, MD), and heat killed as described previously (14). The protein concentration of the bacterial suspension was determined using a BCA protein assay kit (Pierce Biotechnology, Rockford, IL). Female 6- to 8-week-old DBA/2 mice were orally immunized with 100 μg heat-killed bacteria in phosphate-buffered saline (PBS) containing 5% sodium bicarbonate at days 1, 2 and 5. Control mice were orally immunized with PBS containing 5% sodium bicarbonate alone over the same time period. One week after the last immunization, the mice were sacrificed and PPs were obtained and washed, opened via the luminal surface and teased carefully on sterile stainless steel grids. The cells were washed and viable cells were counted. Then, 2×10^5 cells were cultured in triplicate in RPMI-1640 (CSL, Melbourne, Australia) containing 1% glutamine (Sigma, St Louis, MO), 10% heat-inactivated fetal calf serum (CSL) and 5×10^{-3} mol 2-mercaptoethanol (Sigma) in 96-well round-bottomed plates (Nunc, Roskilde, Denmark) for 10 days. PP cells were stimulated with 0.4 μg bacterial suspension per well at day 0. Specific antibody production in the culture supernatants from each well was determined by enzyme-linked immunosorbent assay (ELISA) as previously described (13).

To determine the role of $CD4^+$ and $CD8^+$ T cells *in vivo*, mice were injected with anti-CD4 and anti-CD8 monoclonal antibodies, respectively, as described previously (13, 16). Briefly, mice were injected intraperitoneally with PBS containing 1 mg rat anti-mouse CD4 cell antibodies (clone GK1.5, IgG2b isotype), mouse anti-mouse CD8 cell antibodies (clone 49-11.2, isotype IgG2a), mouse anti-*Fusobacterium nucleatum* antibodies (clone FN4BA4, isotype IgG2a) or purified rat immunoglobulin for 3 consecutive days. Two days after the last injection, mice were sacrificed and PP cells were obtained. Flow cytometric analysis (Becton Dickinson, Mountain View, CA) showed that these treatments resulted in the depletion of the respective $CD4^+$ and $CD8^+$ T-cell subsets in the PPs for at least 2 weeks (data not shown). In one experiment, mice were divided into three groups, each consisting of three to five mice. Group I mice were injected with PBS only for 3 consecutive days. Group II and III mice were injected with rat immunoglobulin and anti-CD4 antibodies, respectively. In another experiment, mice were again divided into three groups. Group A was injected with PBS alone, whereas Groups B and C were injected with anti-*F. nucleatum* and anti-mouse CD8 antibodies, respectively. Two days after the last injection, all mice were orally immunized with *A. viscosus* as above. One week after the last oral immunization, the mice were sacrificed and PP cells were obtained and cultured as described above and specific anti-*A. viscosus* antibody production was determined by ELISA.

The data were analysed by repeat measurement test using a statistical software package (Minitab, Inc., State College, PA).

Results

PP cells from mice orally immunized with *A. viscosus* produced significantly higher levels of specific antibodies than those from sham intragastrically immunized mice ($P < 0.05$) (Fig. 1). Specific IgA and, to a lesser extent, IgM antibodies were the predominant isotypes ($P < 0.05$). No specific antibodies were produced by PP cells taken from mice depleted of $CD4^+$ T cells before intragastric immunization, compared with normal levels in cultures of PP cells taken from mice injected with PBS or purified rat immunoglobulin ($P < 0.05$) (Fig. 2). In contrast, the specific antibody response by PP cells from mice depleted of $CD8^+$ T cells before intragastric immunization remained intact ($P > 0.05$) (Fig. 3).

Discussion

The results of the present study showed that high levels of anti-*A. viscosus* antibodies, predominantly the IgA isotype, were produced by PP cells taken from orally immunized mice. These results are in agreement with previous studies that oral immunization induced a mucosal immune response initiated in the PP in mice (6, 7). Furthermore, PP dendritic cells, which produce cytokines such as IL-10, may preferentially help in the activation of Th2 cells, and hence in the generation of IgA^+ B cells (3, 10). However, whether or not oral immunization with *A. viscosus* would stimulate PP-cell-

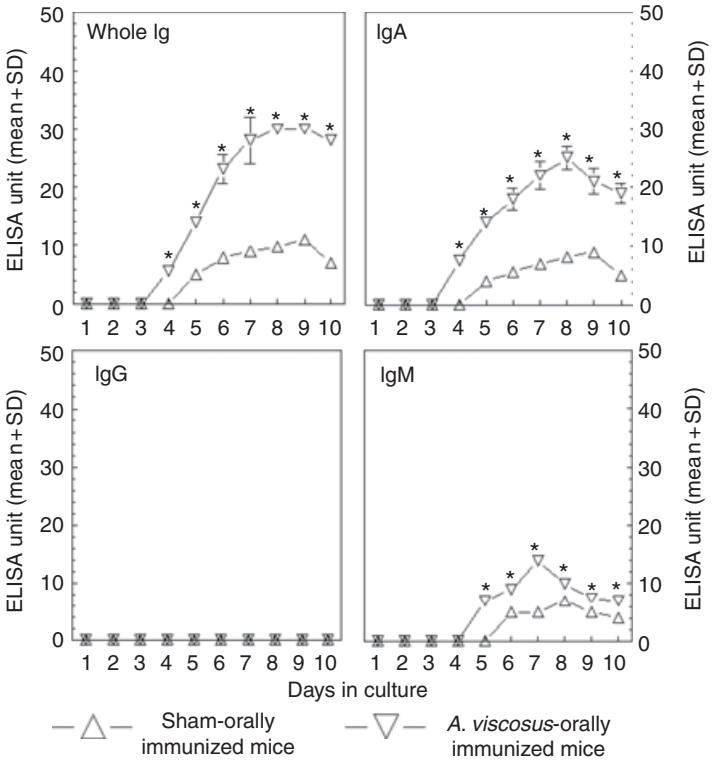

Fig. 1. Specific antibody production by murine PP cells after oral immunization with *Actinomyces viscosus*. Mice were immunized with heat-killed *A. viscosus* and PP cells cultured with antigen and specific antibody production in the culture supernatants determined by ELISA. Sham-orally immunized mice served as the control group. Each group consisted of three to five mice. *Significant difference from the control group at $P < 0.05$.

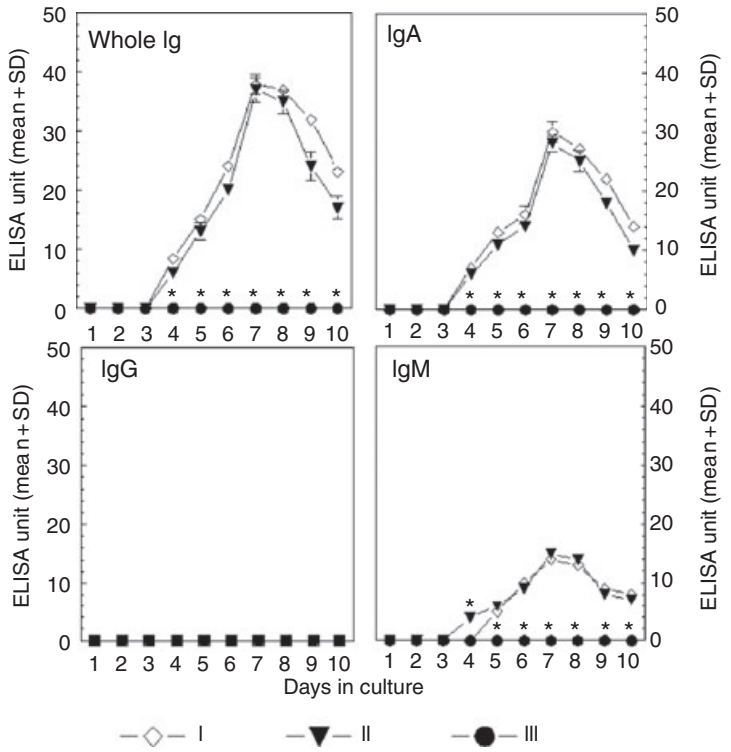

Fig. 2. The effect of CD4 cell depletion on specific antibody production by murine PP cells after oral immunization with *Actinomyces viscosus*. Mice were intraperitoneally injected with PBS (group I), rat immunoglobulin (group II) or rat anti-CD4 antibodies (group III) for 3 consecutive days and 2 days after the last injection, all mice were orally immunized with *A. viscosus*. Each group consisted of three to five mice. *Significant difference from the control (group I) at $P < 0.05$.

derived cytokines, which might in turn enhance *A. viscosus*-specific IgA antibody levels, remains to be investigated further.

Oral immunization of mice with low doses of *A. viscosus* has been shown to activate PP suppressor cells, which migrated to the spleen to induce systemic antigen-specific immune suppression or oral tolerance (14). Therefore, it seems plausible that oral immunization with *A. viscosus* induces both systemic immune tolerance and a local immune response concomitantly. This induction of a concurrent local immune response and systemic immune tolerance after mucosal presentation of certain antigens such as ovalbumin is well documented (9). It has been suggested that T cells are activated at both the local and systemic immune compartments, but T-cell proliferation in the latter immune compartment is gradually decreased (11).

In the present study, the levels of specific IgA and to a lesser extent IgM anti-*A. viscosus* antibodies produced by PP cells *in vitro* from CD4-cell-depleted mice were inhibited, suggesting that specific antibody production by PP cells after oral immunization with *A. viscosus* is a CD4-cell-dependent mechanism. Another study showed that treatment of mice with anti-CD4 antibodies led to a reduction in PP size and in the germinal center although the number of IgA^+ B cells remained the same (8), suggesting that CD4 cells are required for the differentiation of IgA^+ B cells into IgA-producing plasma cells.

The results of the present study also indicated that levels of specific antibody were not decreased in CD8-depleted mice. However, Lagoo et al. (5) demonstrated that murine PP CD8 cells stimulated with anti-CD3 monoclonal antibody may produce IL-5 and IL-10, which provide help for PP B cells to produce IgG and IgA antibodies. This previous study by Lagoo and colleagues may imply that a reduced number of CD8 cells in PP may alter the production of IgA antibodies. Therefore, PP CD8 cell help in the production of IgA and/or IgM antibodies may be dependent upon the nature of the antigen.

Katz and Michalek (4) have demonstrated that transfer of PP cells from mice orally immunized with *Porphyromonas gingivalis* into nude recipients resulted in the production of high levels of specific salivary IgA, which was protective against vertical alveolar bone loss. This suggests that *P. gingivalis*-activated PP CD4 cells may migrate to the salivary glands where they provide help in the production of protective salivary IgA. If this is so, then mucosal challenge by *A. viscosus* may also induce antigen-specific B and/or T cells in the PP, which migrate to the salivary glands where the production of specific salivary IgA may regulate the course of gingivitis (1). Further studies however, are required to determine the role of *A. viscosus*-stimulated PP cells in periodontal disease.

Acknowledgments

This work was supported by NH & MRC grant, Australia. While employed by GMU, W.S. was supported by a fellowship from the World Bank Project XVII, the Indonesian government. The authors are grateful to Drs M. Good (QIMR, Brisbane) and R. Scollay (Walter and Eliza Hall Institute, Melbourne) for kindly providing clone GK1.5 and clone 49-11.2, respectively.

References

1. Crawford PC, Clark WB. Fimbriae-specific antibodies in serum and saliva of mice immunized with *Actinomyces viscosus* T14V fimbriae. Infect Immun 1985: **54**: 507–515.
2. Haffajee AD, Cugini MA, DiBart S, Smith C, Kent RL Jr, Socransky SS. Clinical and microbiological features of subjects with adult periodontitis who responded poorly to scaling and root planing. J Clin Periodontol 1997: **24**: 767–776.
3. Iwasaki A, Kelsall BL. Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. J Exp Med 1999: **190**: 229–239.
4. Katz J, Michalek SM. Effect of immune T cells derived from mucosal or systemic tissue on host responses to *Porphyromonas gingivalis*. Oral Microbiol Immunol 1998: **13**: 73–80.
5. Lagoo AS, Eldridge JH, Lagoo-Deenadayalan S et al. Peyer's patch CD8+ memory T cells secrete T helper type I and type 2 cytokines and provide help for immunoglobulin secretion. Eur J Immunol 1994: **24**: 3087–3092.
6. Lee HO, Cooper CJ, Choi JH, Alnadjim Z, Barrett TA. The state of $CD4^+$ T cell activation is a major factor for determining the kinetics and location of T cell responses to oral antigen. J Immunol 2002: **168**: 3833–3838.
7. McSorley SJ, Asch S, Costalonga M, Reinhardt RL, Jenkins MK. Tracking *Salmonella*-specific CD4 T cells in vivo reveals a local mucosal response to a disseminated infection. Immunity 2002: **16**: 365–377.

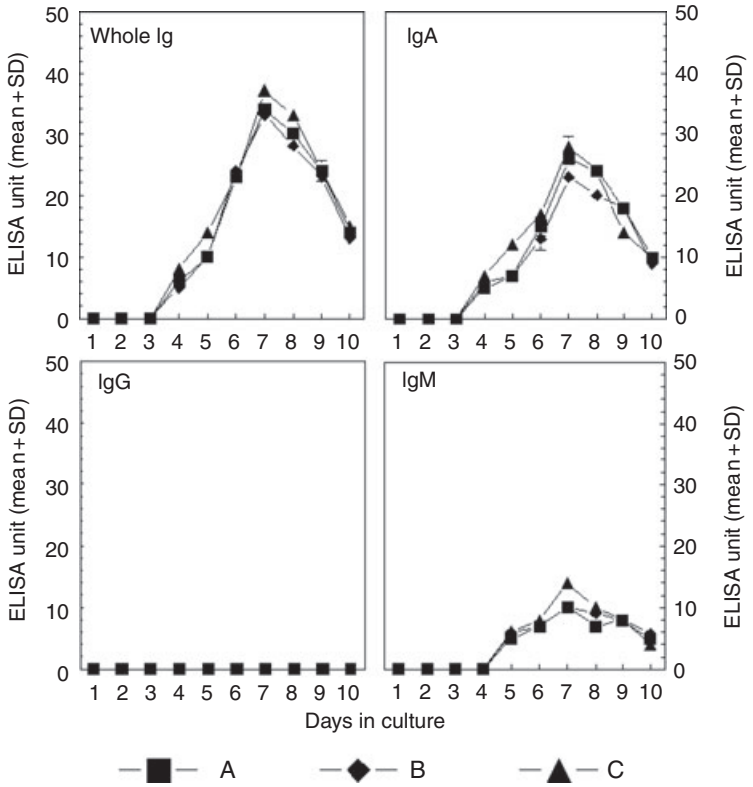

Fig. 3. The effect of CD8 cell depletion on specific antibody production by murine PP cells after oral immunization with *Actinomyces viscosus*. Mice were intraperitoneally injected with PBS (group A), anti-*F. nucleatum* antibodies (group B) or anti-CD8 cell antibodies (group C) for 3 consecutive days and 2 days after the last injection, all mice were orally immunized with *A. viscosus*. Each group consisted of three to five mice.

8. Mega J, Bruce MG, Beagley KW et al. Regulation of mucosal responses by CD4+ T lymphocytes: effects of anti-L3T4 treatment on the gastrointestinal immune system. Int Immunol 1991: **3**: 793–805.
9. Mowat AM. Anatomical basis of tolerance and immunity to intestinal antigens. Nature Rev Immunol 2003: **3**: 331–341.
10. Okahashi N, Yamamoto M, Vancott JL et al. Oral immunization of interleukin-4 (IL-4) knockout mice with recombinant *Salmonella* strain or cholera toxin reveals that CD4+ Th2 cells producing IL-6 and IL-10 are associated with mucosal immunoglobulin A responses. Infect Immun 1996: **64**: 11516–11525.
11. Smith KM, Davidson JM, Garside P. T-cell activation occurs simultaneously in local and peripheral lymphoid tissue following oral administration of a range of doses of immunogenic or tolerogenic antigen although tolerized T cells display a defect in cell division. Immunology 2002: **106**: 139–143.
12. Sosroseno W. Mucosal immunology. In: Seymour GJ, Savage NW, Walsh LJ, ed. Immunology. An introduction for the health sciences. Roseville, Australia: LJ., McGraw-Hill Book Co., 1995: 116–126.
13. Sosroseno W, Bird PS, Gemmell E, Seymour GJ. The role of $CD4^+$ T cells *in vivo* on the induction of immune response to *Porphyromonas gingivalis* in mice. J Periodontol 2002: **73**: 1133–1140.
14. Sosroseno W, Bird PS, Gemmell E, Seymour GJ. The induction of suppressor cells following mucosal presentation of *Actinomyces viscosus* in mice. Oral Microbiol Immunol 2003: **18**: 318–322.
15. Sosroseno W, Bird PS, Gemmell E, Seymour GJ. Oral tolerance to *Actinomyces viscosus*. Oral Disease 2006: **12**: 387–394.
16. Sosroseno W, Bird PS, Gemmell E, Seymour GJ. The role of CD4+ and CD8+T cells on the induction of oral tolerance to *Actinomyces viscosus* in mice. Oral Microbiol Immunol 2006: **21**: 151–158.
17. Yoshida T, Hachimura S, Ishimori M et al. Antigen presentation of Peyer's patch cells can induce both Th1- and Th2-type responses depending on antigen dosage, but a different cytokine response pattern from that of spleen cells. Biosci Biotechnol Biochem 2002: **66**: 963–969.
18. Zambon JJ, Kasprzak SA. The microbiology and histology of human root caries. Am J Dent 1995: **8**: 323–328.