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## ORIGINAL ARTICLE

# The induction of oral tolerance to *Actinomyces viscosus* in mice

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**OBJECTIVES: To determine whether oral tolerance with the oral bacterium *Actinomyces viscosus* was inducible in mice.**

**MATERIALS AND METHODS: Mice were intragastrically (i.g.) and then intraperitoneally (i.p.) immunized with heat-killed *A. viscosus*. A control group of mice received only saline. A delayed type hypersensitivity (DTH) response and the levels of isotype specific antibodies were assessed. Spleen cells from mice that were i.g. immunized with *A. viscosus* were transferred to *A. viscosus*-primed mice *in vivo* and *in vitro*. Furthermore, mice were i.g. immunized with saline or *A. viscosus* and then challenged i.p. with saline, *A. viscosus*, or *Porphyromonas gingivalis*.**

**RESULTS: Intragastric immunization with *A. viscosus* suppressed both DTH and serum specific antibodies to *A. viscosus*. DTH suppression lasted until week 4, while serum immunoglobulin (Ig)A and both IgG and IgM specific antibody levels remained suppressed up to week 8 and 12 respectively. IgG specific antibody suppression was transferable. The DTH response and serum antibodies specific to *A. viscosus* were suppressed in mice after i.g. challenged with *A. viscosus* but not *P. gingivalis*.**

**CONCLUSION: Mucosal presentation of *A. viscosus* in mice led to the suppression of immune response to this bacterium in an antigen-specific fashion. Tolerance of DTH response was short lived, while suppression of antigen-specific IgG antibodies in mucosally tolerized mice was long-lasting.**

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**Keywords:** *Actinomyces viscosus*, oral tolerance, mice

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## Introduction

The classical studies of Wells (1911) and Chase (1946) opened new insights into what is now known as oral tolerance. These early studies demonstrated that if mice were fed with hen's egg protein or hapten and then systemically challenged with the same antigen, suppression of the systemic immune response occurred. Since then, attention has focused upon elucidating the characteristics and mechanisms of this unique phenomenon (Sosroseno, 1995; Faria and Weiner, 1999; Garside and Mowat, 2001). Indeed, studies in humans and animals suggest that the induction of oral tolerance to specific autoantigens may reduce the severity of these autoimmune diseases, indicating the benefit of oral tolerance for treatment of autoimmune diseases (Sosroseno, 1995; Faria and Weiner, 1999; Garside and Mowat, 2001).

It has been previously reported that oral immunization of oral bacteria such as the cariogenic bacterium, *Streptococcus mutans* and the periodontopathic bacteria, *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, resulted in the generation of oral tolerance (Challacombe and Tomasi, 1980; Keys *et al*, 1987; Seymour and Ford, 1990). While characterization of oral tolerance to these periodontopathic bacteria has yet not been reported, oral immunization with *S. mutans* induced an antigen-specific oral tolerance (Challacombe and Tomasi, 1980).

*Actinomyces viscosus* is an important oral bacterium as it is among the first organisms to colonize on the tooth surface and has been associated with root caries (Bowden *et al*, 1990; Nyvad and Killian, 1990) and gingivitis in humans (Haffajee *et al*, 1997). Peripheral blood T cells specific to this bacterium in patients with gingivitis were observed, suggesting that this bacterium may play a role in the immunopathogenesis of periodontal disease (Mahanonda *et al*, 1989). As this bacterium is one of the oral commensal bacteria, a possibility that oral tolerance may be induced by continuous swallowing this bacterium cannot be ruled out. Therefore, the aim of the present study was to examine whether oral tolerance in mice was inducible by

intragastric (i.g.) administration of *A. viscosus* and to determine the duration and specificity of this immune phenomenon.

## Materials and methods

*Animals*

Female 6–8-week-old DBA/2 mice used in this study were supplied by the Central Animal Breeding Centre, The University of Queensland. Institutional Ethics approval was granted for this project.

*Antigen preparation*

*Actinomyces viscosus* T14 was a kind donation from Dr A.C.R. Tanner, The Forsyth Institute, Boston, MA, and *P. gingivalis* ATCC 33277 was a generous gift from Dr D.N. Love, Department of Veterinary Pathology and Bacteriology, The University of Sydney, NSW, Australia. *Actinomyces viscosus* was cultivated in Trypticase Soy Broth (TSB) medium (BBL, Cockeyvile, MD, USA), while *P. gingivalis* was grown in TSB supplemented with 5 g $l^{-1}$ yeast extract (Difco Lab., Detroit, MI, USA), 5 $\mu$g $ml^{-1}$ haemin (Sigma, St Louis, MO, USA), 1 $\mu$g $ml^{-1}$ menadione (Sigma), 0.5 g $l^{-1}$ L-cysteine-hydrochloride and 0.1 g $l^{-1}$ dl-dithiothreitol (BDH Chemicals, Poole, UK), as previously described (Mahanonda *et al*, 1989). The bacteria were incubated at 37°C in atmosphere of 80% $N_2$, 10% $CO_2$ and 10% $H_2$ in an anaerobic jar for 3–5 days. The purity of cultures was monitored by Gram stain and the colony morphology on supplemented TSB agar containing 5% horse blood. The bacteria were harvested and then washed three times in sterile phosphate-buffered saline (PBS) by centrifugation ($1750 \times g$ for 60 min). The bacteria were suspended in PBS and heat killed at 100°C for 15 min, then stored at −20°C. The protein concentration was determined by a BCA protein assay kit (Bio-Rad, Richmond, MI, USA).

*ELISA*

Antibody levels were measured using an indirect enzyme-linked immunosorbent assay (ELISA) method (Sosroseno *et al*, 2002). Briefly, 50 $\mu$l of a 50 $\mu$g $ml^{-1}$ suspension of *A. viscosus* in PBS was pipetted into wells of a 96-well microtiter plate (Nuncl, Roskilde, Denmark). The plate was centrifuged at $1750 \times g$ for 5 min at 4°C, then 100 $\mu$l of cold 0.25% glutaraldehyde in PBS was added to all wells and left for 15 min at room temperature (RT). The plates were washed three times and also washed three times between all other steps (below) and non-specific binding was blocked by the addition of 300 $\mu$l of PBS containing 0.05% Tween-20 (PBS-T) and 1% skim dried milk (SM). The plates were left overnight at 4°C. After washing for three times in PBST, samples were diluted to appropriate dilution in PBS-T-0.1% SM and 100 $\mu$l were added to triplicate wells. A mouse anti-*A. viscosus* hyperimmune serum was used on each plate at a standard dilution of 1:1000. This dilution represented a 50% binding level and was taken as 100 ELISA Units (EU). The total immunoglobulin (Ig), IgA, IgM and IgG antibody specific isotypes were detected using biotinylated sheep anti-mouse whole Ig and anti-mouse IgA at 1:5000 and anti-IgM and IgG at 1:20 000 and the plates were incubated for 60 min at RT. These antibodies were purchased from Promega, Madison, WI. Streptavidin horseradish peroxidase (Amersham International, Buckinghamshire, UK) diluted at 1:10 000 and 100 $\mu$l was added to all wells. Following incubation for 30 min, 150 $\mu$l/well of substrate containing 0.0075% $H_2O_2$ and 2.5 mM *o*-Tolidine hydrochloride substrate in 100 mM phosphate citrate buffer (pH 4.5) were added to all wells. The blue color reaction was stopped after 10 min by 50 $\mu$l/well of 3 M HCl and the optical density (OD) was measured at 450 nm using a Titretek Multiscan (ICN-Flow Lab, Costa Mesa, CA, USA). The OD readings of the background (PBS-TSM instead of sample) were subtracted from all readings. The EU was calculated by dividing the OD of the sample by that of the standard OD and then multiplying by 100 (Sosroseno *et al*, 2002).

*Serum antibody levels*

Mice were killed 24 h after footpad challenge. Blood was collected by cardiac puncture and the serum separated by centrifugation, and stored at −20°C. Serum antibody levels to *A. viscosus* were determined using an ELISA (above). Serum was diluted 1:300 in PBS-SM and assayed in duplicate wells. Positive and negative controls were included in each assay.

*Delayed type hypersensitivity*

Delayed type hypersensitivity (DTH) was measured using footpad swelling as described previously (Sosroseno *et al*, 2002). Briefly, 1 week after the last intraperitoneal (i.p.) injection, mice were challenged intradermally, by giving a 5-$\mu$l injection of a 100 $\mu$g $ml^{-1}$ suspension of *A. viscosus* in PBS into the left hind footpad using a fine needle attached to a Hamilton syringe (Hamilton Co., Reno, NV, USA). Five microlitres of sterile saline were injected into the right hind footpad as a negative control. The dorso-ventral thickness of the hind footpad was measured using a dial micrometer (Mitutoyo, Kawashaki-Shi, Japan). Measurements were taken before challenge and subtracted from reading of footpad swelling after 24 h.

*Experimental procedures*

*Induction of oral tolerance*

*Immunization procedure*. Intragastric challenge was carried out by passing a thin flexible tubing attached to a rounded end needle into the stomach of the mouse. *Actinomyces viscosus* was given as a 100 $\mu$g of bacterial suspension in 0.2 ml of PBS containing 7.5% sodium bicarbonate (PBS-SB). Mice were divided into three groups of eight mice. One group of animals was i.g. challenged with bacterial suspension on day 1, 2 and 5 (group III), while the other two groups of animals (I and II) were sham i.g. challenged with PBS-SB on the same days. Group I and II were the negative and positive control group, respectively. After the last gastric intubation, group II and III mice received two weekly i.p.

injections of 100 $\mu$g of *A. viscosus* in 100 $\mu$l of saline, while group I mice were i.p. challenged with saline only.

*Duration of tolerance*. Mice were given an i.g. challenge of *A. viscosus*, as described above. Following the last i.g. intubation, mice were then divided into six groups of three mice and were immunized by i.p. injection of *A. viscosus* at time intervals of 1, 2, 4, 6, 8, and 12 weeks, followed by an i.p. boost 1 week later. A control group was given saline instead of *A. viscosus*. One week after the last i.p. challenge, the DTH response and serum isotype antibodies to *A. viscosus* were determined.

*Adoptive transfer experiment*
Mice were i.g. primed as above, and 12 weeks after the last i.g. intubation, mice were killed and the spleens removed. Forty million viable cells in 100 $\mu$l of sterile saline solution were injected into the tail vein of systemically primed syngeneic recipients (Group T). Spleen cells of mice that were i.g. sham primed were transferred by passive adoption as above and served as the control group (group C). After 24 h and at 1 week, the recipients were all immunized by an i.p. injection of 100 $\mu$g of an *A. viscosus* suspension in 100 $\mu$l of saline. Footpad reactions (DTH) serum antibody levels to *A. viscosus* were determined as described above.

In vitro *co-culture assay*
Mice were i.g. challenged with *A. viscosus* or saline for two consecutive days and repeated at day 5, while another group of mice were immunized i.p. with *A. viscosus* weekly for 2 weeks. Twelve weeks after the last i.g. immunizations, mice were killed. The systemically immunized mice were killed 1 week after the last i.p. immunization. Spleens were removed and single cell suspensions were prepared. The cell suspensions were washed three times in RPMI-1640 medium (CSL, Melbourne, Australia) containing 50 IU $ml^{-1}$ of penicillin (CSL) and 50 $\mu$g $ml^{-1}$ streptomycin (CSL). Cell were resuspended in complete RPMI medium containing 10% heat inactivated fetal calf serum (CSL), 50 IU $ml^{-1}$ penicillin (CSL) and 50 $\mu$g $ml^{-1}$ streptomycin (CSL), 2 mM L-glutamine (Sigma), and $5 \times 10^5$ mM 2-mercaptoethanol. One million spleen cells from mice i.g. challenged with *A. viscosus* were co-cultured with an equal number of spleen cells from mice i.p. immunized with this bacterium (Group T) in 24 well plates (Nuncl). As a control (group C), spleen cells from sham i.g. primed animals were co-cultured with i.p. primed spleen cells. All cultures were incubated for 10 days in a 37°C humidified incubator in an atmosphere of 5% $CO_2$ in air and stimulated with 4 $\mu$g/well of *A. viscosus* at day 3. The culture supernatants were harvested and splenic antibody levels to *A. viscosus* were determined by an ELISA.

*Antigenic specificity of oral tolerance*
In order to determine if oral tolerance was specific to *A. viscosus*, mice were divided into five groups. Three groups of mice (Groups a, b and e) were primed i.g. with saline while the two remaining groups (c and d) were challenged i.g. with *A. viscosus*, as described above. Subsequently, group a was sham i.p. challenged with saline, groups b and c were challenged with *A. viscosus*. Groups d and e were challenged with *P. gingivalis*, following the immunization procedures as described above. The DTH and serum antibody levels to *A. viscosus* were determined as above.

*Statistical analysis*
The results of different immunizations and duration was analyzed by One way-analysis of variance followed by the Fischer's least square differences. The Student *t*-test was used to analyze the results of the cell transfer. The statistical analysis was calculated by using a statistical package (Minitab Inc., State College, PA, USA).

## Results

*The induction of oral tolerance*
Following gastric and systemic immunization, both DTH and serum antibody levels specific for *A. viscosus* were determined. As seen in Figure 1, the mean footpad swelling of the *A. viscosus* treated mice (III) was significantly depressed when compared with the positive control group (II) ($P < 0.01$). The DTH response of group III was not significantly different from the negative control (group I) ($P > 0.05$). Serum specific isotype antibodies (IgA, IgM and IgG) to *A. viscosus* in group III were significantly suppressed when compared

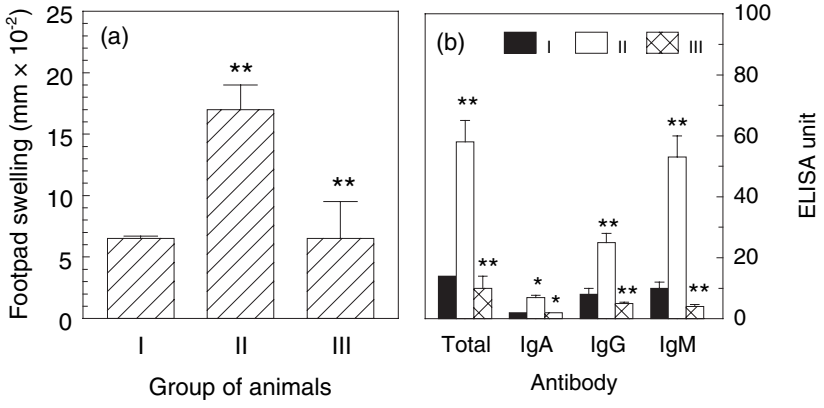


**Figure 1** Standard deviation of delayed type hypersensitivity response (**a**) and serum antigen-specific antibodies (**b**) in mice intragastrically immunized with saline (group I and II) or *Actinomyces viscosus* (group III). All groups were then systemically challenged with *A. viscosus*. $*P < 0.05$; $**P < 0.01$

with those of group II ($P < 0.01$). IgM was the most suppressed at 95%, followed by IgG (86%) and IgA (30%).

*Duration of oral tolerance*

A decreased in footpad swelling was observed for 3 weeks after gastric priming and by week 4, the swelling was elevated but still less than the control ($P < 0.01$) (Figure 2). Unlike the DTH responsiveness, serum isotype levels specific to *A. viscosus* showed a different pattern. Low levels of specific IgM antibodies were detected in the gastric treated animals which persisted for 8 weeks ($P < 0.01$), and levels started to increase at week 12 ($P < 0.05$). Suppression of specific IgA antibodies could be observed up to week 8 ($P < 0.05$). Interestingly, very low level of serum IgG in the gastric immunized mice could still be seen at week 12 ($P < 0.01$) (Figure 2).

*Adoptive transfer experiments*

In order to determine the cellular mechanisms of sustained suppression of specific IgG antibodies, adoptive spleen cell transfer *in vivo* and *in vitro* experiments were carried out. The results of the *in vivo* experiments indicated that spleen cells from mice which had been challenged by administering an i.g. dose of *A. viscosus*, did not transfer suppression of DTH to a naive syngeneic recipient when later challenged by a systemic i.p. injection of *A. viscosus*. When these mice were tested for footpad swelling to measure DTH, no statistical difference between the group T and C were detected ($P > 0.05$), shown in Figure 3. However, specific serum IgM antibodies to *A. viscosus* were elevated significantly when compared with the control group ($P < 0.01$), while no statistical difference could be seen in the IgA specific antibody levels ($P > 0.05$). On the contrary, serum specific IgG antibodies were depressed at a significant level in the group T when compared with the control (group C) ($P < 0.01$) (Figure 3).

Spleen cells from *A. viscosus*- or saline-gastrically primed mice were transferred *in vitro* and co-cultured with spleen cells from mice challenged by a systemic i.p. injection of *A. viscosus*, specific antibody levels in the tissue culture supernatant was measured by an ELISA. Specific total, IgA and IgM antibodies of the systemically immunized cells co-cultured with gastrically immunized cells (group T) showed statistically difference with those of the control group (group C) ($P > 0.05$) (Figure 4). The reverse was observed for the specific IgG antibodies of group T which were significantly lower than those of the control group ($P < 0.01$) (Figure 4).

## Specificity of oral tolerance

When mice were challenged with an i.g. dose of *A. viscosus* followed by a systemic i.p. challenge of the same bacterium (group c), DTH response of this group was

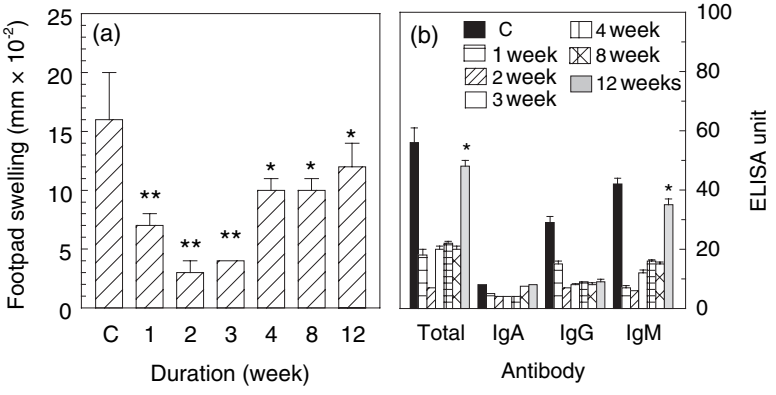


**Figure 2** Duration of oral tolerance to *Actinomyces viscosus*. Mean and standard deviation of delayed type hypersensitivity response (**a**) and serum antigen-specific antibodies (**b**) in mice intragastrically (i.g.) immunized with saline or *A. viscosus*. All groups were then systemically challenged with *A. viscosus* at the indicated time. For group C, systemic challenge was carried out 1 week after the last i.g. immunization. $*P < 0.05$; $**P < 0.01$

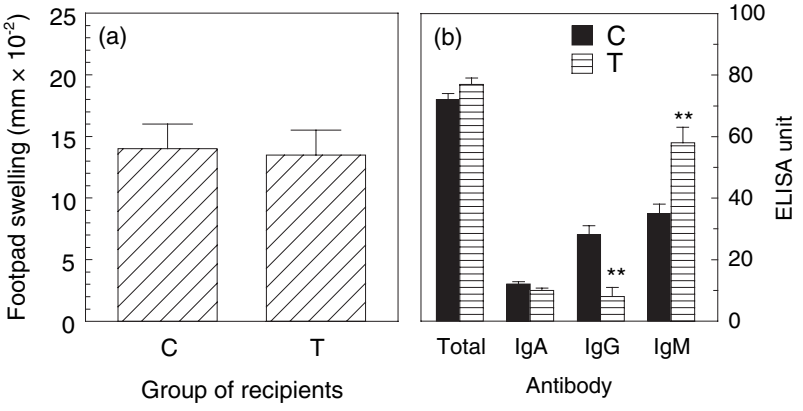


**Figure 3** Mean and standard deviation of delayed type hypersensitivity response (**a**) and serum antigen-specific antibodies (**b**) in the recipients of spleen cells from mice intragastrically (i.g.) immunized with saline (group C) or *Actinomyces viscosus* (group T). Spleen cells from gastrically immunized mice were obtained at 12 weeks after the last i.g. immunization. All recipients were then systemically challenged with *A. viscosus* 24 h after the cell transfer. $**P < 0.01$

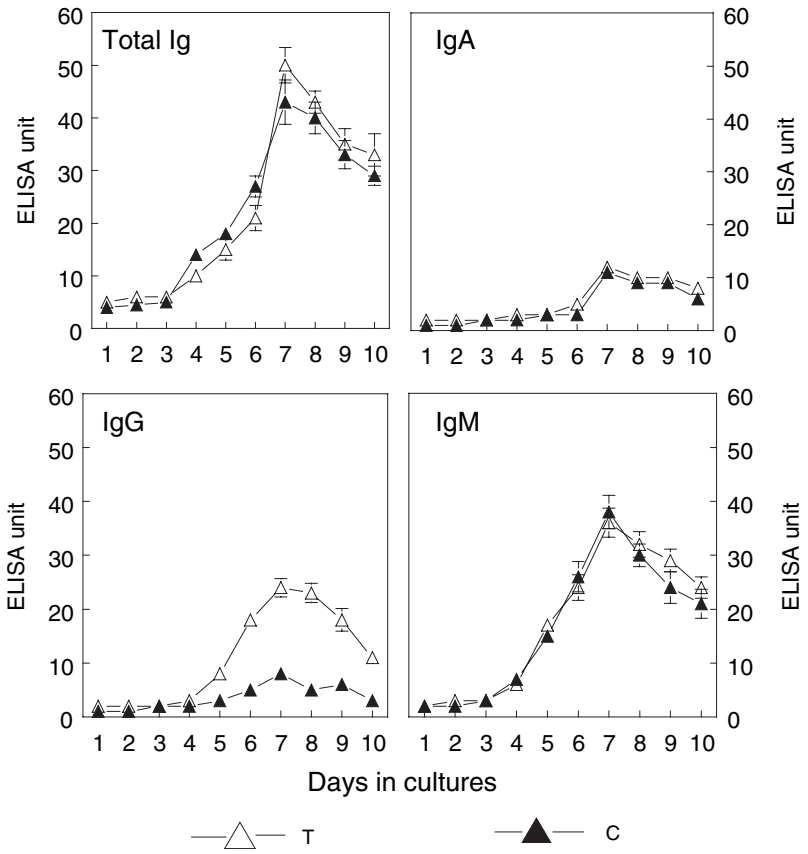


**Figure 4** Mean and standard deviation of *Actinomyces viscosus*-specific antibody production in the spleen cell co-cultures. Spleen cells from systemically primed mice were co-cultured with those from mice intragastrically (i.g.) immunized with saline (group C) or *A. viscosus* (group T). Spleen cells from gastrically immunized mice were obtained at 12 weeks after the last i.g. immunization. The cultures were stimulated with antigen at day 3. $*P < 0.05$; $**P < 0.01$

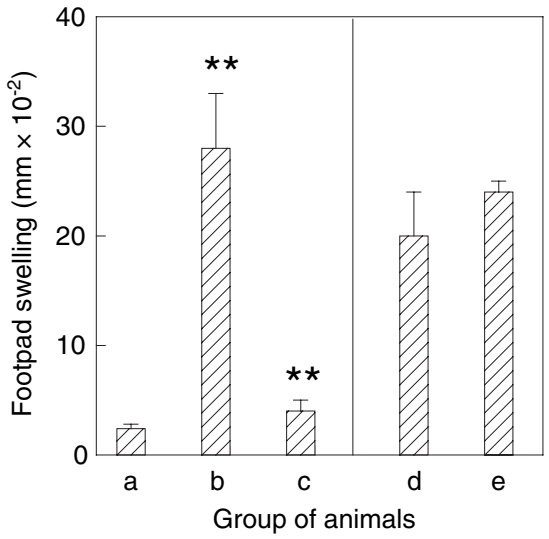


**Figure 5** The antigen specificity of delayed type hypersensitivity response in oral tolerance to *Actinomyces viscosus*. Footpad swelling in group a, b, and c was induced by *A. viscosus*, whereas that in group d and e was induced by *Porphyromonas gingivalis*. Bar represents standard deviation of the mean. $**P < 0.01$

significantly suppressed when compared with that of group b which was i.g. immunized with saline and followed by systemically immunized with *A. viscosus* ($P < 0.01$) (Figure 5). Of interest, DTH response of mice i.g. immunized with *A. viscosus* and then followed by systemically immunized with *P. gingivalis* (group d) was comparable with that of group e which was sham gastrically and then systemically immunized with *P. gingivalis* ($P > 0.05$) (Figure 5). Similarly, when serum antibody isotypes specific to *A. viscosus* were determined, the levels of serum antigen-specific antibodies in group c were significantly suppressed when compared with those in group b ($P < 0.01$) (Figure 6). The levels of serum antibodies specific to *P. gingivalis* in group d were comparable with those in group e ($P < 0.05$).

## Discussion

The present study showed that the mucosal presentation of *A. viscosus* in mice induced oral tolerance with a significant suppression of both cellular and humoral immune responsiveness. Hence, these results are in accordance with the previous findings that oral bacteria are indeed capable of initiating oral tolerance (Challacombe and Tomasi, 1980; Keys *et al*, 1987; Seymour and Ford, 1990).

It is interesting to note in this study that *A. viscosus* specific IgA, IgG and IgM antibody levels were all suppressed. The IgM antibody level was found to be suppressed the most, although the duration of this suppression was limited. However, the IgG antibody level remained suppressed for at least 12 weeks. It is now recognized that IgM-bearing B cells are the most susceptible to tolerization by T-independent (Ti)

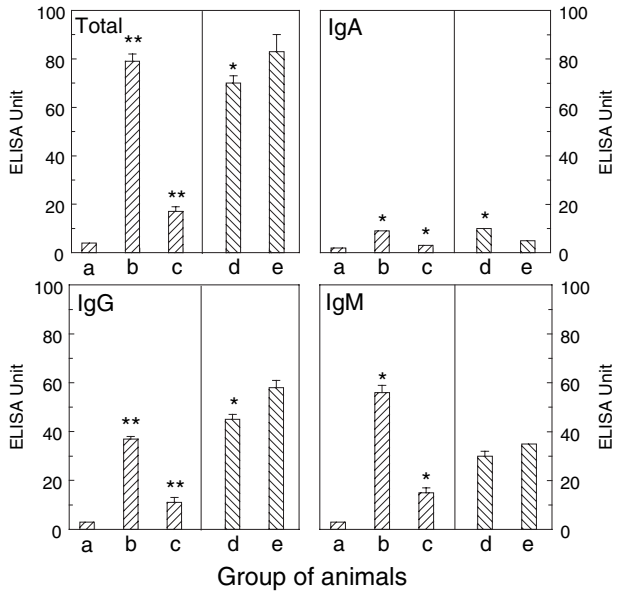


**Figure 6** The antigen specificity of serum antibody levels in oral tolerance to *Actinomyces viscosus*. Serum antibodies in group a, b, and c were specific to *A. viscosus*, whereas those in group d and e were specific to *Porphyromonas gingivalis*. Bar represents standard deviation of the mean. $*P < 0.05$; $**P < 0.01$

antigens (Vivetta *et al*, 1977). *Actinomyces viscosus* has been reported to be a Ti-antigen (Engel *et al*, 1977) and this may explain the effect on IgM in the present study. Furthermore, previous reports have shown that IgG and IgM, but not IgA, are suppressed by oral presentation of OVA and SRBC (Mattingly, 1983; Saklayen *et al*, 1984). The results in the present study showed that there was a 30% suppression of IgA antibodies which lasted for 8 weeks. Therefore, the nature of the antigen may explain the apparent discrepancy between this and other studies (Mattingly, 1983; Saklayen *et al*, 1984). It seems likely that mucosal administration with oral tolerance is able to suppress IgA, IgG and IgM specific antibodies by yet unknown mechanisms. Isotype specific-suppressor T cells do exist in the mouse (Lynch, 1987) and different antigens may activate different suppressor T cells resulting in the different isotype suppression seen.

In the present study, DTH unresponsiveness was abrogated earlier than that of humoral immune response. In contrast, following oral administration of OVA, suppression of DTH was longer than that of humoral immunity (Strobel and Ferguson, 1987). Again, the most likely reason for this difference is the nature of the antigen used. Oral tolerance induced by OVA was abrogated at day 60 compared with day 30 for *S. mutans* (Challacombe and Tomasi, 1980), indicating that food antigens may be more tolerogenic than bacterial antigens. Food antigens have been suggested to possess a unique antigenic epitope that induces T cells but only acts weakly on B cells (Challacombe and Tomasi, 1980; Wold *et al*, 1989) and it is possible that bacterial antigens such as those found on *A. viscosus* may stimulate B cells and, to a lesser degree, T cells (Engel *et al*, 1977). This could explain the long-lasting DTH suppression following oral administration of OVA, whereas *A. viscosus* induces a short-term transient effect on DTH with a more sustained effect on humoral immunity.

The sustained suppression especially of antigen-specific IgG antibodies could be due to isotype-specific suppressor T cells as suggested above. This is supported by the fact that the transfer of mucosally tolerized spleen cells only inhibited the production of IgG antibodies as seen in the present study. Indeed, the IgG-bearing suppressor T cells induced by gastric administration of antigen have been demonstrated previously (Mattingly and Waksman, 1980; Richman *et al*, 1981). FcR-bearing suppressor T cells appear to suppress IgG production markedly, while only weakly with IgM production (Lynch, 1987). Thus, it would seem reasonable to suggest that the long-lasting IgG suppression observed in this experiment might be due to IgG-bearing suppressor T cells. Furthermore, oral tolerance to allergens and OVA involved suppression of both the Th1 and Th2 type response (Hirahara *et al*, 1998; Kang *et al*, 1999); however, mucosally suppressed Th2 type response was more pronounced when compared with mucosally suppressed Th1 type response cells (Kang *et al*, 1999). Feeding with OVA induced high levels of interferon-gamma (IFN-$\gamma$) and the oral tolerance to this antigen remained intact in IFN-$\gamma$ receptor knockout mice (Mowat *et al*, 1999). IFN-$\gamma$ is known to upregulate the DTH response (Young and Hardy, 1995). One may assume, therefore, that mucosal presentation of *A. viscosus* might stimulate IFN-$\gamma$-producing IgG-specific suppressor cells which, in turn, might induce the limited life of the DTH unresponsiveness but long-lasting specific IgG antibody suppression. However, whether or not the pronounced suppression of antigen-specific IgG antibody levels seen in the present study is due to this pathway remains to be investigated. It is not understood at this stage what is the cell phenotype or what is the cytokine profile of the putative suppressor cell population.

The present study showed that suppression of both DTH and serum antibodies specific to *A. viscosus* was observed only when mice were gastrically administered with *A. viscosus* and systemically challenged with the same antigen. However, the immune response to *P. gingivalis* remained high in mice gastrically immunized with *A. viscosus* and systemically challenged with *P. gingivalis*. These results suggest that the induction of oral tolerance to *A. viscosus* is an antigen-specific phenomenon. The results of the present study are in accordance with the previous study showing that systemic immune suppression following oral immunization with *S. mutans* could only be induced by systemic challenged with this bacterium but not OVA (Challacombe and Tomasi, 1980). The results of the present study are not surprising and not only confined to oral bacteria. Other studies have shown that ingestion with different protein antigens such as myelin basic protein and collagen type II in mice also led to the antigen-specific oral tolerance (Sosroseno, 1995; Faria and Weiner,

1999; Garside and Mowat, 2001). The mechanisms for this specificity is unclear and these could include the generation of antigen-specific suppressor T cells (Ishii *et al*, 1993; Chen *et al*, 1995; Ke and Kapp, 1996) or the antigen specific-suppressor factors which may be from the serum (Kagnoff, 1978) or spleen cells (Mattingly and Waksman, 1980).

The extrapolation of these results in oral diseases, particularly periodontal lesions, is still speculative. *Actinomyces viscosus* are abundantly present in subgingival plaque of patients with gingivitis (Haffajee *et al*, 1997; Noiri *et al*, 1997). Furthermore, the number of peripheral blood T cells specific to this bacterium was slightly increased in patients with gingivitis compared with controls (Mahanonda *et al*, 1989). The ability of this bacterium to upregulate the expression of CD83 on gingival B cells and the production of pro-inflammatory cytokines by oral epithelial cells suggests that this bacterium play a role on the immunopathogenesis of periodontal disease (Mahanonda *et al*, 2002; Han *et al*, 2003). As this bacterium is part of the commensal oral flora, continuous swallowing of these bacteria could stimulate an immune response in the lower gastrointestinal tract. Thus, it can be speculated that the induction of oral tolerance to periodontopathic bacteria may contribute to the immune imbalance of the inflamed gingival (Sosroseno and Herminajeng, 1995; Gemmell *et al*, 2002) and the local immune response that may also be induced (Crawford and Clark, 1986; Mahanonda *et al*, 2002; Han *et al*, 2003) would prevent the adherence of these microorganisms on the tooth and mucosal surface.

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